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An Attempt to Map Two Genes for Tuberous Sclerosis Using Novel Two-Point Methods[a]

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There are several theoretical mechanisms by which a mutation at two or more distinct loci might give rise to clinically similar diseases, but relatively few examples have so far been found. The best chance of identifying such loci, if both are autosomal, exists when the mildness of the disorder allows the collection of large families or when candidate loci can be suggested for direct testing. An example of

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the former was the earlier demonstration of two forms of elliptocytosis distinguished at that time only by the presence or absence of linkage to the rhesus blood group.[1] Osteogenesis imperfecta, caused by mutation at one of two Type I collagen genes, is an example of the successful application of the second approach.[2] Recently locus heterogeneity was also demonstrated for a number of other diseases.

Although tuberous sclerosis (TSC) has very variable severity, there was no *a priori* reason to suppose that it would show locus heterogeneity, because the clinical impression is that variation within families is as great as that between families. However, as detailed elsewhere at this conference,[3] conflicting evidence has led to the suggestion that at least two genes determine TSC: TSC1 on chromosome 9 and TSC2 on chromosome 11. In this report we present two attempts to analyze, by pairwise methods, exactly the same data as those analyzed by Janssen.

MATERIALS AND METHODS

Seventy-four families were considered in total, the sources being London, Cardiff, Rotterdam, Irvine, and Boston. The identification of families by source does not imply the origin of the families, only that of the researchers. Details of some of these families have previously been published.[4–8] Because of possibly different criteria used for diagnosis of nonaffected individuals at risk, penetrance was set at the level used in the laboratory of origin of the data. Pairwise analysis was done by LIPED or the Linkage package as determined by the data format. Data were not factored by sex. In both approaches described herein, selection of families to be analyzed for markers on chromosome 9 or 11 was followed by conventional two-point analysis and multiple two-point analysis using the program MAP90.[9] In making the maps and in placing TSC, interference was allowed for a mapping parameter of 0.35. The pairwise lodscores between the markers used for construction of the maps were taken from published data[10,11] and also derived from Julier (personal communication).

Negative Lodscore Method

The first approach, described elsewhere,[12] uses linkage data from a candidate chromosome only if the alternative chromosome in that family is associated with negative lodscores. For a selected group of loci on each chromosome, thought to be close to the putative TSC genes, sex-pooled lodscores at $\theta = 0.1$ were calculated. For each family, lodscores for each locus on chromosome 9 were added together, and the same was done for chromosome 11. In an initial calculation using a program provided by John Edwards, the overall sum of the lodscores for each chromosome was obtained before and after dividing the families as just

described. In an effort to estimate the most likely positions of the putative TSC1 and TSC2, only those families in which a lodscore more negative than −0.3 was obtained were selected for detailed analysis of markers from the other chromosome. The advantage of this method was seen as its lack of bias; the value of θ and the threshold to be used were selected before the calculation was started.

Maximum Lodscore Method

The second method uses the maximum lodscores from both candidate chromosomes to classify the families (Newton Morton, personal communication). If Z_O is the sum of all maximum lods for m informative loci in a given pedigree and Z_E is the sum evaluated at the correct map distance, large-sample theory predicts that 2 (ln 10) ($Z_O - Z_E$) should be distributed as χ^2_{m-1}, the expected value of which is m − 1. Then a reasonable estimate of Z_E is $Z_O - m - 1/2\ln 10$. The same equation would apply to a family with linkage to m′ loci on another chromosome. Thus, $S = Z_O - Z'_O + (m' - m)/2\ln 10$ is a lod for separating the two classes. A positive value suggests linkage to the first chromosome (9) and a negative value to the second (11). To avoid problems from small families and the possibility that in some families TSC is not linked to either chromosome, a threshold value for S of 0.43 (positive or negative) was used. This corresponds to odds of 2.7 : 1 and to Akaike's criterion of $\chi^2 = 2$.

