

# Evidence for genetic heterogeneity in tuberous sclerosis

J R SAMPSON\*, J R W YATES†, L A PIRRIT\*, P FLEURY‡, I WINSHIP§, P BEIGHTON§, AND J M CONNOR\*

From \*the University Department of Medical Genetics, Duncan Guthrie Institute, Glasgow; †the Department of Pathology, University of Cambridge; ‡the Department of Neurology, University of Amsterdam, Holland; and §the Department of Human Genetics, University of Cape Town Medical School, South Africa.

**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 1CJ52.208M 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, analysis of 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, periungual fibromata, and hypopigmented macules. Hamartomata 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 Gomez<sup>1</sup> 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.<sup>2</sup> 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.<sup>3-5</sup> Subsequently others have reported recombination between *TSC* and *ABO*<sup>6,7</sup> and two multifamily studies have failed to confirm linkage between *TSC* and *ABO*.<sup>8,9</sup> Recombination between *TSC* and *ABL* has also been observed.<sup>10</sup> 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.<sup>11</sup>

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 criteria<sup>1</sup> 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

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.*<sup>4</sup> 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.

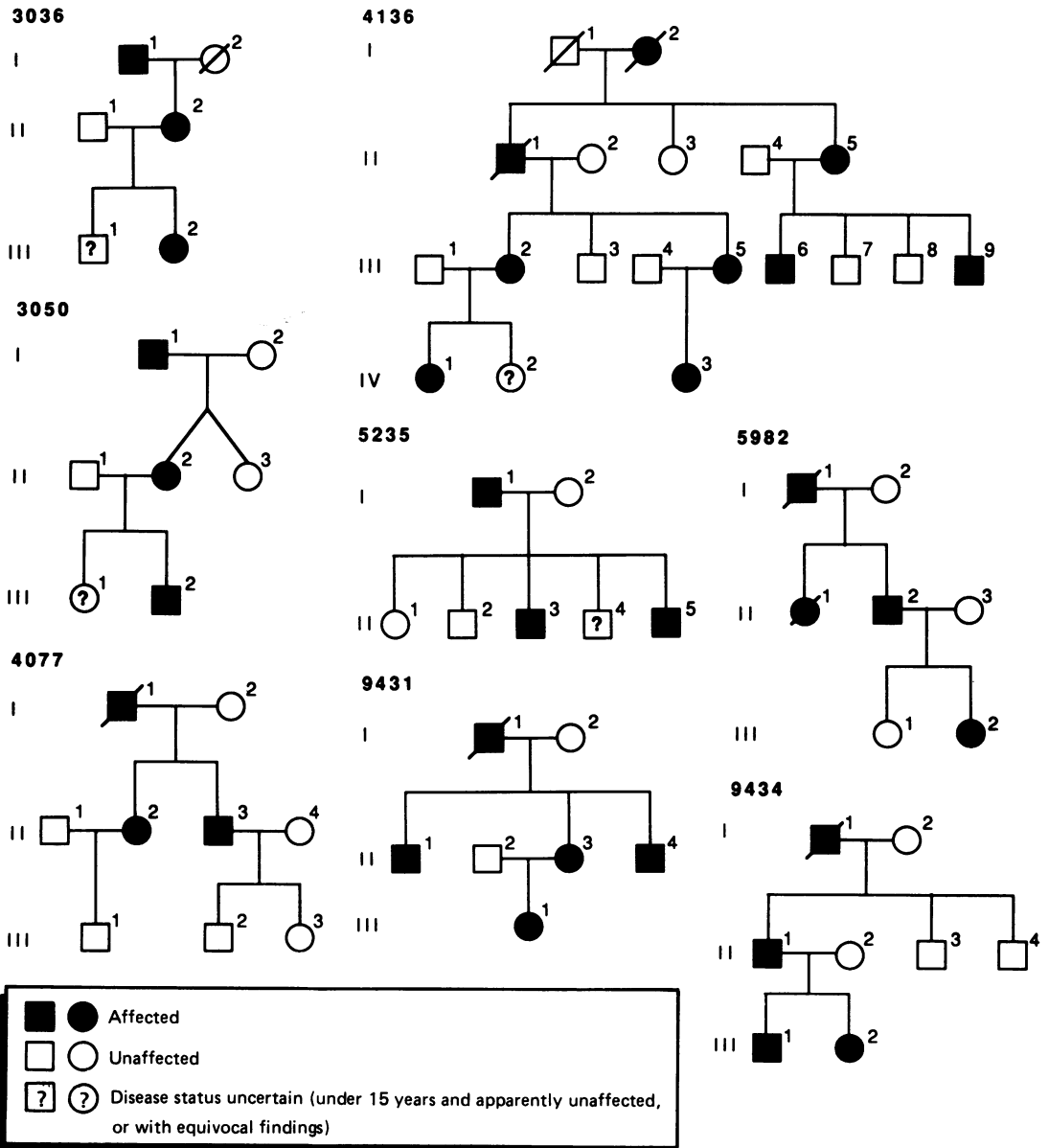


FIG 1 Family pedigrees.

## DNA AND BIOCHEMICAL MARKERS

DNA was extracted from peripheral blood lymphocytes or from lymphoblastoid cell lines according to Kunkel *et al.*<sup>12</sup> 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 <sup>32</sup>P labelled probes under the conditions described by Lathrop *et al.*<sup>13</sup> The probes mapping to distal 9q used in this study were pSA17<sup>14</sup> (*ABL*) which detects a *TaqI* polymorphism with alleles of 7 and 8 kb; MCT136 (*D9S10*)<sup>13</sup> and MCOA12 (*D9S16*)<sup>13</sup> which detect *MspI* RFLPs at 2.0 and 2.2 kb and at 2.5 and 3.0 kb respectively; and EFD126.3 (*D9S7*)<sup>13</sup> 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*)<sup>15</sup> which detects alleles at 2.6 and 2.9 kb; a 1.3 kb *HindIII* subclone of 1CJ52.208M2 (*CJ52*) with alleles at 3.2 and 4.0 kb; and the erythroid porphobilinogen deaminase gene pUSE109 (PBGD)<sup>16</sup> with alleles at 2.2 and 3.0 kb.

