Identification of markers flanking the tuberous sclerosis locus on chromosome 9 (*TSC1*)

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Abstract

Analysis of a large tuberous sclerosis pedigree confirmed linkage to a locus on the long arm of chromosome 9, with recombination events placing the disease gene distal to gelsolin and proximal to dopamine β-hydroxylase.

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Linkage analysis in tuberous sclerosis (TSC) is complicated by locus heterogeneity,[1 2] making it difficult to distinguish recombination from non-linkage and to identify appropriate flanking markers. The problem is exacerbated by the paucity of large affected pedigrees.

Despite these difficulties a number of studies have established the existence of a TSC locus on distal 9q (*TSC1*) and have used a variety of analytical approaches to define confidence intervals for the position of this locus.[1-6] However, the validity of these approaches is undetermined and therefore haplotype analysis in large pedigrees which independently support linkage remains the simplest and least error prone method for defining the interval which contains the TSC1 gene.

We have used 16 polymorphic markers from distal 9q to study a four generation chromosome 9 linked family segregating for tuberous sclerosis (two point analysis, TSC versus ASS, generates a lod score of 3·86 at θ=0, assuming 98% penetrance). Markers flanking the *TSC1* locus have been identified by key recombination events in the haplotypes generated.

Methods

CLINICAL ASSESSMENT OF FAMILY MEMBERS

All family members have been clinically assessed for signs of tuberous sclerosis. At risk subjects without definitive diagnostic signs (as defined by Gomez[7]) were investigated by dermatological examination with Wood's light, indirect ophthalmoscopy, renal ultrasound scan, brain CT scan, and skeletal survey before being ascribed normal status. Apparently un-
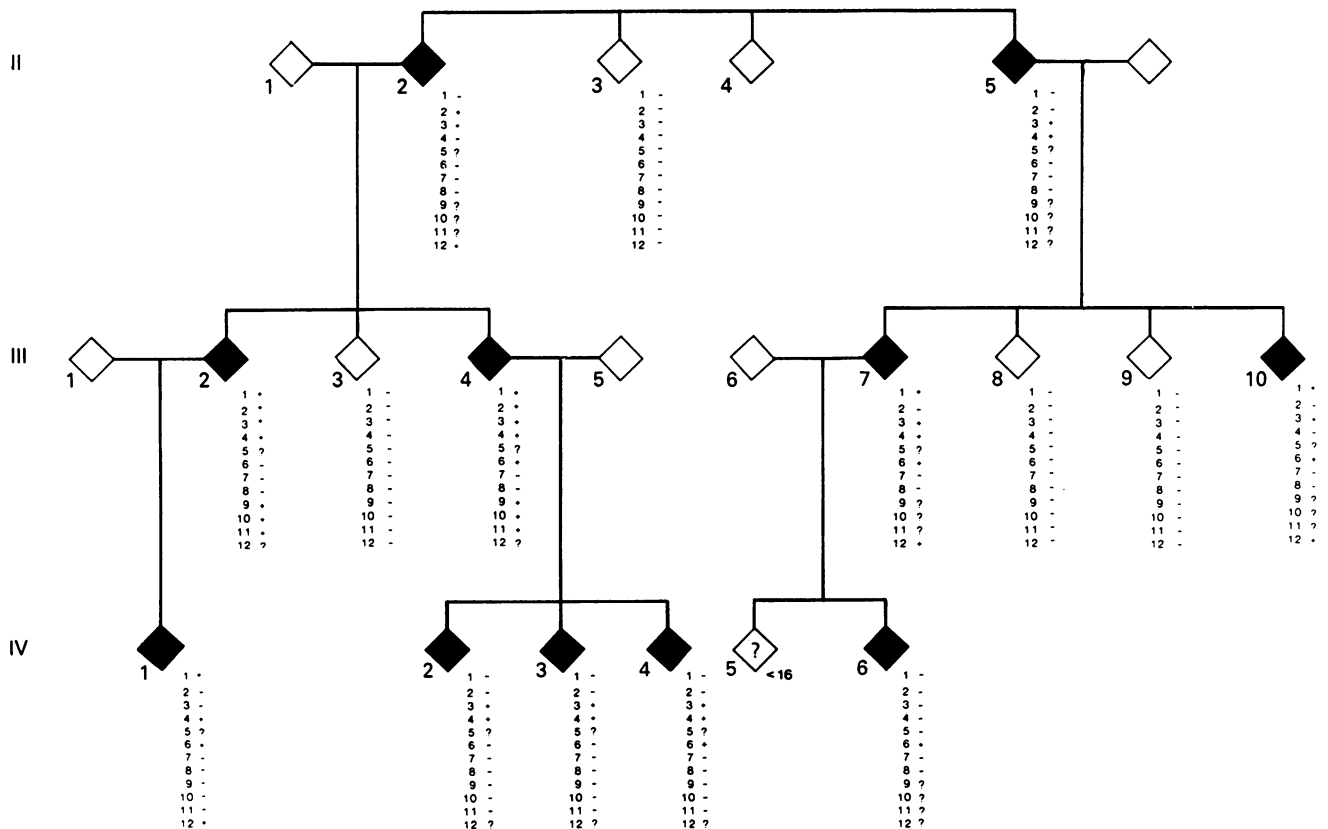

