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Mapping of a Gene Determining Tuberous Sclerosis to Human Chromosome 11q14-11q23

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Tuberous sclerosis (TSC) is a dominantly inherited disorder characterized by hamartomas and hamartias in one or more organs, most often in skin, brain, and kidneys. Analysis of the basic genetic defect in tuberous sclerosis would be greatly expedited by definitive determination of the chromosomal location of the TSC gene or genes. We have carried out genetic linkage studies in 15 TSC families, using 34 polymorphic markers including protein markers and DNA markers. Pairwise lod scores were calculated using LIPED, and multipoint analyses were carried out using MENDEL. In the pairwise linkage analysis, using a penetrance value of 90%, a significant positive lod score was obtained with MCT128.1 (D11S144), 11q22-11q23, $Z_{\max}$ 3.26 at $\theta = 0.08$. The tyrosinase probe TYR (11q14-11q22) gave a maximum lod score of 2.88 at $\theta = 0$. In the multipoint analyses the most likely order is (TYR,TSC)-MCT128.1-HHH172. Homogeneity analysis was carried out using the USERM9 subprogram of MENDEL, which conducts the admixture test of C. Smith (1963, *Ann. Hum. Genet.* 27: 175-182). This test provided no evidence for genetic heterogeneity (that is, non-11-linked families) in this data set.

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INTRODUCTION

Tuberous sclerosis (TSC) is a dominantly inherited disorder characterized by hamartomas and hamartias in one or more organs, most often the skin, brain, and kidneys (Gomez, 1988). Both hamartias and hamartomas represent developmental abnormalities characterized by abnormal cell or tissue arrangement and abnormal function. Hamartomas are distinguished by the additional feature of abnormal cellular proliferation. Dominant inheritance of this disorder was first

reported by Gunther and Penrose (1935). Criteria for definitive and presumptive diagnoses of TSC have been established (Gomez, 1988). A definitive diagnosis is made if one or more of the following features are present: cortical tuber, subependymal glial nodules, retinal hamartoma, facial angiofibroma, ungual fibroma, forehead or scalp fibrous plaque. A presumptive diagnosis of TSC is made if two or more of the following features are present: infantile spasms; myoclonic, tonic, or atonic seizures; hypomelanotic macules; peripapillary retinal hamartoma; gingival fibromas; enamel pits; renal tumors; renal cysts; cardiac rhabdomyoma; pulmonary lymphangiomyomatosis; wedge-shape cortical or subcortical calcification in the brain; multiple subcortical hypomyelinated lesions in brain CT scans; subcortical hyperintense lesions in magnetic resonance scans of the head; first-degree relative with TSC (Gomez, 1988).

Although TSC is known to be inherited as an autosomal dominant mutation, a large percentage of cases are thought to represent new mutations, since manifestations occur in children whose parents show no manifestations of the disorder. However, unaffected parents may have more than one affected child. It is not clear whether such cases represent examples of gonadal mosaicism or reduced penetrance in one of the parents (Baraitser and Patton, 1985).

The population frequency of tuberous sclerosis was estimated by Wiederholt *et al.* (1985) as 1 in 10,000. Estimations of the frequency of new mutations have varied from 1 in 60,000 (Gunther and Penrose, 1935) to 1 in 84,000 live births (Singer, 1971). Accurate assessments of the percentage of cases that represent new mutations and of the degree of penetrance of TSC are not realistic at this time, given the limitations of clinical examination, CT scans, and magnetic resonance imaging. Considerable variation in severity of

FIG. 1. Pedigrees of TSC families used in these studies. ●, Affected individual; ○, unaffected individual; ◐, likely obligate heterozygote or gonadal mosaic; ⊖, individual whose clinical status was not clear at the time of examination and who was not included in the present study. SZ, seizures; HM, hypomelanotic macules; AL or LS, ash leaf spots; AF, angiofibroma; UF, ungual fibromas; PF, perioral fibroma; SP, shagreen patch; FP, forehead plaque; LP, lipoma; ST, skin tags; RH, retinal hamartoma; CC, cerebral calcifications; CT, cortical tubers; GA, giant cell astrocytoma; DD, developmental delay; MR, mental retardation; BT, brain tumor; RA, renal angiomyolipoma; KT, kidney tumors; TA, thyroid adenoma.

TSC may be encountered among affected members of a particular family, providing evidence of variable expressivity.

Analysis of the basic genetic defect in tuberous sclerosis would be greatly expedited by definitive determination of the chromosomal location of the TSC gene

or genes. Fryer *et al.* (1987) published evidence for location of a TSC gene on chromosome 9q34, based on a peak cumulative lod score of 3.85 at $\theta = 0$, between TSC and ABO. Connor *et al.* (1987a,b) reported linkage between TSC and another chromosome 9q34 marker ABL in three Scottish families. The maximum lod score

FIG. 1—Continued

was 3.18 at $\theta = 0$. Subsequently, there have been reports of families for which data analysis did not support linkage between TSC and ABO (Northrup *et al.*, 1987; Renwick, 1987). Povey *et al.* (1988) published evidence

for recombination between TSC and ABL in two families. Kandt *et al.* (1989) carried out two-point and multilocus linkage analyses of TSC and three chromosome 9q markers: ABO, MCT136, and ABLK2.

