

# Report of a critical recombination further narrowing the TSC1 region

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### Abstract

**A large tuberous sclerosis multigenerational family segregating with markers on chromosome 9q from the TSC1 region was studied with a new highly polymorphic marker (designated A6) from the region. A critical affected person showed recombination with the marker, eliminating approximately 100 kilobases from the telomeric end of the critical region, which contains three genes and three to four additional exons for which the associated genes have not been delineated. This information serves to further the search for the TSC1 gene.**

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Key words: tuberous sclerosis complex (TSC); TSC1; chromosome 9q; polymorphism.

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Tuberous sclerosis complex (TSC) is an autosomal dominantly inherited disease with a population frequency of 1 in 6000 to 1 in 10 000.<sup>1-4</sup> Efforts to isolate the causative gene(s) by positional cloning have been under way for a number of years with initial linkage reported to chromosome 9q in 1987.<sup>5</sup> TSC is well documented as exhibiting locus heterogeneity, with one locus on chromosome 9q (TSC1) and another on 16p (TSC2).<sup>6-9</sup> The TSC2 gene has been cloned<sup>10</sup> and work towards characterisation is in progress.<sup>11-13</sup> The TSC1 gene on chromosome 9q, despite being localised first, has continued to elude investigators. One difficulty with cloning the TSC1 gene has been in narrowing its location to facilitate the isolation and testing of candidate genes. There are few large kindreds available with recombinant subjects to aid in narrowing the region.

We have studied a large kindred, previously reported in less detail,<sup>6</sup> with 12 DNA markers from the TSC1 region and five DNA markers (including an intragenic TSC2 polymorphism) from the TSC2 region. Studies of the family yield a maximum two point lod score of 4.68 at  $\theta=0.0$  with a marker from the TSC1 region (table). Linkage of the family is excluded by

markers from the TSC2 region, including an intragenic TSC2 polymorphism. By studying the family with a previously undescribed polymorphic marker (designated A6) on chromosome 9q, we have eliminated a large portion of the TSC1 region from consideration, aiding in the continuing search for the TSC1 gene.

### Methods

#### CLINICAL ASSESSMENT OF FAMILY MEMBERS

All family members have been carefully examined for signs of TSC. Before assignment of TSC gene status, at risk subjects underwent careful physical examination including Wood's light examination of the skin, dilated retinal examination, CT scan or MRI study of the brain, renal ultrasound, and echocardiogram. Some family members have been followed and re-examined several times over the course of an eight year period (II-4, II-6, II-10, III-13, III-14, III-15, III-16, III-17, III-19, III-21, III-22, III-23). Disease status has been assigned in all family members. Results of diagnostic studies are summarised in fig 1.

#### LINKAGE STUDIES FOR CHROMOSOMES 9 AND 16

Twelve dinucleotide repeat markers were tested including four associated with genes (gelsolin (GSN),<sup>14</sup> the Abelson oncogene (ABL),<sup>15</sup> argininosuccinate synthetase (ASS),<sup>16</sup> and dopamine  $\beta$ -hydroxylase (DBH)<sup>17</sup>). Of the remaining eight tested, five (D9S65, D9S64, D9S66, D9S114, and D9S67) map to 9q34<sup>18</sup> and the other three (D16S525, D16S291, and D16S283) map to 16p.<sup>19</sup>

Five additional markers were tested including ABO,<sup>20</sup> Col5A1,<sup>21</sup> TSC2EcoRV (TSC2 intragenic marker),<sup>13</sup> D16S83, and A6. ABO, Col5A1, and the TSC2EcoRV polymorphism were all tested by PCR amplification, restriction enzyme digestion, and electrophoresis on an agarose gel. D16S83 and A9 were tested by Southern blotting analysis (modified from Martin *et al*<sup>22</sup>).