Figure 1 Chromosome 9 linked tuberous sclerosis pedigree. Affected subjects are represented by solid diamonds and +/− refers to the diagnostic signs as follows. (1) Adenoma sebaceum. (2) Periungual fibroma(s). (3) Shagreen patch(es). (4) Hypopigmented macules. (5) Retinal phakoma(ta). (6) Seizures. (7) Mental retardation. (8) Brain tumour. (9) Renal cysts. (10) Renal angiomyolipoma(ta). (11) Renal adenocarcinoma. (12) Periventricular calcification on brain CT scan.

affected family members under 18 years of age were not radiologically investigated and have been ascribed unknown disease status. The diagnostic findings are summarised in fig 1.

DETERMINATION OF HAPLOTYPES

Eleven dinucleotide repeat polymorphisms were typed, including those associated with the genes for gelsolin (*GSN*),[8] argininosuccinate synthetase (*ASS*),[9] the Abelson oncogene (*ABL*),[10] and dopamine β-hydroxylase (*DBH*).[11] The remaining dinucleotide repeats, *D9S59*, *D9S60*, *D9S61*, *D9S63*, *D9S64*, *D9S65*, and *D9S66*, have all been mapped to distal 9q and ordered with respect to *D9S15*, *ASS*, and *ABL*.[12]

Six additional markers were included in the study: *AK-1* (two polymorphisms), *D9S49*, *D9S10*, *ABO*, and *DBH* (a 19 bp deletion polymorphism very close to the dinucleotide repeat[13]). Information from the AK-1 protein polymorphism was combined with data from the *Taq*I RFLP at the same locus to increase informativity. *D9S49* (33.1) and *D9S10* (MCT136) are both VNTR polymorphisms and were resolved using *Taq*I and *Pst*I digested genomic DNA, and standard Southern blotting and hybridisation techniques.[14] Denaturing gradient gel electrophoresis (DGGE) was used to increase the informativity at the ABO blood group locus[15] which was also typed serologically and by PCR.[16]

Loci were ordered as shown in fig 2 according to existing published data[12 17] and consistent with the consensus map agreed at the First International Workshop on Chromosome 9.[18]

Results

The haplotypes generated are indicated in fig 3. The disease conferring chromosome is boxed and recombination events are arrowed. Brackets indicate where informativity precludes positioning of a recombinant to a single marker-marker interval.

Key recombination events on the disease conferring chromosome occur between *ABO* and *DBH*, and *GSN* and *D9S60*, in unaffected subjects III·3 and III·9 respectively, positioning *TSC1* proximal to *DBH* and distal to *GSN*. *DBH* is the closest distal flanking marker so far proposed for *TSC1*. Fig 4 shows the raw data for polymorphic markers defining the recombination event between *ABO* and *DBH* on the disease conferring chromosome.

Additional recombinants on non-disease conferring chromosomes support the established marker order[18] and suggest that *ABO* lies distal to *D9S64*. No recombination occurred in the group *DBH-D9S10-D9S66*.

Discussion

The value of individual recombination events in positioning the TSC1 gene depends on several factors. Most important of these is the probability that the family in question does indeed segregate for a *TSC1* locus, rather than a *TSC2* locus. In a recent collaborative study

of 128 TSC families it was estimated that the proportion of chromosome 9 linked (TSC1) families was 50% (manuscript in preparation). This is the prior probability of linkage and the lod score in an individual family must be interpreted with this in mind.