TS-21

TS-24

TS-26

TS-28

FIG. 1—Continued

These analyses allowed them to exclude TSC for a distance of 20 cM adjacent to the ABO locus. Results of their two-point and multipoint data were subjected to analysis using the HOMOG program of Ott (1974). There was insufficient evidence for rejection of the null hypothesis of homogeneity.

In this paper we describe results of linkage analysis carried out in 15 families with tuberous sclerosis, using 34 polymorphic markers, including protein markers and DNA markers.

MATERIALS AND METHODS

TSC Families Studied

A total of 15 families were analyzed. These included one 5-generation, three 4-generation, eight 3-generation, and three 2-generation families. Data are derived from 150 individuals. Documentation of the pedigrees of families analyzed in this study is provided in Fig. 1. The clinical manifestations in the affected individuals are documented on the pedigrees. An explanation of the abbreviations used is provided in the legend to Fig. 1.

Sample Collection and Derivation of Cell Lines

Blood samples were collected in ACD B tubes and in heparin tubes. Heparinized blood was used for isolation of plasma and red cells for analysis of blood groups, plasma protein markers, and red cell enzyme markers. Blood collected in ACD was used for isolation of peripheral blood lymphocytes by means of Ficoll-Paque (Pharmacia) density gradient centrifugation. The lymphocyte sample was used for direct preparation of DNA. In 9 of the 15 families lymphoblastoid cell lines were established by seeding lymphocytes, at a concentration of approximately 2 million cells/ml, in RPMI 1640 medium containing 10% fetal calf serum, 2 μ g/ml cyclosporin, and Epstein-Barr virus. The virus was present in filtered frozen medium derived from a marmoset cell line which carries the virus. This medium was added at a concentration of 10%.

Preparation of Genomic DNA

Pellets of peripheral blood lymphocytes or lymphoblastoid cell lines were resuspended in lysis buffer containing 10 mM Tris-HCl, pH 8.0, 50 mM EDTA, 0.2% SDS (ultra-pure sodium dodecyl sulfate, Sigma). They were then digested using 50 μ g/ml proteinase K for 4

TABLE 1
Lod Scores at Different Values of Recombination Fraction (θ) for Tuberous Sclerosis
and 24 Polymorphic Markers

Marker/locus	Chromosomal location	Ref.	θ				
			0.05	0.10	0.20	0.30	0.40
Rh	1p36	(34)	-4.07	-2.44	-0.99	-0.42	-0.15
PGMI	1p22.1	(6)	-3.47	-2.13	-0.96	-0.42	-0.14
FY	1p12-q23	(49)	-3.28	-1.06	0.98	0.75	0.43
JK	2p	(48)	-3.96	-1.45	-0.57	-0.67	-0.15
TGFA*	2p13	(5)	-2.07	-1.05	-0.26	-0.01	0.03
ACPI	2p25	(27)	-8.23	-5.14	-2.33	-0.99	-0.29
EDF64.2* (D3S46)	3	(37)	-1.18	-0.67	-0.25	-0.09	-0.02
pHM6*	4p11-q21	(47)	-0.54	0.11	0.45	0.37	0.14
pF47*	4p11-q21	(36)	-1.44	-0.89	-0.39	-0.15	-0.03
pEGF121*	4q21-q25	(36)	-0.72	-0.44	-0.19	-0.08	-0.02
PADH74 and 36*	4q21-q25	(36)	-4.70	-2.61	-0.90	-0.24	-0.10
GC	4q21	(38, 12)	-1.95	-0.88	-0.14	0.09	0.10
MNS	4q28	(12, 36)	-1.82	-1.43	0.25	0.40	0.24
BF	6p21	(41)	-2.05	-1.23	-0.51	-0.18	-0.04
GLO	6p21	(32)	-4.14	-2.72	-1.40	-0.97	-1.14
HHH171* (D6S38)	6p	(24)	-2.18	-1.37	-0.65	-0.31	-0.12
P	6p	(13)	0.01	0.00	-0.01	-0.01	-0.02
PSW50*	8p23	(59)	-0.72	-0.44	-0.20	-0.07	-0.02
ESD	13q14	(53)	-0.86	-0.54	-0.23	-0.08	-0.01
pAW101*	14q13	(11)	-2.51	-1.18	-0.67	-0.25	-0.09
HP	16q22	(35)	-3.38	-2.73	-0.86	-0.33	-0.08
Alphoid*	17 cen	(26)	-0.46	-0.23	-0.06	-0.01	0.00
ADA	20q13	(23)	0.32	0.33	0.30	0.24	0.14
pMSI-27* (D20S4)	20	(18, 25)	-0.46	-0.23	-0.06	-0.01	0.00

* RFLP marker.

h at 37°C. Following digestion, DNA was extracted from these solutions by using phenol-chloroform, according to the method described by Maniatis *et al.* (1982).

