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A Comparative Study on Genetic Heterogeneity in Tuberous Sclerosis: Evidence for One Gene on 9q34 and a Second Gene on 11q22–23[a]

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LINKAGE STUDIES IN TUBEROUS SCLEROSIS

Tuberous sclerosis (TSC) is a dominantly inherited disorder. Although the variability in expression is high, complete penetrance may be assumed.

Following a suggestion of positive lod scores between TSC and ABO,[1] Fryer and coworkers reported linkage between TSC and the ABO blood group locus with a peak cumulative lod score of 3.85 at $\Theta = 0$[2]. This was supported by Connor *et al.*, who found linkage to the abl oncogene with a lod score of 3.18 at $\Theta = 0$.[2b] These findings enabled Connor and coworkers to perform a first-trimester exclusion of TSC in a pregnancy at risk. The abl oncogene maps approximately 10 cM proximal of ABO on 9q34 (FIG. 1). Subsequent studies mapped the TSC locus between MCT136 and MCOA12.[4]

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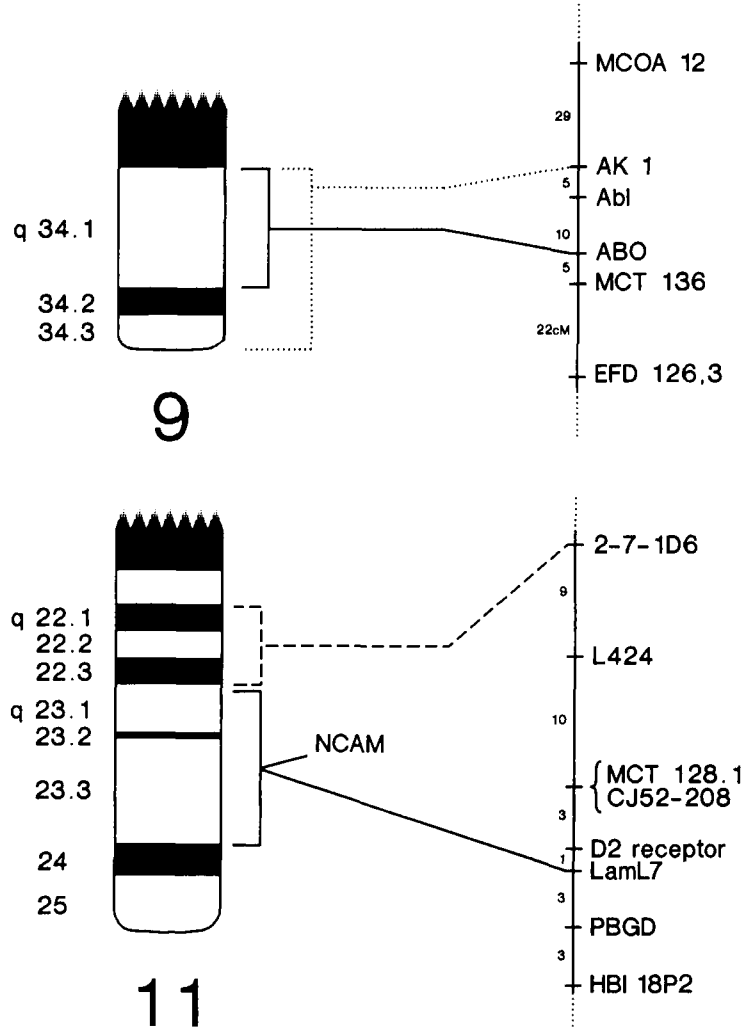

FIGURE 1. Genetic maps of chromosome 9 (*top*) and 11 (*bottom*) with physical locations of selected markers indicated on the karyogram. The positions of the markers used in our study (MCOA12, AK1, abl, ABO, MCT128.1, CJ52.208, LamL7 and PBGD) are indicated as are their positions towards other markers of interest. The order of ABO and MCT136 is in doubt (J. L. Haines, personal communication). Scale is in centimorgans (cM).