Secondly the possibility of clinical misclassification (for example, because of non-penetrance or the existence of phenocopies) must be considered. Both key recombination events in this study occurred in unaffected subjects who had undergone the extensive protocol of investigation outlined above. This comprehensive clinical and radiological workup is required because of the highly variable expression exhibited by the TSC gene. Non-penetrance is very unusual in TSC, but were either of the key recombinant subjects non-penetrant gene carriers the recombination events would place *TSC1* proximal to *GSN* or distal to *DBH*. Previous attempts at defining flanking markers are consistent with *TSC1* mapping between *GSN* and *DBH*.[1 2 4–6]

Lastly, false recombination events may be detected because of typing errors. The use of multiple polymorphic systems at key loci in combination with typing of multiple informative markers either side of key recombination events and the exclusion of double recombi-
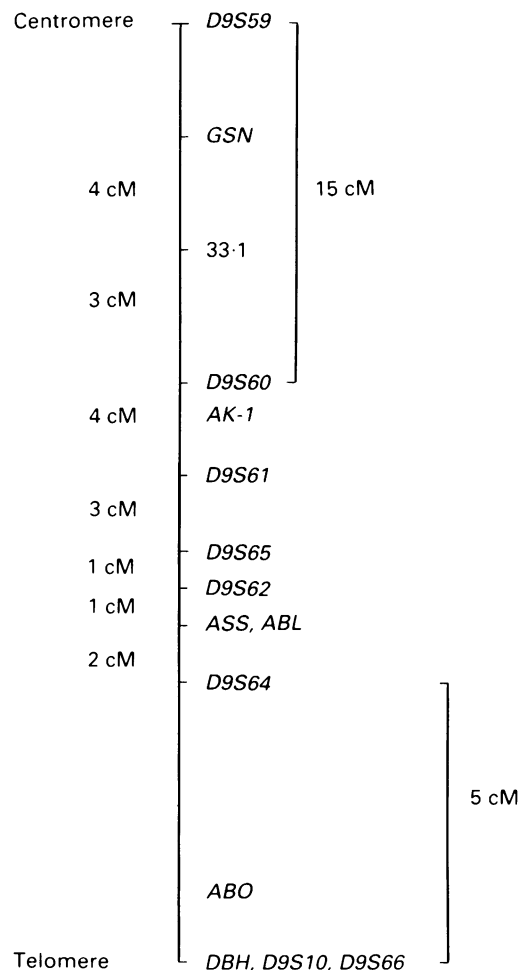
Figure 2   Genetic map of chromosome 9q. Sex averaged intermarker distances (in centimorgans) are indicated in the intervals.
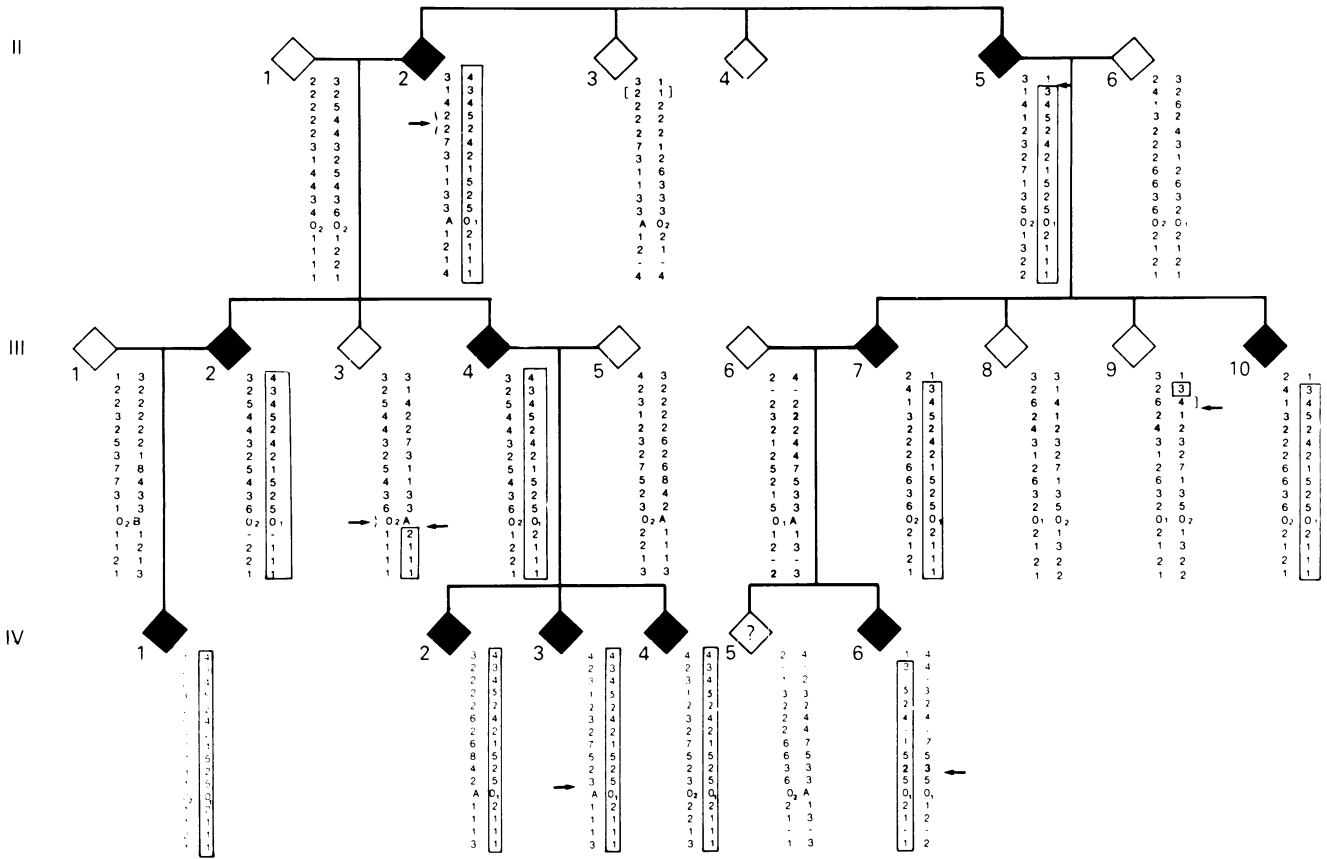
Figure 3   Haplotypes for markers from distal 9q in a chromosome 9 linked tuberous sclerosis pedigree. Affected subjects are represented by solid diamonds and recombination events are arrowed. Markers are listed proximal (top) to distal (bottom) as follows: D9S59, GSN, 33.1, D9S60, AK-1, D9S61, D9S65, D9S62, ASS, ABL, D9S64, ABO, DBH dinucleotide repeat, DBH deletion polymorphism, D9S10, D9S66.