RESULTS

Negative Lodscore Method

Preliminary analysis of the families gave encouraging results, summarized in TABLE 1. Fifty-three families had at least a small amount of data for both chromosomes 9 and 11. Of those, 21 were positive for 9 and negative for 11, 18 were positive for 11 and negative for 9, 2 were positive for both, and 12 were negative for both. On the basis of the more stringent criteria previously decided upon, 30 families were selected for analysis of 9 markers and 24 for 11 markers, 8 families being included in both analyses. TABLE 2, column 1, lists the individual families selected by this method. TABLES 3 and 4 show maximum lodscores, and FIGURES 1 and 2 show the most likely positions of TSC1 and TSC2 obtained from these data.

Maximum Lodscore Method

TABLE 2, column 2, shows the individual families, 18 being selected for analysis on chromosome 9, 14 for analysis on chromosome 11, and 42 unclassified. The

TABLE 1. Preliminary Analysis of 74 Families using Negative Lodscore Method

Sum of lods with TSC on chromosome 9	− 9.17
Sum of lods with TSC on chromosome 11	− 7.71
Sum of lods on chromosome 9 when lods with 11 negative	+ 6.76
Sum of lods on chromosome 11 when lods with 9 negative	+ 6.19

TABLE 2. Grouping of TSC Families by Three Separate Methods

Family	Neg	Max	IC	Family	Neg	Max	IC
Rot 2079	9	9	9	Lon 5214	9	9	9
Rot 2046	9	–	9	Lon 5372	–	–	9
Rot 2067	–	–	11	Lon 5386	9	9	9
Rot 2068	11	11	11	Lon 5272	–	–	9
Rot 2077	–	11	–	Lon 5301	9 + 11	–	11
Rot 1222	11	11	11	Lon 5275	9	9	9
Rot 1219	–	9	9	Lon 5349	9	9	9
Rot 1264	–	9	9	Lon 5235	9	–	9
Car 0001	9	9	9	Lon 5477	9	9	9
Car 0002	–	–	–	Lon 5379	9	9	9
Car 0003	11	–	11	Lon 5252	9	9	9
Car 0004	11	–	11	Lon 5385	9 + 11	–	11
Car 0005	9	9	9	Lon 5241	–	–	9
Car 0006	–	–	9	Lon 5274	11	–	9
Car 0007	9	9	9	Lon 5350	11	11	11
Car 0008	–	9	11	Lon 5388	9 + 11	11	11
Irv 0004	11	–	11	Lon 5441	–	–	9
Irv 0008	11	11	11	Lon 5404	9	–	9
Irv 0011	9	9	9	Lon 5412	9	–	9
Irv 0015	11	11	11	Lon 5298	–	–	9
Irv 0016	11	11	11	Bos 1	–	–	9
Irv 0019	–	–	11	Bos 2	–	–	9
Irv 0020	–	–	11	Bos 3	–	–	11
Irv 0021	9	9	9	Bos 4	9 + 11	–	11
Irv 0023	–	–	11	Bos 5	–	–	11
Irv 0024	11	11	11	Bos 6	–	9	9
Irv 0026	–	11	11	Bos 7	9 + 11	–	11
Irv 0028	–	–	11	Bos 8	9 + 11	–	9
Irv 0029	9	–	9	Bos 9	9	9	9
Irv 0033	9	–	11	Bos 10	9	–	9
Irv 0101	–	–	11	Bos 11	9	–	11
Lon 5400	11	11	11	Bos 12	9 + 11	–	9
Lon 5348	11	11	11	Bos 13	–	–	11
Lon 5431	9 + 11	–	11	Bos 14	11	–	9
Lon 5406	11	11	11	Bos 15	–	–	9
Lon 5244	11	11	11	Bos 16	–	–	11
Lon 5384	11	–	11	Bos 17	–	–	11

ABBREVIATIONS: NEG = families selected for analysis with 9 and/or 11 markers by negative lodscore approach; MAX = separation of families by maximum lodscore approach; IC = results from imaginary chromosome approach.[3] Rot = Rotterdam. Car = Cardiff, Irv = Irvine, Lon = London, and Bos = Boston.

maximum lodscores obtained in selected families are again given in TABLES 3 and 4, with the most likely map positions of TSC1 and TSC2 in FIGURES 1 and 2.