Markers Analyzed

We analyzed 34 polymorphic markers, including 13 serum protein and red cell markers and 21 polymorphic DNA markers. The markers analyzed are distributed on 13 of the 22 human autosomes. Not all families were analyzed for each marker. Fourteen families were analyzed for the chromosome 9q markers, and 10 families were analyzed for the chromosome 11q markers. The difference in the number of families analyzed is because DNA supplies were depleted prior to commencement of certain of the analyses, where lymphoblastoid cell lines were not established. The markers analyzed are listed in Tables 1, 2, and 3. Methods used for characterization of markers are referenced in the tables.

The chromosome 11 probes MCT128.1, CJ52.208M2, HHH172, HBI18P1, and HBI18P2 were provided by Drs. Y. Nakamura and C. Julier. These latter probes are random DNA probes that have been assigned to the distal half of chromosome 11q (Nakamura and Julier, personal communication; Junien and McBride,

1989). The probe for tyrosinase, pMel 34-1, was provided by Dr. B. Kwon (Kwon *et al.*, 1987).

Linkage Analysis

The two-point linkage data were analyzed used the program LIPED (Ott, 1985). In order to facilitate data entry into the LIPED system, the LIPIN program (Trofatter *et al.*, 1986) was used. Since there is clear evidence that some individuals who may carry the TSC gene have no clinical manifestations and may also have negative findings in special examinations such as head CT scan, abdominal ultrasound, and X-ray, it is important that a penetrance factor less than 100% be included in the linkage calculations. In the two-point linkage analysis, lod scores were computed in two ways: first, setting the penetrance at 90%; and second, setting the penetrance at 97%. In the first model we postulate that an individual has a 90% conditional probability of being affected given that he has a copy of the TSC gene. In the second model an individual has a 97% conditional probability of being affected given that he has a copy of the TSC gene. The 90% penetrance value was initially selected on the basis of information on TSC cases accumulated by Dr. K. Dumars at the University of California, Irvine. Analysis was subsequently

TABLE 2
(a) Results of Pairwise Linkage Analysis of TSC and Chromosome 9q34 Markers
Using a 90% Penetrance Rate of TSC

Locus	Lod score at $\theta =$							Families
	0.00	0.05	0.10	0.15	0.20	0.30	0.40	
ABO	$-\infty$	-4.35	-2.27	-1.07	-0.46	-0.08	0.14	All except 11
ASSG3	-3.66	-0.21	-0.01	0.06	0.09	0.08	0.05	1,16,26
AK1	-10.05	-2.89	-1.87	-1.29	-0.91	-0.41	-0.14	8,16
ABL	-5.16	-0.72	-0.44	-0.29	-0.19	-0.08	-0.02	8

Note. ASSG3 is a probe derived from the ASS gene (40). AK1 was typed using the enzyme polymorphism (21) and a DNA probe (4). ABL was typed using a DNA probe ABL 3 (Baumann and Smith, personal communication).

(b) Allele Frequencies of Chromosome 9 Markers

(i) ABO blood groups					Ref. (16)
A1	A2	B	O		
0.2090	0.0696	0.0612	0.6602		
(ii) DNA markers					
Marker	Allele 1		Allele 2		
ASSG3	0.50		0.50		
AK1	0.79		0.21		
ABL	0.20		0.80		

Note. Allele 1 corresponds to larger molecular weight polymorphic DNA fragment.

carried out at 97% penetrance based on the observation that in the pedigrees collected for linkage studies only 4 of 150 cases were suggestive of nonpenetrance. The two-point linkage data were analyzed using the program LIPED (Ott, 1985). Allele frequencies for the marker loci are given in Tables 2b and 3b. The frequency for the TSC allele in all analyses is 0.0001, based on the population frequency of TSC, 1 in 10,000 (Wiederholt *et al.*, 1985). Two-point analyses were conducted using penetrances of both 90 and 97%. No age of onset correction was applied.

Multipoint Analyses

Multipoint analyses were performed using the USERM7 routine of the MENDEL package of computer programs (Lange and Boehnke, 1983) on a Microvax computer. A penetrance value of 90% was used in all multipoint analyses. Log likelihoods were calculated for each of the possible locations of the TSC locus relative to a map of three markers having a known order. For each location, the log likelihood is compared with the log likelihood calculated for another location. The odds of alternative locations of the TSC locus, compared with the location identified by the maximum likelihood, are computed by deriving the difference in their log likelihoods. Comparisons of these differences allow one to assess the strength of the evidence for the most likely order.