## LINKAGE ANALYSIS

The LIPED computer program<sup>17</sup> was used to compute two point lod scores and LINKMAP from version 4.7 of LINKAGE was used for multipoint analysis.<sup>18</sup> The LINKSYS program was used for data management.<sup>19</sup> 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.<sup>20</sup> 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.*<sup>13</sup>

## 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.<sup>21</sup> Using HOMOG the recombination fraction  $\theta$  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  $\alpha$  of families are linked at recombination fraction  $\theta'$  and the remaining families are unlinked, the likelihood being maximised as a function of  $\alpha$  and  $\theta'$ . 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.<sup>3-5</sup> Since then, subjects, II.3, III.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

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

Marker locus	Chromosome	Recombination fraction								$\hat{Z}$	$\hat{\theta}_m$	$\hat{\theta}_f$
		0	0.001	0.05	0.1	0.2	0.3	0.4				
<i>D9S7</i>	Zm 9	—∞	-7.54	-1.05	-0.16	0.39	0.42	0.26	1.43	0.32	0.00	
	Zf	1.20	1.20	1.09	0.98	0.75	0.50	0.25				
<i>D9S10</i>	Zm 9	—∞	-0.72	0.77	0.85	0.71	0.47	0.21	2.01	0.10	0.00	
	Zf	1.16	1.16	1.06	0.96	0.72	0.45	0.18				
<i>ABO</i>	Zm 9	—∞	-1.32	0.24	0.40	0.40	0.28	0.12	0.45	0.15	0.33	
	Zf	-0.40	-0.40	-0.29	-0.20	-0.08	-0.01	0.01				
<i>ABL</i>	Zm 9	—∞	-0.03	1.42	1.44	1.17	0.74	0.30	3.01	0.08	0.09	
	Zf	—∞	-0.30	1.18	1.25	1.04	0.68	0.28				
<i>D9S16</i>	Zm 9	—∞	-1.92	-0.27	-0.05	0.05	0.02	-0.01	0.24	0.17	0.24	
	Zf	—∞	-2.10	-0.45	-0.19	0.00	0.06	0.05				
<i>PBGD</i>	Zm 11	—∞	-2.48	-0.75	-0.44	-0.16	-0.05	-0.01	0	0.5	0.5	
	Zf	—∞	-5.27	-1.89	-1.30	-0.75	-0.44	-0.21				
<i>D11S144</i>	Zm 11	—∞	-5.68	-2.19	-1.53	-0.86	-0.47	-0.20	0	0.5	0.5	
	Zf	—∞	-2.13	-0.50	-0.26	-0.10	-0.05	-0.03				
<i>1CJ52.208M</i>	Zm 11	—∞	-1.60	-0.03	0.14	0.18	0.10	0.03	0.18	0.16	0.5	
	Zf	—∞	-3.10	-1.30	-0.93	-0.54	-0.29	-0.12				