DISCUSSION

Progress in understanding and in possibly preventing TSC would be greatly helped by a single localization for the disease gene. Unfortunately it now seems beyond reasonable doubt that this is not the case and that a solution to the problem of locus heterogeneity must be found. Each approach used by ourselves

TABLE 3. Maximum Lodscores for TSC1 against Markers on Chromosome 9

Markers	Negative Lodscore Method		Maximum Lodscore Method	
	$\hat{\theta}$	$\hat{Z}$	$\hat{\theta}$	$\hat{Z}$
ASSP3	0.50	0.00	0.50	0.00
ORM	0.35	0.06	0.50	0.00
D9S16	0.10	0.69	0.05	1.05
ALAD	0.30	0.18	0.10	0.68
AK1 (protein)	0.00	1.51	0.00	1.51
AK1 (DNA)	0.30	0.33	0.00	1.52
ABL	0.05	4.18	0.00	5.91
ASS (ASG1)	0.25	0.83	0.00	3.34
ASS (ASG3)	0.20	0.71	0.05	1.66
D9S10	0.05	1.65	0.10	1.98
ABO	0.10	2.70	0.05	3.96
D9S7	0.25	0.46	0.20	0.83

and others to analyze the available data has some good and bad points. It is not appropriate to discuss all the theoretical aspects here. However, in summary the negative lodscore approach, although not claiming great precision in selection of families, is entirely free from bias. The maximum lodscore method does not require a knowledge of marker order or distance, or the proportion of families

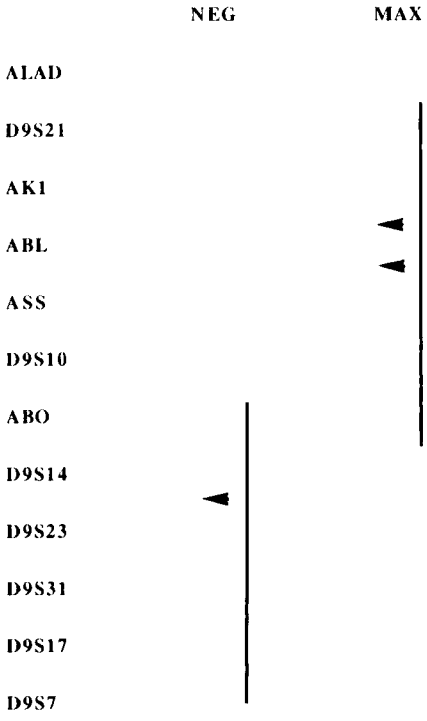

FIGURE 1. Pairwise lodscores from 25 loci extending from D9S19 to D9S11 were used to make a basic genetic map. The *arrows* indicate the best intervals in which TSC1 can be fitted by the negative lodscore method (NEG) and by the maximum lodscore method (MAX). TSC1 could not be excluded with odds of greater than 10:1 from the regions defined by the *bars*.

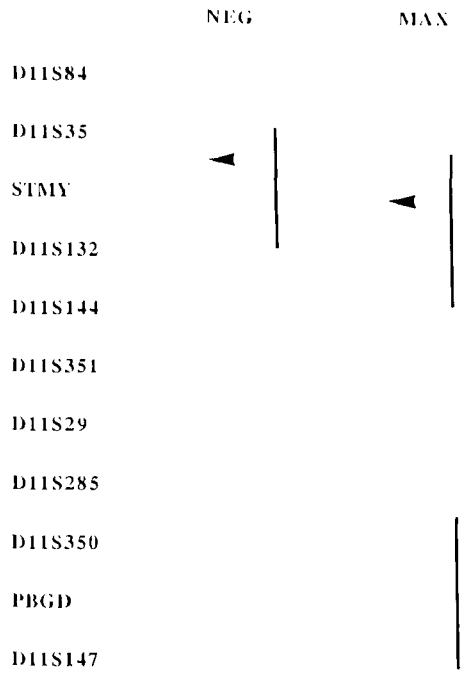

FIGURE 2. Pairwise lodscores from 11 loci extending from D11S84 to D11S147 were used to make a basic genetic map. The *arrows* indicate the best intervals in which TSC2 can be fitted by the negative lodscore method and by the maximum lodscore method. TSC2 could not be excluded with odds of greater than 10:1 from the regions defined by the bars.