The newly discovered marker, A6, is a 1.7 kb HindIII/EcoRI DNA fragment isolated from cosmids c01 and c02 located in the TSC1 critical region, as reported by Murrell *et al*.<sup>23</sup> The physical mapping information indicates that A6 is located approximately 100 kb proximal to D9S114 and 200 kb distal to D9S66. On testing 22 unrelated subjects, A6 detects a HindIII polymorphism with at least 12 alleles ranging in size from 4.5 to 10 kb (data not shown). Eighteen of 23 members of HOU-4 were tested with A6 (fig 2). No result was

Two point lod scores in family HOU-4 for TSC and chromosome 9q markers

Locus	Lod scores at indicated recombination fraction ( $m=f$ )				
	0.00	0.05	0.10	0.20	0.30
ASS	4.68	4.31	3.92	3.07	2.11
D9S64	3.48	3.20	2.90	2.26	1.53
DBH	4.68	4.31	3.92	3.07	2.11
D9S66	0.90	0.81	0.72	0.52	0.30
D9S114	— $\infty$	2.49	2.47	2.07	1.46
D9S67	— $\infty$	0.47	1.05	1.27	1.04

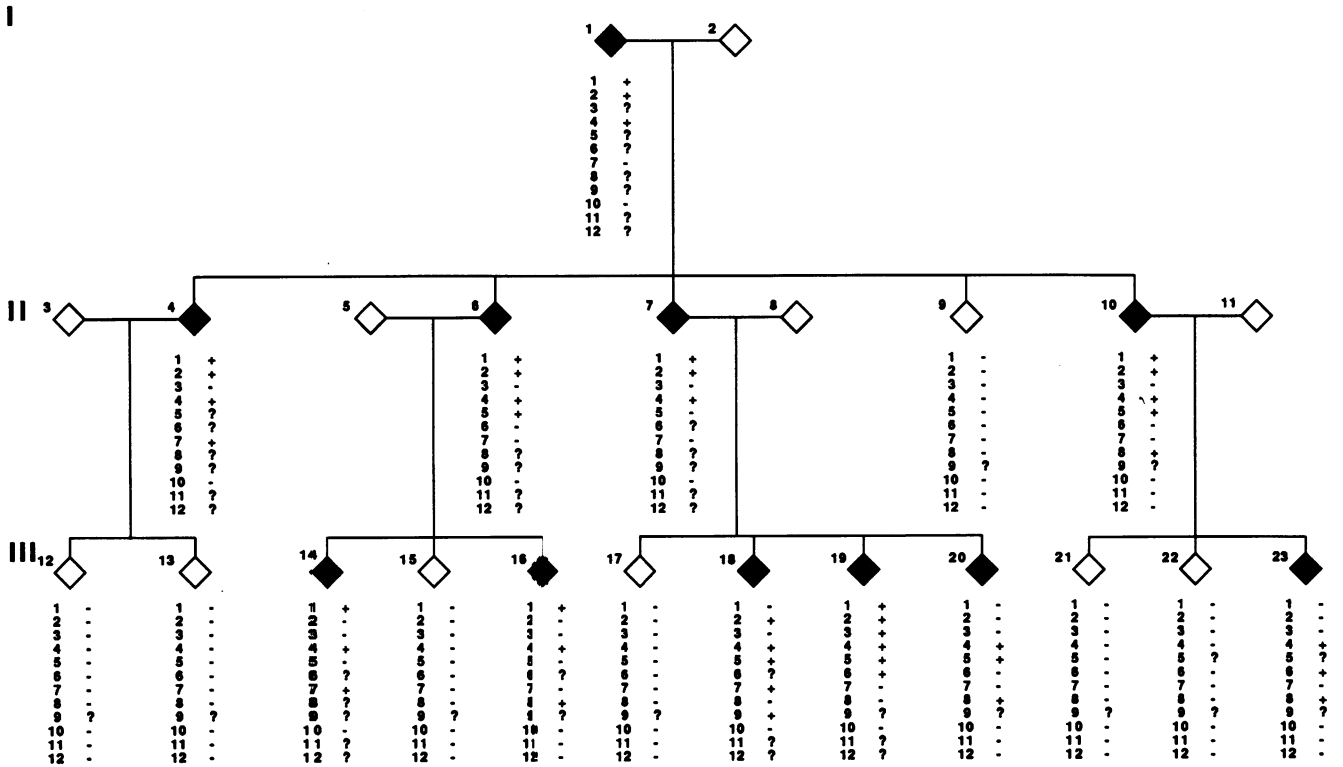


Figure 1 Chromosome 9 linked tuberous sclerosis pedigree. Affected people are indicated by solid diamonds and +/– refers to diagnostic findings as follows: (1) facial angiofibroma, (2) periungual fibromas, (3) shagreen patches, (4) hypopigmented macules, (5) retinal lesions (phakomata or achromic patches or both), (6) cardiac