Heterogeneity Testing

Heterogeneity testing was performed using the USERM9 subprogram of the MENDEL package, which conducts the admixture test (or A-test) of Smith (1963), on a Microvax computer. The penetrance of TSC was set equal to 0.9. The null hypothesis tested was that all families analyzed have a TSC gene on chromosome 11 vs the alternative hypothesis that TSC in a percentage of families (α) is not caused by this gene. The log likelihood and estimates of θ in males and females were obtained when α equaled 1 (i.e., homogeneity) and when α was estimated.

RESULTS

Two-Point Linkage Analysis

Linkage analysis between TSC and the 24 marker loci listed in Table 1 provided no evidence of linkage. Results of two-point linkage analysis between TSC and the chromosome 9q34 markers ABO, ABL, ASSG3, and AK1, listed in Table 2, do not support assignment of the TSC gene to this region. Results of two-point linkage analysis between TSC and 6 markers that map to chromosome 11q14-qter (tyrosinase, MCT128.1, CJ52.208M2, HBI18P1, HBI18P2, and HHH172) are listed in Table 3. A significant positive lod score was obtained with the probe MCT128.1 ($Z_{\max}$ 3.26 at θ

TABLE 3
(a) Results of Pairwise Linkage Analysis of TSC and Chromosome 11q Markers
(Penetrance Value for TSC 0.9)

Locus	Lod score at $\theta =$							Families
	0.00	0.05	0.10	0.15	0.20	0.30	0.40	
TYR	2.88	2.58	2.27	1.97	1.64	1.04	0.49	16,21,15
128.1	-3.25	3.20	3.23	3.0	2.64	1.8	0.87	3,8,21,24,15,16,26,28
208M2	$-\infty$	0.91	1.01	0.95	0.84	0.56	0.27	1,3,15,16,8,11,20,21,26
HHH172	-3.22	0.26	0.56	0.65	0.62	0.40	0.15	21,3,26,11,24,15
18P1	1.39	1.24	1.09	0.96	0.81	0.68	0.54	16,26
18P2	-7.02	-0.91	-0.44	-0.21	-0.09	0	0.01	21,11

References for chromosomal assignments:

- 128.1 or MCT128.1 (D11S144) (28)
- 208M2 or CJ52.208M2 (D11S351) (28)
- H172 or HHH172 (D11S350) (28)
- 18P2 or HBI18P2 (D11S147) (28)
- 18P1 or HBI18p1 Nakamura and Julier, personal communication
- TYR or pMel 34-1 (54)

(b) Allele Frequencies of Chromosome 11 DNA Markers

Marker	Allele 1	Allele 2
TYR	0.52	0.48
128.1	0.52	0.48
208M2	0.41	0.59
HHH172	0.60	0.40
18P1	0.60	0.40
18P2	0.25	0.75

Note. Allele 1 corresponds to higher molecular weight polymorphic DNA fragment.

= 0.08). The lod score obtained with the tyrosinase probe TYR was 2.88 at $\theta = 0$. These scores were obtained when the penetrance was calculated at 90%. When a 97% penetrance factor was used in calculating the lod scores, only very minimal changes were noted: the peak lod score with TSC and TYR was 2.92 at $\theta = 0$, i.e., a change of 0.04; the peak lod score between TSC and MCT128.1 was 2.96 at $\theta = 0.05$.

Multipoint Linkage Analysis

Since two-point linkage analysis provided evidence of linkage of a TSC gene on chromosome 11q, multipoint analyses were performed to infer the most likely location of the TSC gene with respect to three markers, tyrosinase, MCT128.1, and HHH172. These markers were selected for multipoint analyses because previous linkage and physical mapping studies (Junien and McBride, 1989) had determined their order on chromosome 11q to be centromere-tyrosinase-MCT128.1-HHH172-telomere.

Estimates of male and female recombination fractions and the log likelihoods under each location of the disease gene and the three markers listed above are shown in Table 4. Also shown are the odds of alternative locations of the TSC locus compared with the location identified by the maximum log likelihood.

Since there are no recombinants between tyrosinase and the disease gene, we cannot differentiate between two of the possible orders of these loci. We observed the largest log likelihood (-128.1) with the TSC locus centromeric to MCT128.1 and HHH172. As the TSC locus is moved away from tyrosinase, we observed a decrease in log likelihoods, as anticipated given the zero recombination observed between tyrosinase and TSC. In these data we find an odds ratio of 63.1 against the location of the TSC locus being telomeric to HHH172. However, we cannot exclude the possibility of the TSC locus lying between MCT128.1 and HHH172.

Heterogeneity Testing

The USERM9 subprogram of MENDEL was run using the three regionally assigned markers on chromosome 11q, TYR, MCT128.1, and HHH172. The maximum likelihood estimate of α for each marker tested is 1, with no difference in the log likelihood or estimates of θ_{male} and θ_{female} in each case. No formal test of heterogeneity could be conducted, as α was estimated at 1, the boundary value. We conclude that there is no evidence of genetic heterogeneity in the sample.