nants helps to minimise the likelihood of such errors.

Given the substantial difficulties encountered in linkage analysis under locus heterogeneity, refinement of the position of the *TSC1* locus is likely to be made by careful study of the few large families segregating for mutations at this locus.
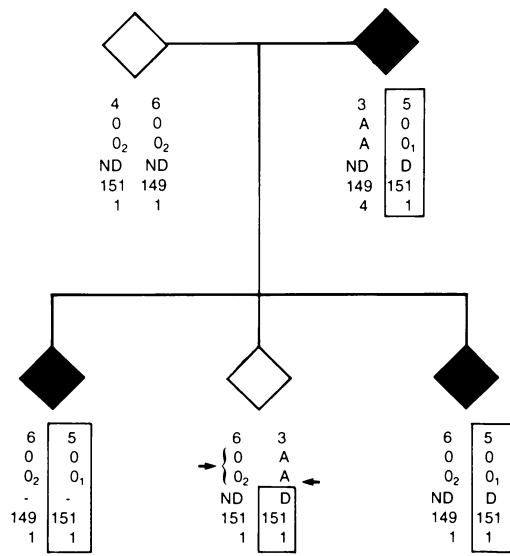
Figure 4   Raw data identifying DBH as a probable distal flanking marker for TSC1. The disease conferring chromosome is boxed. Recombination events are arrowed and correspond to those indicated in subject III·3 in fig 3. The polymorphisms are listed proximal (top) to distal (bottom) as follows: D9S64, ABO by serotyping, ABO by DGGE, DBH 19 bp deletion polymorphism (D = deletion present, ND = not deleted), DBH CA repeat allele size, D9S66.

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