In contrast with these reports, a substantial number of families were described with free recombination between TSC and the chromosome 9 markers.[5,6] Recently Kandt *et al.* excluded TSC from an area of 20 cM encompassing the ABO locus.[7]

In 1988 Clark *et al.* described a TSC patient with trisomy for 11q23.3-qter.[8] The mother was reported to have a balanced t(11q23.3; 22q11.2) rearrangement,

but there was no evidence of TSC in either parent. The gene for neural cell adhesion molecule, which is located on 11q23, was postulated as a candidate locus. Linkage studies in 15 North American families using four chromosome 9q markers and six chromosome 11q markers revealed evidence for linkage to chromosome 11.[9] A maximum two-point lod score of 3.26 at $\Theta = 0.08$ was obtained with the marker MCT128.1.

The present and other[11] collaborative efforts aim at ending the controversy by combining data from several studies, including those in which linkage to either chromosome 9 or 11 has been established. Data were combined without selection for any type of families. We compared different statistical strategies to investigate the possibility of locus heterogeneity.

LOCUS HETEROGENEITY

In the last decade reverse genetics has become an important tool to localize and identify disease genes. This approach is based on the search for co-segregation of a disease trait and genetic markers of known location. The growing number of mapped informative polymorphic markers enhanced the success of this approach. Several genes causing diseases have been mapped and identified this way.

This approach is based, however, on the assumption that data from informative meioses in different families can be combined. This assumption would be invalid if the disease-causing locus is not identical in all families, that is, if locus heterogeneity exists. Given the complexity of biochemical pathways, it is expected that locus heterogeneity will prove to be a feature of a significant proportion of genetic diseases.

Of interest, then, is the analysis of diseases that have been mapped in some families to a locus, although in other phenotypically similar families a mutation at the mapped locus cannot account for the observed data.

Tuberous sclerosis may very well be a member of this small group of diseases[4,10,11,11a] showing locus heterogeneity. Other examples of diseases belonging to this group are elliptocytosis, X-linked retinitis pigmentosa, Charcot-Marie-Tooth disease type 1, adult polycystic kidney disease, and manic depression. Only for Charcot-Marie-Tooth and X-linked retinitis pigmentosa has more than one locus been mapped.[12,13,14]

In some diseases the assignment of families to a certain locus is facilitated by the type of segregation. This is the case when one or more loci are X-linked, while other loci are autosomal. Diseases like limb girdle muscular dystrophy and manic depression are phenotypically indistinguishable from their X-linked counterparts, Becker dystrophy and X-linked manic depression.

Most inherited diseases show autosomal inheritance, however. For autosomal diseases with locus heterogeneity, arguments for classification apart from linkage data are hard to obtain. Lack of linkage data may explain why the diseases have not yet been recognized as being heterogeneous. Even when linkage data are available, a locus may be overlooked, if the statistical analysis does not allow for locus heterogeneity. Lander and Botstein[15] estimated that a trait-causing locus that accounts for 60% of all cases may be missed completely in a linkage study under the false assumption of homogeneity. If the tested marker lies within 1 cM of the disease locus, linkage may still be excluded from a region of about 20 cM encompassing the marker. This implies that the recent exclusion data of Kandt *et al.*[7] do not undermine the validity of the previous assignment of a TSC locus to 9q34.

The power of reverse genetics is clearly reduced in the case of locus heterogeneity. This complicated problem requires the development and use of powerful statistical approaches.

LOCUS HETEROGENEITY IN TUBEROUS SCLEROSIS

For most heterogeneous traits only one candidate region is known. Since there are two candidate regions for TSC, TSC1 (9q) and TSC2 (11q), our problem is quite exceptional. We compared various approaches that allow for locus heterogeneity. These methods may be divided into two groups: The first group contains approaches that start with a separation of families, followed by conventional two-point analyses. Results obtained with these methods are presented in detail elsewhere.[20] Approaches from the second group start with conventional two-point or multipoint analyses, followed by weighted family assignment to the putative disease loci. Results obtained this way are presented in this paper.