TABLE 4
Log Likelihoods of Multipoint Analyses

Order							Log likelihood	Odds
1 (TSC, TYR)	—	MCT128.1	—	HHH172			—128.1	
θ_f	0.096		0.220					
θ_m	0.0		0.066					
2 TYR	—	MCT128.1	—	TSC	—	HHH172	—128.7	4:1*
θ_f	0.0		0.071		0.317			
θ_m	0.0		0.0		0.025			
3 TYR	—	MCT128.1	—	HHH172	—	TSC	—129.9	63:1*
θ_f	0.204		0.148		0.170			
θ_m	0.00		0.00		0.00			

* Odds against this gene order compared with gene order 1.

DISCUSSION

Results of our studies strongly support the location of a tuberous sclerosis locus on chromosome 11. Our two-point linkage analysis data did not support localization of a TSC gene to human chromosome 9q34 region. The studies of Fryer *et al.* (1987) and Connor *et al.* (1987a,b) did not utilize chromosome 11q markers.

The assignment of a TSC gene to the vicinity of the tyrosinase gene is of interest in view of the abnormalities of pigmentation seen in this disorder. Tyrosinase in melanocytes is responsible for the conversion of tyrosine to DOPA and of DOPA to the DOPA quinones which give rise to melanin (Witkop *et al.*, 1978). One of the most common abnormalities in TSC is the occurrence of hypomelanotic macules, which may include typical ashleaf spots, large areas of depigmentation, or multiple confetti spots (Gomez, 1988). Cafe au lait spots also occur more frequently in TSC patients than in the normal population. We have noted that hypopigmented macules with darkly pigmented regions at their center occur frequently in TSC patients. In certain patients, white hair patches also occur. Electron microscopic examination of hypomelanotic macules from TSC patients has revealed that the melanosomes within melanocytes are reduced in number and size and in the degree of melanization (Rogers, in Gomez, 1988). It is possible that these pigmentation abnormalities in TSC may be due to the proximity of the TSC gene to the gene encoding tyrosinase.

The assignment of a TSC gene to human chromosome 11 is also of interest in light of two literature reports that describe the simultaneous occurrence of TSC and other genetic disorders. The ataxia telangiectasia gene was recently mapped to the 11q22–11q23 region by Gatti and co-workers (1988). The first report in the literature of increased radiation sensitivity in ataxia telangiectasia involved a patient with this disorder who was found on autopsy to have tuberous sclerosis (Gotoff *et al.*, 1967). TSC has been reported in two patients with features of multiple endocrine neo-

plasia type 1 (MEN1). The gene responsible for MEN1 was mapped to the chromosome 11q13 region (Bale *et al.*, 1987). It is of interest to note that in reviewing the significance of the cases with TSC and MEN1, Zimmerman (1988; see Gomez, 1988) considered two possibilities: first, that the fullest expression of TSC may include a form of multiple endocrine neoplasia resembling MEN 1; and second, that TSC and MEN1 are genetically linked.

We initiated a study of chromosome 11q markers following the finding of an infant who was the first case of TSC in a family and who had in addition an unbalanced 11/22 chromosomal translocation with a breakpoint in the 11q22–11q23 region (Clark *et al.*, 1988).

Further studies aimed at refining the regional localization of the TSC gene on chromosome 11 are in progress. Refined regional localization will open the way for detailed analysis of the molecular genetic defect in tuberous sclerosis. It will also be important to carry out linkage studies in additional families with TSC to establish whether there is genetic heterogeneity in this disorder. Families who have demonstrated linkage of TSC and chromosome 9q34 region should also be analyzed using a series of chromosome 11q markers. Additional studies should be carried out to exclude the possibility that chromosomal translocations are present in certain TSC families.