Zm and Zf indicate, respectively, the male and female sex specific lod scores calculated at each recombination fraction.  $\hat{Z}$  indicates the maximum lod score obtained, and  $\hat{\theta}_m$  and  $\hat{\theta}_f$  the sex specific recombination fractions corresponding to  $\hat{Z}$ .

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

Family	Recombination fraction					
	0.001	0.05	0.1	0.2	0.3	0.4
5235	0.901	0.814	0.720	0.517	0.298	0.094
4077	1.177	1.071	0.961	0.733	0.496	0.253
4136	2.998	2.736	2.455	1.847	1.179	0.479
9431	-5.097	-1.721	-1.143	-0.592	-0.298	-0.115

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

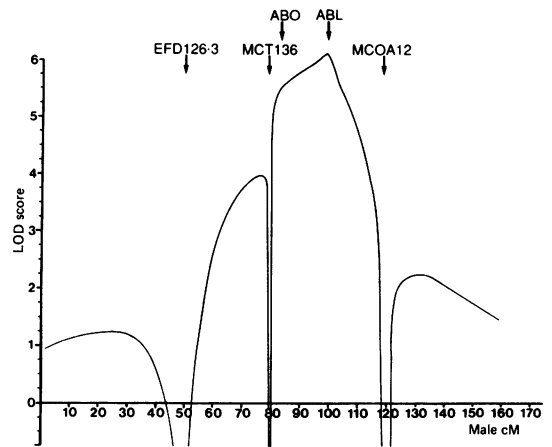


FIG 2 *Multipoint analysis indicating likelihood for localisation of TSC in relation to male genetic map of distal 9q.*

*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

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

Marker locus	Chromosome	Recombination fraction								$\hat{Z}$	$\hat{\theta}_m$	$\hat{\theta}_f$
			0	0.001	0.05	0.1	0.2	0.3	0.4			
<i>D9S7</i>	Zm 9	∞	-7.94	-1.39	-0.45	-0.20	0.33	0.34	1.35	0.36	0.00	
	Zf	1.20	1.20	1.09	0.98	0.75	0.50	0.25				
<i>D9S10</i>	Zm 9	∞	-0.95	0.58	0.69	0.62	0.42	0.20	1.82	0.12	0.00	
	Zf	1.13	1.12	1.03	0.93	0.70	0.44	0.17				
<i>ABO</i>	Zm 9	1.08	1.08	0.96	0.84	0.59	0.35	0.13	1.42	0.00	0.00	
	Zf	0.04	0.04	0.08	0.11	0.12	0.11	0.07				
<i>ABL</i>	Zm 9	2.38	2.38	2.14	1.89	1.36	0.81	0.31	5.09	0.00	0.00	
	Zf	2.40	2.40	2.18	1.95	1.44	0.90	0.37				
<i>D9S16</i>	Zm 9	∞	-2.06	-0.39	-0.15	-0.01	-0.01	-0.02	0.18	0.20	0.25	
	Zf	∞	-2.10	-0.45	-0.19	0.00	0.05	0.05				

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 multi-point 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.<sup>11</sup> 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 (EFD126.3, 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.