rhabdomyomata, (7) seizures, (8) brain CT findings of TSC (calcified subependymal nodules), (9) brain MRI findings of TSC (tubers), (10) mental retardation, (11) renal angiomyolipomata, (12) renal cysts. A ‘?’ indicates that the person’s status for a finding is unknown.

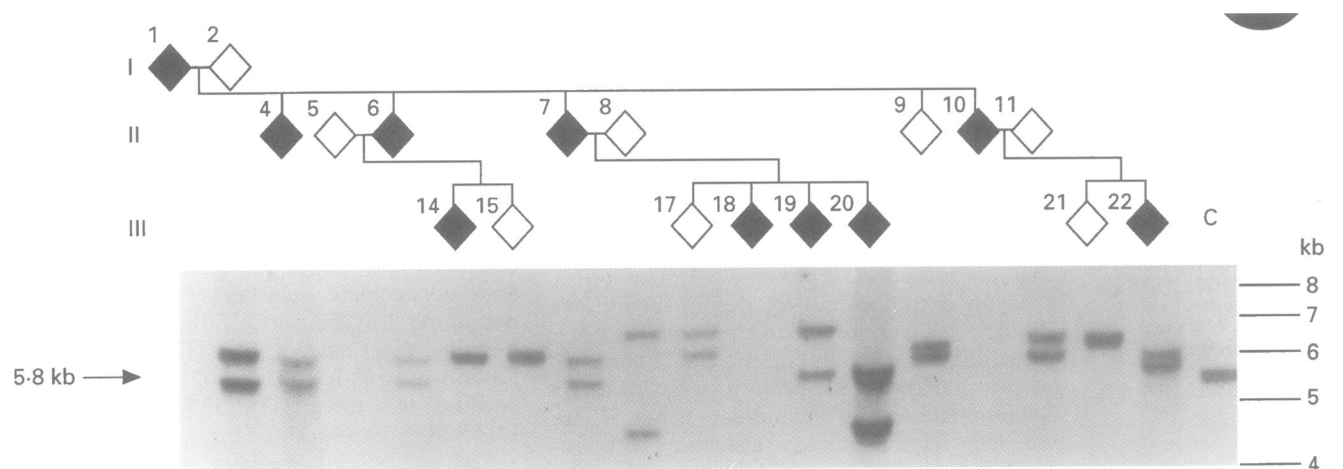


Figure 2 Southern blot of DNA from members of family HOU-4. All affected subjects have a band at 5.8 kb with the exception of III-14 whose chromosomes have undergone a recombinational event.

obtained on four subjects (I-1, II-5, II-10, and III-18); however, genotypes on these people are not critical to our conclusions. We were able to determine the genotype on seven affected subjects including the critical recombinant subject, III-14. Map position for all markers was as indicated in recent published reports for chromosomes 9 and 16.<sup>19,24,25</sup>

**Results**

Loci tested on chromosome 16p included (centromere to telomere): D16S283, D16S291, the TSC2 gene (exon 40), D16S83, and D16S525. D16S83 excluded linkage to  $\theta=0.07$ , the

TSC2 gene excluded linkage to  $\theta=0.025$ , and D16S291 excluded linkage to  $\theta=0.16$ . Haplotyping the five markers showed no crossovers, excluding the entire region causing TSC in this family.