A well-known test for heterogeneity is the "classic" admixture test.[16] It has been used in the analysis of almost all genetically heterogeneous diseases mentioned above. For each map location (x) and for each proportion of linked families (α) a lod score under heterogeneity [$Z_i(\alpha, x)$] is computed.

$$Z_i(\alpha, x) = \log_{10}[\alpha \cdot 10^{Z_i(x)} + (1 - \alpha)].$$

The combination of α and x for which $Z_i(\alpha, x)$ is maximal is evaluated in a Chi square test, using the maximum value of $Z_i(\alpha, x)$, assuming homogeneity ($\alpha = 1$) as a reference. The admixture tests were performed by using the HOMOG computer program.[17]

An alternative approach is also based on the admixture test. Instead of one position (x), two positions (x_1 and x_2) are evaluated. The equation

$$Z_i(\alpha, x_1, x_2) = \log_{10}[\alpha \cdot 10^{Z_i(x_1)} + (1 - \alpha) \cdot 10^{Z_i(x_2)}]$$

is similar to the equation given above, where x_2 is at $\Theta = 0.5$. This alternative approach can be taken to test for two distinct loci on one chromosome. Such a test has been performed successfully for X-linked retinitis pigmentosa.[14] If both loci are not on the same chromosome, a so-called "imaginary chromosome" has to be constructed, as described previously.[10] We composed the imaginary chromosome by combining the results of two multipoint analyses (one for each candidate region) in a head-to-tail orientation. The most distal marker on chromosome 9 and the most proximal marker on chromosome 11 were flanking the junction. The recombination fraction between these markers and the junction was set at 0.5. By the use of this imaginary construct, the TSC linkage problem to markers on separate chromosomes was reduced to a linkage problem with two regions of interest on a single (imaginary) chromosome. The imaginary chromosome was analyzed with the programs HOMOG2 and POINT4.[17] Both programs are part of the HOMOG package. This method is only valid in the absence of further heterogeneity in the data set. Therefore a final check using the HOMOG3 program was performed. This program allows for a third locus, which may be unlinked to either candidate region.

Seventy-three families were analyzed, the majority of which have been included in previous publications.[4,9,10] For two-point and multipoint analyses the various programs of the LINKAGE package were utilized.[19]

RESULTS OBTAINED WITH THE "CLASSIC" ADMIXTURE TESTS

The simplest application of the "classic" admixture test is the analysis of two-point linkage data using HOMOG. Two of these tests were performed, one for the ABO locus on chromosome 9 and one for the anonymous chromosome 11 marker MCT128.1. The results of these tests are shown in Table 1. Significant support for heterogeneity could only be obtained with the ABO linkage data. A similar test using MCT128.1 revealed only insignificant support for heterogeneity.

A more advanced application of the admixture test uses a map of markers. The markers MCOA12 (D9S28, *Msp*I), AK1 (*Taq*I or protein polymorphism), abl (*Pst*I or *Taq*I), and ABO were used as chromosome 9 markers in a five-point analysis. Another five-point analysis was carried out using the chromosome 11 markers

TABLE 1. Results of the Various Admixture Tests

Analysis: Marker(s)/ Programs	Components of Chi Square test for H_2 versus H_1			Most Likely Location Assuming Heterogeneity
	X^2	p Value	α	
ABO/ MLINK-HOMOG	4.593	0.0161 (1 df)	TSC1: 0.38	TSC1: at ABO
MCT128.1/ MLINK-HOMOG	1.057	0.1520 (1 df)	TSC2: 0.47	TSC2: 35 cM of MCT128.1
Map of chromosome 9 markers/ LINKMAP/HOMOG	10.172	0.0007 (1 df)	TSC1: 0.45	TSC1: 6 cM prox. of ABO
Map of chromosome 11 markers/ LINKMAP-HOMOG	0.505	0.2386 (1 df)	TSC2: 0.30	TSC2: 35 cM dist. of PBGD
Combined map (imaginary chromosome)/ LINKMAP-HOMOG	15.448	0.0002 (2 df)	TSC1: 0.48 TSC2: 0.52	TSC1: 6 cM prox. of ABO TSC2: 35 cm dist. of PBGD

NOTE: H_1 = hypothesis of linkage under homogeneity; H_2 = hypothesis of linkage under heterogeneity; α = proportion of families linked with indicated locus.