linked to a particular chromosome. The imaginary chromosome method is powerful and was able to classify a higher percentage of the families than was either of the other approaches. However, it is very dependent on the precise basic map used and is vulnerable (as is the negative lodscore method) to problems arising from further heterogeneity. With all these problems it is encouraging that a large measure of agreement exists between the methods. Substantial agreement also exists with results obtained by Haines[13] using a larger combined data set which includes all the families described herein. In all cases evidence is strong that a locus TSC1 is present on chromosome 9 and that the most likely position is near

TABLE 4. Maximum Lodscores for TSC2 against Markers on Chromosome 11

	Negative Lodscore Method		Maximum Lodscore Method	
Markers	$\hat{\theta}$	$\hat{Z}$	$\hat{\theta}$	$\hat{Z}$
D11S84	0.10	0.50	0.075	0.75
D11S35	0.50	0.00	0.50	0.00
STMY	0.00	0.66	0.00	0.70
D11S132	0.00	1.23	0.00	2.16
D11S144	0.25	1.24	0.125	2.39
D11S351	0.25	1.08	0.00	3.44
D11S29	0.30	0.38	0.25	0.44
D11S350	0.30	0.21	0.175	0.48
PBGD	0.20	0.27	0.00	1.25
D11S147	0.00	0.27	0.00	0.85

ABL, although a position slightly distal to ABO cannot be excluded. In about an equal number of families there is evidence for a locus on chromosome 11. Although the data from Irvine, taken alone,[8] provide convincing evidence for the existence of a locus on chromosome 11, over the whole data set (as evidenced by the lodscores in TABLES 3 and 4) the support for this locus is not as unequivocal as that for TSC1 on chromosome 9.

The best position for TSC2 by both pairwise approaches is in the region of D11S132, proximal to D11S144 (MCT128) and distal to D11S35; this is only marginally preferred to a position distal to PBGD. By the imaginary chromosome approach the best positions for TSC are either proximal or distal to the whole set of markers, reflecting a lack of critical recombinants indicating position. However, in this analysis the markers used included only PBGD, D11S29, D11S351, and D11S144; thus, a localization of TSC proximal to this set of markers is consistent with the localization by MAP90 with the negative lodscore method or the maximum lodscore method. Of course, the possibility of a third locus cannot be excluded. This might have a greater effect on chromosome 11 data than on chromosome 9 data for artefactual reasons. At least in the London data set many more markers on chromosome 9 than on 11 have been tested; hence a family in which TSC has been excluded from a long section of chromosome 9 may be allocated for analysis on chromosome 11 despite the fact that whatever information is available for markers on chromosome 11 is negative.

Those families that appear to have negative lodscores for both chromosomes are clearly of crucial importance. They are good families in which to look for a third locus; alternatively they may contain critically important recombinants. There are four families in this category in the London data. In one of the families the recombination is dependent on the correct diagnosis of a fully investigated 25-year-old adult as unaffected. In the three other families the negative scores do not depend on unaffected members, but in all three only a limited number of markers on chromosome 11 have been tested, so that assignment of TSC to chromosome 11 in these families cannot be entirely excluded.

FUTURE PROSPECTS

The collection of new large families well characterized for tuberous sclerosis and the generation of many new markers on 9q34 and 11q23 would be desirable. However, even with the material available, a major limitation of the analysis was that so few families have been typed for a good range of available markers on each chromosome. The laboratories participating in this collaboration have agreed to make every effort to test all families for markers ABL, ABO, AK1, MCT126, MCOA12, and ASS on chromosome 9, and CRI424, D11S35, MCT128, APOA4, CJ52.208, D11S29, and PBGD on chromosome 11. It would also be of interest for experienced clinicians to see if, with the powerful tool of hindsight, any differences can be detected in those families showing linkage to different chromosome regions.

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