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REFERENCES

1. BADER, S. A., WAGNER, C., SMITH, M., NAKAMURA, Y., LA-LOUEL, J.-M., DOWDY, S. F., AND STANBRIDGE, E. J. (1989). Regional assignment of four probes on chromosome 11q. Manuscript submitted.
2. BALE, S. J., MARX, S. J., LANGFIELD, D., BALE, A. E., AND GREEN, J. (1987). Multiple endocrine neoplasia type 1 (MEN1): Genetic linkage studies. *Cytogenet. Cell Genet.* **46**: 575.
3. BARAITSER, M., AND PATTON, M. A. (1985). Reduced penetrance in tuberous sclerosis. *J. Med. Genet.* **22**: 29-31.
4. BECH-HANSEN, N. T., MARSHALL, K. J., AND KRAUS, S. L. (1989). TaqI RFLP in human adenylate kinase-1 region on chromosome 9. *Nucleic Acids Res.*, in press.
5. BRISSENDEN, J. E., DERYNK, R., AND FRANCKE, U. (1985). Mapping of transforming growth factor alpha gene on human chromosome 2 close to the breakpoint of the Burkitt's lymphoma t(2;8) variant translocation. *Cancer Res.* **45**: 5593-5597.
6. CARRITT, B., KING, J., AND WELCH, H. M. (1982). Gene order and localization of enzyme loci on the short arm of chromosome 1. *Ann. Hum. Genet.* **46**: 329-335.
7. CLARK, R. D., SMITH, M., PANDOLFO, M., FAUSEL, R. E., AND BUSTILLO, A.-M. (1988). Tuberous sclerosis in a liveborn infant with trisomy due to t(11q23.3:22q-11.2) translocation. *Amer. J. Hum. Genet.* **43**: 44 (abstract).
8. CONNOR, J. M., YATES, J. R. W., MANN, L., AITKEN, D. A., AND STEPHENSON, J. B. P. (1987a). Tuberous sclerosis: Analysis of linkage to red cell and plasma protein markers. *Cytogenet. Cell Genet.* **44**: 63.
9. CONNOR, J. M., PIRRI, L. A., YATES, J. R. W., FRYER, A. E., AND FERGUSON-SMITH, M. (1987b). Linkage of the tuberous sclerosis locus to DNA polymorphism detected by v-abl. *J. Med. Genet.* **24**: 544-546.
10. COOK, P. J. L., ROBSON, E. B., BUCKTON, K. E., SLAUGHTER, C. A., GRAY, J. E., BLANK, C. E., JAMES, F. E., RIDLER, M. A. C., INSLEY, J., AND HULTEN, M. (1978). Segregation of ABO, AK1 and ACONS in families with abnormalities of chromosome 9. *Ann. Hum. Genet.* **41**: 365-377.
11. DONLON, T. A., LITT, M., NEWCOM, S. R., AND MAGENIS, R. E. (1983). Localization of the restriction fragment length polymorphism D14S1 (pAW-101) to chromosome 14q32.1-32.2 by in situ hybridization. *Amer. J. Hum. Genet.* **35**: 1097-1106.
12. FALK, C. T., AND HUSS, J. (1985). Estimates of recombination frequencies between the chromosome 4 markers MNS, GC, and PLG. *Cytogenet. Cell Genet.* **40**: 626.
13. FELLOUS, M., BILLARDON, C., DAUSSET, J., AND FREZAL, J. (1971). Linkage probable entre les locus 'HL-A' et 'P'. *C. R. Acad. Sci. Paris* **272**: 3356-3359.
14. FRYER, A. E., CHALMERS, A., CONNOR, J. M., FRASER, I., POVEY, S., YATES, A. D., YATES, J. R. W., AND OSBORNE, J. P. (1987). Evidence that the gene for tuberous sclerosis is on chromosome 9. *Lancet* **1**: 659-661.
15. GATTI, R., BERKEL, I., BODER, E., BRAEDT, G., CHARMLEY, P., CONCANNON, P., ERSOY, F., FOROUD, T., JASPERS, N., LANGE, K., LATHROP, G. M., LEPPERT, M., NAKAMURA, Y., O'CONNELL, P., PATERSON, M., SALSER, W., SANAL, O., SILVER, J., SPARKES, R., SUSI, E., WEEKS, D., WEI, S., WHITE, R., AND YODER, F. (1988). Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature (London)* **336**: 557-560.
16. GIBLETT, E. R. (1969). "Genetic Markers in Human Blood," Blackwell, Oxford.
