Evidence for genetic heterogeneity in tuberous sclerosis

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SUMMARY The question of genetic heterogeneity in tuberous sclerosis (TSC) was addressed by genetic linkage studies in eight affected families using nine polymorphic markers (EFD126.3, MCT136, ABO, ABL, AK1, and MCOA12 from distal 9q, and PBGD, MCT128.1, and 1CI52.208M from distal 11q). The data as a whole supported a TSC locus on distal 9q, the peak lod score on multipoint analysis being 3.77 at 6 cM proximal to the Abelson oncogene locus (ABL). However, as two point lod scores using the HOMOG programs showed significant evidence for genetic heterogeneity (p = 0.01), linkage to ABL being unlikely in one family. After exclusion of the unlinked family, multipoint analysis gave a peak lod score of 6.1 in the vicinity of ABL. The family unlinked to ABL showed no recombinants with two chromosome 11 probes, but was too small to provide significant evidence for linkage. Genetic heterogeneity in TSC will complicate efforts to clone the causative genes and severely limit the use of linked probes for carrier detection and prenatal diagnosis.

Tuberous sclerosis (TSC) is an autosomal dominant disorder characterised by multisystem hamartosis. Dermatological and neurological features most frequently suggest the diagnosis. Almost 50% of patients are mentally retarded and 80% have a history of seizures. Skin lesions include adenoma sebaceum, shagreen patches, periangual fibromata, and hypopigmented macules. Hamartomatous tubers and cysts of the kidneys and lungs may occur and rhabdomyomata of the heart are common in affected infants. The visceral abnormalities occasionally have serious consequences but are usually asymptomatic. Diagnosis is based on clinical criteria defined by Gomez1 and exclusion of the disease requires rigorous investigation for asymptomatic manifestations. Attempted total ascertainment in the west of Scotland yielded a prevalence of 1 in 12 000 in the under 10 age group. Expression varies greatly but non-penetrance of the disease gene is exceptional.2

The tuberous sclerosis gene has been provisionally mapped to chromosome 9 by family linkage studies showing absence of recombination with the ABO blood group, the red cell enzyme polymorphism adenylate kinase (AK1), and a restriction fragment length polymorphism at the Abelson oncogene locus (ABL), all these markers being localised to 9q34.3-5 Subsequently others have reported recombination between TSC and ABO6 7 and two multigenerational studies have failed to confirm linkage between TSC and ABO.8 9 Recombination between TSC and ABL has also been observed.10 This raises the question of genetic heterogeneity, that is, the existence of more than one locus at which mutation can lead to TSC. More recently an infant with TSC who was trisomic for 11q23.3-qter has been reported and the possibility of a disease locus in this chromosomal region suggested.11

In this study we have investigated eight rigorously assessed multigeneration families with TSC using markers from distal 9q and from distal 11q in order to address the question of genetic heterogeneity.

Patients and methods

FAMILY ASSESSMENT

Eight families in which several subjects were affected by TSC on accepted diagnostic criteria1 were identified as suitable for linkage studies. Family members with no signs of TSC after clinical assessment, including Wood’s light examination of the skin and

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indirect ophthalmoscopy, were investigated with cranial CT scan and renal ultrasound and were scored as unaffected if the findings were normal. Pedigrees of the families studied in this investigation are shown in fig 1. Families 3036, 3050, 4077, 4136, 5235, and 5982 had been assessed for the study of Fryer et al. Further members of four of these families were investigated to meet the criteria for inclusion in this study. Apparently unaffected minors (under 15 years) and those with clinical findings of uncertain significance (for example, a solitary renal cyst) were excluded.

FIG 1 Family pedigrees.
**Evidence for genetic heterogeneity in tuberous sclerosis**

**DNA and Biochemical Markers**

DNA was extracted from peripheral blood lymphocytes or from lymphoblastoid cell lines according to Kunkel et al. After digestion with restriction enzymes (Bethesda Research Laboratories), under conditions recommended by the manufacturer, fragments were fractionated by electrophoresis using 0.8% agarose gels, blotted onto Hybond N filters (Amersham), and hybridised with 32P labelled probes under the conditions described by Lathrop et al. The probes mapping to distal 9q used in this study were pSA1714 (ABL) which detects a TagI polymorphism with alleles of 7 and 8 kb; MCT136 (D9S10) and MCOA12 (D9S16) which detect MspI RFLPs at 2-0 and 2-2 kb and at 2-5 and 3-0 kb respectively; and EFD126.3 (D9S57) with five MspI alleles between 1-5 and 2-0 kb. All family members were also typed for the protein markers ABO and AK1 which map in this region. The probes mapping to distal 11q, all of which are polymorphic with MspI, were a 0-9 kb EcoRI subclone of MCT128.1 (D11S144) which detects alleles at 2-6 and 2-9 kb; a 1-3 kb HindIII subclone of 1C52.208M2 (C52) with alleles at 3-2 and 4-0 kb; and the erythroid porphobilinogen deaminase gene pUSE109 (PBGD) with alleles at 2-2 and 3-0 kb.