MCT128.1 (D11S144, *Msp*I), CJ52.208 (D11S351, *Msp*I), LamL7 (D11S29, *Taq*I) and PBGD (PBGD, *Msp*I or *Pst*I). All inter-marker distances are given in FIGURE 1. The results obtained with HOMOG are presented in TABLE 1. The use of a map of four chromosome 9 markers instead of one resulted in an increased significance when heterogeneity was tested versus homogeneity. However, a similar study on the chromosome 11 region showed no increased significance.

RESULTS OBTAINED WITH THE IMAGINARY CHROMOSOME APPROACH

By combining the results of both five-point analyses into one larger structure an imaginary chromosome was constructed. The results obtained with this approach are shown in TABLES 1, 2, and 3. The outcome of the analysis showed strong support for locus heterogeneity, with one putative TSC locus 6 cM proximal to ABO (TSC1) and another putative TSC locus 35 cM distal to PBGD

TABLE 2. The 74 TSC Families and Their Assignment to Chromosome 9 or 11

Family	Assignment to Chromosome by Method			Lod Scores at Putative TSC Loci	
	ICA	MLSM	NLSM	TSC1	TSC2
Rot 2079	9 (0.80)	9	9	0.41	−0.24
Rot 2046	9 (0.61)	—	9	0.23	0.00
Rot 2067	11 (0.56)	—	—	0.00	0.07
Rot 2068	11 (0.95)	11	11	−1.03	0.24
Rot 2077	—	11	—	0.24	0.21
Rot 1222	11 (0.89)	11	11	−0.67	0.22
Rot 1219	9 (0.87)	9	—	0.79	−0.06
Rot 1264	9 (0.68)	9	—	0.38	0.02
Car 0001	9 (0.98)	9	9	1.07	−0.63
Car 0002	—	—	—	0.00	−0.03
Car 0003	11 (1.00)	—	11	−3.64	−0.01
Car 0004	11 (0.58)	—	11	−0.14	−0.03
Car 0005	9 (0.66)	9	9	0.21	−0.11
Car 0006	9 (0.87)	—	—	0.84	−0.01
Car 0007	9 (1.00)	9	9	2.79	−0.59
Car 0008	11 (0.52)	9	—	0.00	0.00
Irv 0004	11 (0.90)	—	11	−0.92	0.00
Irv 0008	11 (1.00)	11	11	−3.10	0.15
Irv 0011	9 (0.60)	9	9	0.00	−0.21
Irv 0015	11 (1.00)	11	11	−3.09	0.44
Irv 0016	11 (0.61)	11	11	−0.26	−0.10
Irv 0019	11 (0.52)	—	—	0.00	0.00
Irv 0020	11 (0.57)	—	—	−0.14	−0.05
Irv 0021	9 (0.75)	9	9	0.49	−0.03
Irv 0023	11 (0.52)	—	—	0.00	0.00
Irv 0024	11 (0.80)	11	11	−0.41	0.15
Irv 0026	11 (0.71)	11	—	0.01	0.38
Irv 0028	11 (0.55)	—	—	0.00	0.06
Irv 0029	9 (0.55)	—	9	0.00	−0.12
Irv 0033	11 (0.56)	—	9	0.00	0.07
Irv 0101	11 (0.53)	—	—	0.23	0.25
Lon 5400	11 (1.00)	11	11	−2.05	0.26
Lon 5348	11 (0.95)	11	11	−1.05	0.20
Lon 5431	11 (0.73)	—	9&11	−0.49	−0.08
Lon 5406	11 (0.89)	11	11	−0.86	0.00
Lon 5244	11 (0.86)	11	11	−0.76	0.00
Lon 5384	11 (0.87)	—	11	−0.58	0.21
Lon 5214	9 (0.60)	9	9	0.00	−0.20
Lon 5372	9 (0.75)	—	—	0.52	0.00
Lon 5386	9 (0.79)	9	9	0.53	−0.08
Lon 5272	9 (0.72)	—	—	0.52	0.07
Lon 5301	11 (0.79)	—	9&11	−0.59	−0.07
Lon 5275	9 (0.63)	9	9	0.00	−0.27
Lon 5349	9 (0.65)	9	9	0.24	−0.07
Lon 5235	9 (0.66)	—	9	0.25	−0.06
Lon 5477	9 (0.52)	9	9	0.00	−0.08
Lon 5379	9 (0.53)	9	9	0.00	−0.08
Lon 5252	9 (0.97)	9	9	1.19	−0.29
Lon 5385	11 (0.75)	—	9&11	−0.52	−0.06
Lon 5241	9 (0.68)	—	—	0.52	0.15
Lon 5274	9 (0.71)	—	11	0.42	0.00
Lon 5350	11 (0.88)	11	11	−0.66	0.15