Haplotypes generated for chromosome 9q are indicated in fig 3. The grandparental haplotype segregating with TSC is boxed. Crossover events have occurred in II-10, III-14, and III-19. II-10 and III-19 indicate recombination with Col5A1, eliminating it as a candidate gene. III-14 has had a crossover event detected with the new polymorphic marker (fig 2), eliminating the DNA telomeric as harbouring the TSC1 gene.

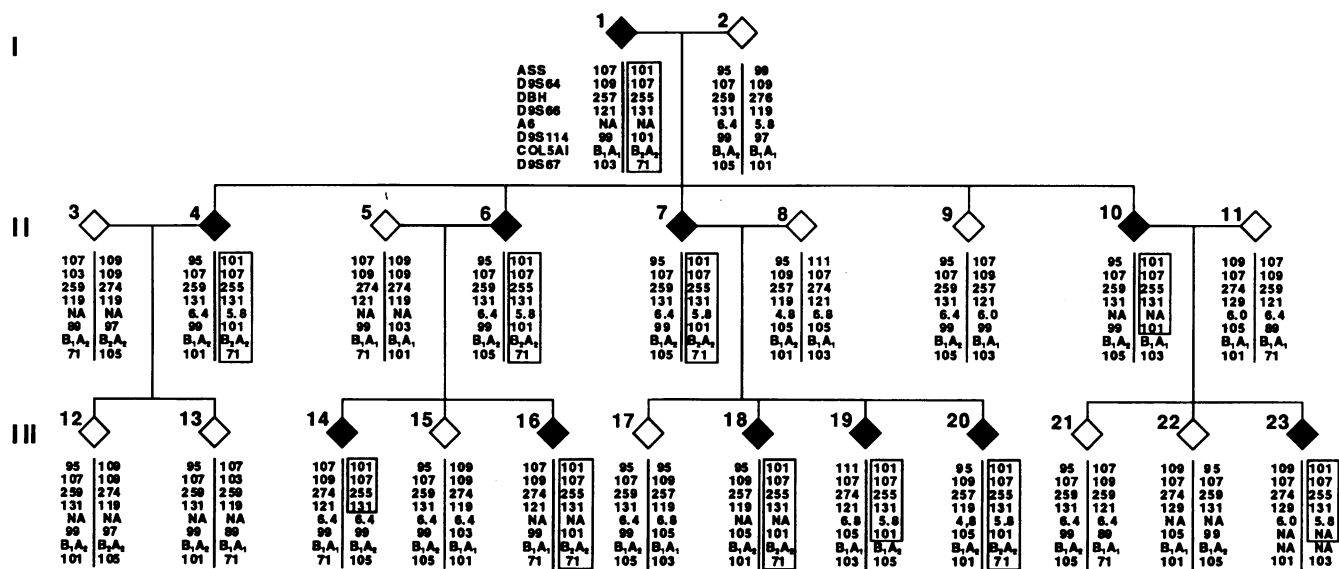


Figure 3 Pedigree of family HOU-4 with results of chromosome 9q markers haplotyped beneath each person. The affected haplotype has been boxed indicating the recombinational event.

**Discussion**

The large family described here shows strong evidence of linkage to the TSC1 gene while eliminating the TSC2 gene as causative. Unlike a previous study seeking to accomplish a similar aim, narrowing of the TSC1 critical region using a large chromosome 9 linked family and critical recombinants,<sup>26</sup> the key recombinant subject (III-14) in our family is definitely affected with TSC. It is therefore valid to use the information generated to narrow the TSC1 critical region further. By testing A6, a newly discovered polymorphic marker from the region, we have eliminated 100 kilobases and three genes from the telomeric end of the critical region (Murrell *et al*, submitted). The reported findings help in the continuing search for the TSC1 gene.

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