**Linkage Analysis**

The LIPED computer program was used to compute two point lod scores and LINKMAP from version 4-7 of LINKAGE was used for multipoint analysis. The LINKSYS program was used for data management. The disease gene frequency was assumed to be 0-0001 with complete penetrance. Confidence intervals specified correspond to the recombination fractions at a lod score one unit less than the maximum. For the LINKMAP analyses the order of marker loci on chromosome 9 was taken to be centromere-MCOA12-ABL-ABO-MCT136-EFD126.3-qter. Intermarker recombination fractions used were 0-16, 0-14, 0-04, and 0-22 respectively for males with a female/male ratio of genetic distance of 1-1, these being derived from the data of Lathrop et al.

**Tests for Genetic Heterogeneity**

Two point lod scores for informative families were analysed for evidence of genetic heterogeneity using the HOMOG and HOMOG 2 programs. Using HOMOG the recombination fraction 0 is varied to maximise the likelihood on the assumption that all families are linked (homogeneity). This is compared with a simple model of heterogeneity in which a proportion α of families are linked at recombination fraction 0' and the remaining families are unlinked, the likelihood being maximised as a function of α and 0'. HOMOG 2 considers the possibility of more than one linked locus, each having a different recombination fraction with the marker under examination.

**Results**

**Linkage Analysis, All Families**

Segregation of ABO and ABL in families 4077, 4136, and 5235 has already been reported. Since then, subjects, II.3, II.3, III.7, and III.8 from family 4136 and II.2 from family 5235 have been fully investigated and confirmed as unaffected. They are included in this analysis. Subject II.4 from

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**Table 1 Two point lod scores, versus TSC, obtained on analysis of all families.**

<table>
<thead>
<tr>
<th>Marker locus</th>
<th>Chromosome</th>
<th>Recombination fraction</th>
<th>0</th>
<th>0-001</th>
<th>0-05</th>
<th>0-1</th>
<th>0-2</th>
<th>0-3</th>
<th>0-4</th>
<th>Z</th>
<th>0m</th>
<th>0f</th>
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<td>-7-54</td>
<td>-1-05</td>
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<td>0-42</td>
<td>0-26</td>
<td>1-43</td>
<td>0-32</td>
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<tr>
<td>D9S10</td>
<td>Zm 9</td>
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<td>0-77</td>
<td>0-85</td>
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<td>0-40</td>
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<td>0-12</td>
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<td>0-18</td>
<td>0-16</td>
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Zm and Zl indicate, respectively, the male and female sex specific lod scores calculated at each recombination fraction. Z indicates the maximum lod score obtained, and 0m and 0f the sex specific recombination fractions corresponding to Z.
family 5235 was found to have a single renal cyst but no other signs to suggest TSC and was excluded as disease status uncertain. Two point linkage analysis gave positive lod scores between TSC and all five informative markers on chromosome 9; AK1 was uninformative. On chromosome 11 the marker CJ52 gave a small positive score of 0.18 in males at a recombination fraction of 0.16, but was negative in females. MCT128.1 and PBGD gave entirely negative scores. The sex specific two point lods at standard recombination fractions and the maximum lods and corresponding recombination fractions are shown in table 1. Multipoint analysis of TSC in relation to the chromosome 9 probes using the program LINKMAP gave a peak lod score of 3.77 with TSC proximal to ABL at a recombination fraction of 0.06 in males and 0.04 in females.

The raw data for each of the markers are available on request from the authors.

Evidence of genetic heterogeneity
Two point lod scores obtained for each family informative for ABL are shown in table 2. Three families showed no recombinants and gave a combined lod score of 5.09, equivalent to no recombinants in 17 phase known meioses. In contrast, family 9431 showed a minimum of two recombinants (one male and one female meiosis) among four meioses. Formal analysis for genetic heterogeneity using

![Image](http://jmg.bmj.com/ on December 29, 2017 - Published by group.bmj.com)

**FIG 2** Multipoint analysis indicating likelihood for localisation of TSC in relation to male genetic map of distal 9q.