(TABLE 2 *Continued*)

Family	Assignment to Chromosome by Method			Lod Scores at Putative TSC Loci	
	ICA	MLSM	NLSM	TSC1	TSC2
Lon 5388	11 (0.81)	11	9&11	−0.66	−0.08
Lon 5441	9 (0.53)	—	—	0.09	0.00
Lon 5404	9 (0.78)	—	9	0.52	−0.07
Lon 5412	9 (0.66)	—	9	0.25	−0.06
Lon 5298	9 (0.62)	—	—	0.25	0.00
Bos 1	9 (0.81)	—	—	0.69	0.04
Bos 2	9 (0.57)	—	—	0.15	0.00
Bos 3	11 (0.52)	—	—	0.00	0.00
Bos 4	11 (0.81)	—	9&11	−0.66	−0.07
Bos 5	11 (0.60)	—	—	0.00	0.14
Bos 6	9 (0.79)	g	—	0.51	−0.10
Bos 7	11 (0.81)	—	9&11	−0.64	−0.04
Bos 8	9 (0.68)	—	9&11	0.00	−0.36
Bos 9	9 (0.91)	g	9	1.07	0.01
Bos 10	9 (0.52)	—	9	0.00	−0.07
Bos 11	11 (0.71)	—	9	−0.41	−0.05
Bos 12	9 (0.54)	—	9&11	0.00	−0.10
Bos 13	11 (0.54)	—	—	0.00	0.03
Bos 14	9 (0.67)	—	11	0.40	0.06
Bos 15	9 (0.62)	—	—	0.25	0.00
Bos 16	11 (0.83)	—	—	−0.66	0.00
Bos 17	11 (0.70)	—	—	−0.33	0.00

NOTE: ICA: imaginary chromosome approach; MLSM: family assignment by maximum lod score method; NLSM: possible family assignments by negative lod score method. (Since families can be selected twice by using the NLSM, all possible assignments are indicated instead of a definitive assignment.) Next to the family assignment by the ICA, the posterior probability of the assignment of each family (by ICA) is given in parentheses. The last two columns show the lod scores at the putative TSC1 and TSC2 loci, as calculated using the imaginary chromosome approach.

Rot = Rotterdam, Car = Cardiff, Irv = Irvine, Lon = London and Bos = Boston.

TABLE 3. Odds against Possible Orders When Compared to the Most Likely Order[a]

Locus Order	Odds against Locus Order
MCOA12–AK1–abl–TSC1–ABO	1
TSC1–MCOA12–AK1–abl–ABO	$4.8 \cdot 10^4$
MCOA12–TSC1–AK1–abl–ABO	199
MCOA12–AK1–TSC1–abl–ABO	1.32
MCOA12–AK1–abl–ABO–TSC1	2.95
MCT128–CJ52–L7–PBGD–TSC2	1
TSC2–MCT128–CJ52–L7–PBGD	1.22
MCT128–TSC2–CJ52–L7–PBGD	$9.3 \cdot 10^9$
MCT128–CJ52–TSC2–L7–PBGD	$1.3 \cdot 10^8$
MCT128–CJ52–L7–TSC2–PBGD	$2.5 \cdot 10^3$

[a] Given at the top of each list.