## References

- Gomez MR. Criteria for diagnosis. In: Gomez MR, ed. *Tuberous sclerosis*. New York: Raven Press, 1988:9-19.
- Sampson JR, Scahill SJ, Stephenson JBP, Mann L, Connor JM. Genetic aspects of tuberous sclerosis in the west of Scotland. *J Med Genet* 1989;26:28-31.
- Connor JM, Yates JRW, Mann L, Aitken DA, Stephenson JBP. Tuberous sclerosis: analysis of linkage to red cell and plasma protein markers. *Cytogenet Cell Genet* 1987;44:63-4.
- Fryer AE, Connor JM, Povey S, et al. Evidence that the gene for tuberous sclerosis is on chromosome 9. *Lancet* 1987;ii: 659-61.
- Connor JM, Pirrit LA, Yates JRW, Fryer AE, Ferguson-Smith MA. Linkage of the tuberous sclerosis locus to a DNA polymorphism detected by *v-abl*. *J Med Genet* 1987;24:544-6.
- Northrup H, Beaudet AL, O'Brien WE, Herman GE, Lewis RA, Pollack MS. Linkage of tuberous sclerosis to ABO blood group. *Lancet* 1987;ii:804-5.
- Renwick JH. Tuberous sclerosis and ABO. *Lancet* 1987;ii: 1096-7.
- Kandt RS, Pericak-Vance MA, Hung WY, et al. Multilocus linkage analysis in tuberous sclerosis. *Am J Hum Genet* 1988;43(suppl 3):148A.
- Smith M, Haines J, Trofatter J, Dumars K, Pandolfo M, Conneally M. Linkage studies in tuberous sclerosis. *Cytogenet Cell Genet* 1987;46:694.
- Povey S, Burley M, Fryer AE, Osborne J, Al-Gazali LI, Mueller R. Genetic recombination between tuberous sclerosis and oncogene *v-abl*. *Lancet* 1988;ii:279-80.
- Clark RD, Smith M, Pandolfo M, Fausel RE, Bustillo AM. Tuberous sclerosis in a liveborn infant with trisomy due to t(11q23.2;22q11.2) translocation: is neural cell adhesion molecule a candidate gene for tuberous sclerosis? *Am J Hum Genet* 1988;43(suppl 3):44A.
- Kunkel LM, Smith KD, Boyer SH, et al. Analysis of human Y chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977;74:1245-9.
- Lathrop M, Nakamura Y, O'Connell P, et al. A mapped set of genetic markers for human chromosome 9. *Genomics* 1988;3: 361-6.
- Dale B, Ozanne B. Characterization of mouse cellular deoxyribonucleic acid homologous to Abelson murine leukaemia virus-specific sequences. *Mol Cell Biol* 1981;1:731-42.
- Carlson M, Nakamura Y, Krapcho K, et al. Isolation and mapping of a polymorphic DNA sequence MCT128.1 on chromosome 11 (D11S285). *Nucleic Acids Res* 1988;16:378.

- <sup>16</sup> Llewellyn DH, Kalsheker NA, Elder GH, et al. A MspI polymorphism for the human porphobilinogen deaminase gene. *Nucleic Acids Res* 1987;**15**:1349.
- <sup>17</sup> Ott J. Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. *Am J Hum Genet* 1974;**26**:588-97.
- <sup>18</sup> Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;**81**:3443-6.
- <sup>19</sup> Attwood J, Bryant S. A computer program to make linkage analysis with LIPED and LINKAGE easier to perform and less prone to input errors. *Ann Hum Genet* 1988;**52**:259.
- <sup>20</sup> Conneally PM, Edwards JH, Kidd KK, et al. Report of the committee on methods of linkage analysis and reporting. *Cytogenet Cell Genet* 1985;**40**:356-9.
- <sup>21</sup> Ott J. Linkage analysis and family classification under heterogeneity. *Ann Hum Genet* 1983;**47**:311-20.

Correspondence to Dr J R Sampson, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF4 4XN.