HOMOG and HOMOG 2 reached statistical significance for ABL (p=0.01).

**Linkage analysis with exclusion of family 9431**
As significant evidence for genetic heterogeneity owing to family 9431 had been found, the data for the chromosome 9 probes were reanalysed after exclusion of this family. The two point lod scores are summarised in table 3. Multipoint analysis with chromosome 9 markers gave a peak lod score of 6.1 coincident with ABL (fig 2). TSC was placed between MCOA12 and MCT136 with odds of 140:1 against the next most likely order, in which TSC was distal to MCT136. In the absence of any recombination, ordering with respect to ABL and ABO was not resolved. The likelihood curve was broad with confidence limits extending 19 cM distal and 8 cM proximal to ABL. Limiting factors in the precision

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**TABLE 2 Lod scores for families informative for ABL.**

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<thead>
<tr>
<th>Family</th>
<th>Recombination fraction</th>
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<td></td>
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<td>9431</td>
<td>0.097</td>
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</table>

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**TABLE 3 Two point lod scores obtained with chromosome 9 markers after exclusion of family 9431.**

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<th>Marker locus</th>
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<th>Recombination fraction</th>
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<th>0.001</th>
<th>0.05</th>
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<th>0.4</th>
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<td>0.177</td>
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<td>0.177</td>
<td>0.177</td>
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</table>
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of this analysis are uncertainties about the genetic map in this region and the constraint of a fixed ratio of female and male recombination demanded by LINKMAP.

Discussion

Linkage analysis in TSC is hampered by the lack of large affected families and by the difficulty of excluding the condition in those at risk. Apparent discrepancies in previous linkage data might have arisen through misclassification of minimally affected subjects or because of wide confidence limits for the recombination fractions obtained or as a result of genetic heterogeneity. In this study the inclusion of only rigorously investigated family members and the consideration of genetic heterogeneity should have minimised errors owing to these factors.

In one family (9431) segregation of TSC with ABL was atypical of the data as a whole. This family showed recombinants in at least two of four meioses, while no recombinants were seen in the other families, which gave a lod score equivalent to 17 phase known meioses. Formal testing provided statistically significant evidence for genetic heterogeneity and for exclusion of family 9431 (p=0.01). Clinical features in this family were diagnostic of tuberous sclerosis; all affected subjects had adenoma sebaceum and multiple calcified subependymal nodules on CT scan in addition to secondary diagnostic signs. All meioses in family 9431 were scored in affected subjects.

As well as providing evidence for genetic heterogeneity in TSC our findings provide support for the localisation of one disease locus to distal 9q. Positive lod scores were obtained with each of five markers forming a linkage group in this region and multipoint analysis placed TSC between MCOA12 and MCT136 in the vicinity of the Abelson oncogene locus, a possible candidate gene.

At present the only clue to the possible localisation of other TSC gene(s) comes from the report of an infant with tuberous sclerosis who was trisomic for 11q23.3–qter.11 We therefore investigated our families with the probes MCT128.1, 1CJ52.208M2, and PBGD which map to this region. These probes did not show evidence of linkage to TSC in our families but it was interesting to note that the family unlinked to ABL gave small positive scores with MCT128.1 and CJ52. Large collaborative studies are needed to confirm genetic heterogeneity and to determine whether loci on chromosome 11 or elsewhere are involved. Genetic heterogeneity will complicate efforts to isolate the TSC genes and severely limit the application of linked probes for genetic counselling in the condition.

ABO and AK1 typing for family 9431 was kindly provided by Drs E de Toit and R Martell (Provincial Laboratory for Tissue Immunology, Medical School, University of Capetown), and for family 9434 by Professor P Meera Khan (Institut voor Anthropogenetica, University of Leiden). DNA probes were donated by Professor Y Nakamura of Howard Hughes Medical Institute, Salt Lake City (EF126.3, MCT136, MCOA12) and Dr M Smith of the Department of Pediatrics, University of California, Irvine (subclones of MCT128.1 and 1CJ52.208M). Financial support for this work was provided by the Medical Research Council, Action Research, the Scottish Hospital Endowments Research Trust, and the Foundation to Promote Scientific Research for the Benefit of the Child (University of Amsterdam). The probe 1CJ52.208M was isolated by C Julier in the laboratory of Y Nakamura.

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