NOTE: The odds are calculated using the imaginary chromosome approach.

(TSC2). Additional tests revealed that other possible orders could not always be significantly excluded (TABLE 3). The most likely location of both putative TSC loci was confirmed by a HOMOG3 analysis. These results revealed no evidence for a third locus in our data set.

THE VALIDITY OF THE MODEL

Although the quality of the model we tested permitted locus heterogeneity to be proven, we may still question whether we tested the optimal model. The model can be regarded to be less optimal if we used false inter-marker distances or orders, if we did not test markers from the real TSC regions, if further locus heterogeneity is the case, or if the penetrance (100%) is not correct. Testing a less optimal model might result in reduced significance and incorrect putative locations. The putative TSC2 locus is not flanked by a distal marker. The additional tests on the chromosome 11 region revealed an alternative TSC2 locus 30 cM proximal to MCT128.1. This alternative position is almost as likely as the position 35 cM distal to PBGD (TABLE 3). The validity of the tested region on chromosome 11 seems questionable.

Exclusion of relatives without symptoms in additional analyses did not alter the outcome. Therefore the assumed full penetrance did not influence the model to a large extent (results not shown). Furthermore the maps of chromosome 9 and chromosome 11 markers are rigorously tested by several groups.[21,22] Therefore analysis of the data under the assumption of further locus heterogeneity or the analysis of more distant chromosome 11 markers are the best candidate strategies for improving the model.

COMPARISON WITH RESULTS OBTAINED USING ALTERNATIVE METHODS

Although locus heterogeneity may not be an uncommon feature, only a few examples are known. So far it has not been possible to test methods for heterogeneity analysis on a large scale. Therefore it is wise to use multiple methods in each study, in order to avoid biases. Povey and coworkers used two alternative methods in the analysis of the same family material.[20] Both methods start with a separation of families, followed by conventional two-point analysis.

The first approach is called the Negative Lod Score Method (NLSM).[20] The method only uses linkage information from a candidate region if the alternative region is associated with negative lod scores.[23] This analysis is followed by conventional two-point analysis on the selected families. This way the problem is simplified into two segments for which homogeneity is assumed. No linkage data are used twice.

The second method is called the Maximum Lod Score Method (MLSM).[20] It uses the maximum lod scores from both candidate regions to classify the families (N. Morton, personal communication).

Part of the results obtained by these methods are included in TABLE 2. The results on the chromosome 11 localization support a TSC2 locus proximal of MCT128.1.[20] A position between 2-7-1D6 and L424 seems likely. There seems to be an inconsistency within the results on the localization of the TSC1 locus. The NLSM supports a locus between ABO and EFD126.3, which is in contrast with the position near abl as supported by the MLSM[20] and ICA. This discrepancy is not more than a seeming discrepancy since none of the resulting locus orders are inconsistent with the odds against these orders as given in TABLE 3.

All approaches used have shown their value. The imaginary chromosome approach has increased power in heterogeneity analysis when compared to the "classic" admixture tests. The imaginary chromosome approach seems to be more refined and less biased, since data from all of the families are used to identify the two most likely locations, without prior selection. However, misleading results could be generated by using an incorrect model. For instance, if one of the putative locations was inaccurate, then the probabilities of linkage assigned to each family would be incorrect, and the associated lod scores would be low. Because of these potential problems, the use of an alternative method such as the NLSM or the MLSM to verify the results is also recommended.

CONCLUDING REMARKS

Our studies revealed evidence for a model with two different loci independently causing TSC. The first locus (TSC1) maps on chromosome 9 between the abl oncogene and the ABO blood group locus. Several methods confirmed a position about 6 cM proximal to ABO. The second locus (TSC2) maps on chromosome 11q22–23. The exact position of the TSC2 locus remains unclear.

Our current studies aim at finding closer linked markers to both TSC1 and TSC2 loci. On chromosome 11 a set of informative markers that span a wider range is under investigation. On chromosome 9 our studies focus on new markers, closely linked to abl and ABO.

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