17. GOMEZ, M. (1988) "Tuberous Sclerosis," Raven Press, New York.
18. GOODFELLOW, P. J., DUNCAN, A. M. V., FARRER, L. A., HOLDEN, J. J. A., WHITE, B. N., KIDD, J. R., KIDD, K. K., AND SIMPSON, N. E. Localization and linkage of 3 polymorphic DNA sequences on human chromosome 20. (1987). *Cytogenet. Cell Genet.* **44**: 112-117.
19. GOTOFF, S. P., AMIRMOKRI, E., AND LIEBNER, E. J. (1967). Ataxia telangiectasia: Neoplasia, untoward responses to X irradiation and tuberous sclerosis. *Amer. J. Dis. Child.* **114**: 617-625.
20. GUNTHER, M., AND PENROSE, L. S. (1935). The genetics of epiloia. *J. Genet.* **31**: 413-430.
21. HARRIS, H., AND HOPKINSON, D. A. (1975). "Handbook of Enzyme Electrophoresis in Human Genetics," North Holland, Amsterdam.
22. HENRY, I., GALLANO, P., BESMOND, C., WEIL D., MATTEI, M. G., TURLEAU, C., BOUE, J., KAHN, A., AND JUNIEN, C. (1985). The structural gene for aldolase B (ALDB) maps to 9q13-q32. *Ann. Genet.* **49**: 173-180.
23. HERBSCHLEB-VOOGT, E., VOSSEN, J. M., PEARSON, P. L., AND MEERA KHAN, P. (1979). The gene determining the basic molecular defect in adenosine deaminase (ADA) deficiency associated severe combined immunodeficiency (SCID) is on chromosome 20. *Cytogenet. Cell Genet.* **25**: 164.
24. HOFF, M., NAKAMURA, Y., HOLM, T., PAYSON, R., O'CONNELL, P., LEPPERT, M., LATHROP, G. M., LA LOUEL, J.-M., AND WHITE, R. (1988). Isolation and mapping of a polymorphic DNA sequence (pHHH171) on chromosome 6p (D6S38). *Nucleic Acids Res.* **16**: 4742.
25. JORGENSEN, A. L., BOSTOCK, C. J., BOK, A. L., HOLDEN, J. J. A., WHITE, B. N., KIDD, J. R., KIDD, K. K., AND SIMPSON, N. E. (1987). Localization and linkage of three polymorphic DNA sequences on human chromosome 20. *Cytogenet. Cell Genet.* **44**: 112-117.
26. JORGENSEN, A. L., BOSTOCK, C. J., AND BOK, A. L. (1986). Chromosome specific sub-families within human aliphoid repetitive DNA. *J. Mol. Biol.* **187**: 185-196.
27. JUNIEN, C., KAPLAN, J.-C., BERNHEIM, A., AND BERGER, R. (1979). Regional assignment of red cell acid phosphatase locus to band 2p25. *Hum. Genet.* **48**: 17-21.
28. JUNIEN, C., AND MCBRIDE, O. W. (1989). Report of the committee on the genetic constitution of chromosome 11. *Cytogenet. Cell Genet.* **51**: 226-250.
29. KANDT, R. S., PERICAK-VANCE, M. A., HUNG, W.-H., GARDNER, R. J. M., NELLIST, M., PHILLIPS, K., WARNER, K., SPEER, M. C., CROSSEN, P. E., LAING, N. G., AND ROSES, A. D. (1989). Absence of linkage of ABO blood group locus to familial tuberous sclerosis. *Exp. Neurol.* **104**: 223-228.
30. KWON, B. S., HAQ, A. K., POMERANTZ, S. H., AND HALABAN, R. (1987). Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. *Proc. Natl. Acad. Sci. USA* **84**: 7473-7477.
31. LANGE, K., AND BOEHNKE, M. (1983). Extensions to pedigree analysis. V. Optimal calculations of Mendelian likelihoods. *Hum. Hered.* **33**: 291-301.
32. LOPEZ-LARREA, C., BOOTELLO, A., AND ARNAIZ-VILLENA, A. (1981). Linkage of GLO with HLA in a sample of the Spanish population. *Hum. Genet.* **57**: 317-320.
33. MANIATIS, T., FRITSCH, E. F., AND SAMBROOK, J. (1982). "Molecular Cloning, a Laboratory Manual" Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
34. MARSH, W. L., CHAGANTI, R. S. K., GARDNER, F. H., MAYER, K., NOWELL, P. C., AND GERMAN, J. (1974). Mapping human

- autosomes: Evidence supporting assignment of rhesus to the short arm of chromosome no. 1. *Science* **183**: 966-968.
35. MCGILL, J. R., YANG, F., BALDWIN, W. D., BRUNE, J. L., BARNETT, D. R., BOWMAN, B. H., AND MOORE, C. M. (1987). Localization of the haptoglobin alpha and beta genes (HPA and HPB) to human chromosome 16q22 by in situ hybridization. *Cytogenet. Cell Genet.* **38**: 155-157.
 36. MURRAY, J. C., BUETOW, K. H., SMITH, M., CARLOCK, L., CHAKRAVARTI, A., FERRELL, R., GEDAMA, L., GILLIAM, C., SHIANG, R., AND DE HAVEN, C. R. (1988). Pairwise linkage analysis of 11 loci on human chromosome 4. *Amer. J. Hum. Genet.* **42**: 490-497.
 37. NAKAMURA, Y., LEPPERT, M., O'CONNELL, P., WOLFF, R., HOLM, T., CULVER, M., MARTIN, C., FUJIMOTO, E., HOFF, M., KUMLIN, E., AND WHITE, R. (1987). Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* **235**: 1622.
 38. NAYLOR, S. L., MCCOMBS, J. L., MOORE, C. M., YANG, F., PARKER, J., AND BOWMAN, B. H. (1985). Mapping of human group specific component to chromosome 4. *Cytogenet. Cell Genet.* **40**: 710.
 39. NORTHROP, H., BEAUDET, A. L., O'BRIEN, W. E., HERMAN, G. E., LEWIS, R. A., AND POLLACK, M. (1987). Linkage of tuberous sclerosis and ABO blood group. *Lancet* **2**: 804-805.
 40. NORTHROP, H., LATHROP, M., LU, S.-Y., DAIGER, S., BEAUDET, A., AND O'BRIEN, W. E. (1989). Multilocus linkage analysis with the human arginosuccinate synthetase gene. *Genomics* **5**: 442-444.
 41. OLASEN, B., TEISBERG, P., GEDDE-DAHL, T., JR., AND THORSBY, E. (1975). The Bf locus in the HLA region of chromosome 6: Linkage and association studies. *Humangenetik* **30**: 291-296.
 42. OTT, J. (1974). Estimation of the recombination fraction in human pedigrees: Efficient computation of the likelihood for human linkage studies. *Amer. J. Hum. Genet.* **26**: 585-597.
 43. OTT, J. (1985). "Analysis of Human Genetic Linkage," Johns Hopkins Press, Baltimore.
 44. PANDOLFO, M., WAGNER, C., AND SMITH, M. (1988). An anonymous DNA probe (LAMP 92) detects a Pvu II polymorphism on human chromosome 9 (D9S29). *Nucleic Acids Res.* **16**: 14.
 45. POVEY, S., BURLEY, M. W., FRYER, A. E., OSBORNE, J., ALGAZALI, L. I., AND MUELLER, R. (1988). Genetic recombination between tuberous sclerosis and oncogene v-abl. *Lancet* **2**: 279-280.
 46. RENWICK, J. H. (1987) Tuberous sclerosis and ABO. *Lancet* **2**: 1096-1097.
 47. SCHMIDT, C. J., HAMER, D. H., AND MCBRIDE, D. W. (1984). Chromosomal location of human metallothionein genes: Implications for Menkes' disease. *Science* **224**: 1104-1106.
 48. SHERMAN, S. L., AND SIMPSON, S. P. (1985). Evidence for the location of JK and CO on chromosome 2 based on family studies. *Cytogenet. Cell Genet.* **40**: 143.
 49. SHERMAN, S. L., LEWIS, M., MCALPINE, P. J., AND KING, J. (1987). Comparison of orders involving FY among physically located chromosome 1 markers. *Cytogenet. Cell Genet.* **46**: 691.
 50. SINGER, K. (1971). Genetic aspects of tuberous sclerosis in a Chinese population. *Amer. J. Hum. Genet.* **23**: 33-40.
 51. SMITH, C. (1963). Testing for heterogeneity of recombination fraction values in human genetics. *Ann. Hum. Genet.* **27**: 175-182.
 52. SMITH, M., HAINES, J., TROFATTER, J., DUMARS, K., PANDOLFO, M., AND CONNEALLY, M. (1987). Linkage studies in tuberous sclerosis. *Cytogenet. Cell Genet.* **46**: 694-695.
 53. SPARKES, R. S., SPARKES, M. C., WILSON, M. G., TOWNER, J. W., BENEDICT, W., MURPHREE, A. L., AND YUNIS, J. J. (1980). Regional assignment of genes for human esterase D and retinoblastoma to chromosome 13q14. *Science* **208**: 1042-1044.
 54. SPRITZ, R., STRUNK, K., OETTING, W., AND KING, R. (1988). RFLP for Taq 1 at the human tyrosinase locus. *Nucleic Acids Res.* **16**: 9890.
 55. SU, T.-S., NUSSBAUM, R. L., AIRHART, S., LEDBETTER, D. H., MOHANDAS, T., O'BRIEN, W. E., AND BEAUDET, A. L. (1984). Human chromosomal assignments for 14 arginosuccinate synthetase pseudogenes: Cloned DNAs as reagents for cytogenetic analysis. *Amer. J. Hum. Genet.* **36**: 954-964.
 56. TROFATTER, J. A., HAINES, J., AND CONNEALLY, M. (1986). Lipin, an interactive data entry and management for Liped 1986. *Amer. J. Hum. Genet.* **39**: 147-148.
 57. WIEDERHOLT, W. C., GOMEZ, M. R., AND KURLAND, L. T. (1985). Incidence and prevalence of tuberous sclerosis in Rochester, Minnesota, 1950 through 1982. *Neurology* **35**: 600-603.
 58. WITKOP, C., QUEVEDO, W. C., AND FITZPATRICK, T. (1978). Albinism and other disorders of pigment metabolism. In "The Metabolic Basis of Inherited Disease" (J. Stanbury, J. Wyngaarden, and D. Frederickson, Eds.), 4th ed., pp. 283-316, McGraw-Hill, New York.
 59. WOOD, S., POON, R., RIDDELL, D. C., ROYLE, N. J., AND HAMERTON, J. L. (1986). A DNA marker for human chromosome 8 that detects alleles of differing sizes. *Cytogenet. Cell Genet.* **42**: 113-118.
 60. ZIMMERMAN, D. (1988). The endocrine system. In "Tuberous Sclerosis" (M. R. Gomez, Ed.), 2nd ed., pp. 169-178, Raven Press, New York.