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INTRODUCTION

Tuberous sclerosis is a disorder of cell migration, cell proliferation and cell differentiation which can potentially involve almost every organ in the body, (Gomez 1988). The organ systems most commonly involved in Tuberous sclerosis (TSC)include the skin, central nervous system, kidney and heart.The lung , liver and gastrointestinal tract may also be involved. The most common TSC lesions in the brain include cortical tubers, subependymal nodules and giant cell astrocytomas, Reagan, (1988). There is evidence also for the occurrence of heterotopias and cellular migration defects in TSC . These are seen on cerebral imaging as bands extending radially from the cerebral mantle to the cortex , as cerebellar bands or as non-specific conglomerate foci (Braffman et al., 1992). The most common lesions in the kidney are angiomyolipomas, cystic lesions and renal cell carcinoma sometimes occurs, (Bernstein and Robbins, 1991). Approximately 50% of infants with tuberous sclerosis have cardiac rhabdomyomas, which may lead to outflow obstruction or cardiac arrhythmias. Skin lesions in TSC include hypomelanotic macules, facial angiofibromas, ungual fibromas, shagreen patch of the lumbar area or forehead. There is evidence the aneurysms particularly of the abdominal aorta may complicate TSC. There is also evidence that liver hamartomas are much more common in TSC than was previously recognized, (Jozwiak et al., 1992).

Genetics

Gunther and Penrose (1935) first reported that TSC is inherited as a dominant trait. It is clear however that there is a high frequency of new mutations.This mutation frequency has been reported as 50% (Wilson et al., 1978), although some studies have indicated that the frequency of new mutations may be as high as 75%, (Jozwiak et al., 1992). Variable penetrance is observed in TSC, individuals who carry this mutation may have few or no signs on clinical examination and marked intra and interfamilial variation in the type and the degree of symptoms.

It is clear therefore that a true idea of the frequency of new mutations will not be available until the disease gene is cloned, since individuals who carry the TSC gene may not be identified by clinical studies.

It is important to note the TSC occurs in all races. The population frequency in Caucasians has been estimated as 1 in 10,000 in Caucasians (Wiederholt, 1985). It is clear however, that the population frequency estimation is dependent upon the clinical methods and imaging used for disease ascertainment and that the frequency of the TSC mutation will likely turn out to be much

higher than is estimated on the basis of clinical examination, given the variation in penetrance.

It is also important to note that lesions may arise throughout the life of an individual. Lesions such as peri-ungual fibromas and angiofibromas are not present in early infancy but appear later in childhood or in adult life. There are reports in the literature documenting the appearance of a renal angiomyolipoma in a kidney which was previously documented as normal, (Munjal and Schultz 1992).

At this time it is difficult to know if mutations in tuberous sclerosis genes play a role in the origin of lesions such as isolated TSC type tumors occurring in the absence of other findings of TSC e.g. isolated cases of subpependymal giant cell or of renal angiomyolipoma or infantile cardiac rhabdomyoma.

At the present time the only finding which can be used with some degree of reliability, for prenatal diagnosis of TSC is the finding of fetal cardiac rhabdomyomas., (Green et al., 1991, Giacoia 1992). One difficulty in using this observation for prenatal diagnosis is the time of onset of these lesions and a second difficulty relates to the fact that these lesions do not occur on 100% of affected individuals with TSC.

A possible explanation for this variable expression and variable age of onset, is that TSC represents a premutation and that additional somatic events are required for the development of the actual lesion. One possibility which should be considered is that the TSC genes lie in regions where the DNA is meiotically and mitotically unstable. The second or mitotic event leading to the origin of a TSC lesion may occur on the same chromosome as the premutation, for example a specific repetitive element which represents a premutation, may undergo further amplification. Amplification of trinucleotide repeats has been documented as comprising the disease gene mutation in a number of different diseases e.g. fragile X mental retardation (Yu et al., 1991), in Huntington's chorea (Gusella et al., 1993) and in myotonic dystrophy, where there is evidence that different tissues in an individual patients differ with respect to the degree of amplification of the repeat (Fu et al., 1992). It is possible that the second (somatic) event may occur on the homologous chromosome and that this event may be for example a gene deletion.

If one postulates that the TSC gene is a premutation it becomes easier to explain the finding that monozygotic twins may differ in their expression of TSC lesions. There have been several publications dealing with the discordant expression of TSC in monozygotic twins. Kondo et al., (1992) described a set of monozygotic twins with TSC; one twin had facial angiofibromas, shagreen patch, cerebral lesions renal angiomyolipomas, while the other twin had only a few hypopigmented macules.

Proof of the theory that the TSC mutation represents a premutation will only be obtained once the TSC gene is isolated and analyzed in a number of different tissues, in normal and affected individuals and in TSC lesions. Isolation of the TSC gene may be approached by the positional cloning strategy once the TSC gene(s) are definitively mapped.

Linkage studies in TSC

Linkage studies in TSC have provided evidence that there is more than one TSC locus in the human genome. This genetic heterogeneity in Tuberous sclerosis has led to difficulties with the linkage analysis approach (Conneally 1991). Some of these difficulties have been counteracted by the pooling of family resources from a number of different centers. The development of highly polymorphic marker systems throughout the genome (Weber and May 1989) has also facilitated linkage studies. In addition during the past 5 years linkage programs to analyze heterogeneity have improved (Ott 1991).

In 1987 Fryer et al., published evidence for linkage of TSC and the ABO blood group locus: they observed a peak cumulative lod score of 3.85 at theta=0. Subsequent studies failed to confirm this initial linkage . A large data set was then pooled and analyzed. Results

of this collaborative data analysis determined that there was overwhelming evidence in favor of a locus on chromosome 9q34; from this analysis it was estimated that in 38% of families the TSC locus mapped to chromosome 9q34.

In April 1992 at the First International Workshop on chromosome 9, collaborative data were presented which clearly indicated that in certain families linkage to chromosome 9 or any other proposed TSC loci was clearly excluded (Povey et al., 1992). Following this the TSC collaborative group in the USA pooled resources. Each group contributed DNA from a multigeneration family which had had extensive clinical work-up, and extensive linkage analysis and which clearly indicated that the TSC gene segregated independently of chromosome 9q. Analysis of microsatellite repeat polymorphisms in DNA samples from this pooled resource, revealed that a second TSC locus was present on chromosome 16pl3.3 in close proximity to the polycystic kidney disease region. Results of this linkage analysis, reported by Kandt et al., (1992) revealed that the cumulative lod score between TSC and the marker SM7 (D16S283) was 9.52 at theta 0.02.

Subsequently linkage studies carried out in other chromosome 9 unlinked families, have confirmed the linkage of TSC and chromosome 16pl3.3. In one multigeneration family with TSC the cumulative lod score between TSC and D16S283 was 5.98 (Smith et al., 1992). The segregation of chromosome 16pl3.3 markers in this family is illustrated in Fig. 1.

Current indications are that linkage to chromosome 9 or 16 accounts for more than 90% of cases of familial TSC. Additional data pooling and analysis will need to be carried out to prove that there is not a third TSC locus. It is still possible that isolated families may not map to these chromosomes and that there are families which map to chromosome 11, (Smith et al., 1990), Janssen et al., 1991 or 12 (Fahsold et al. 1991), or that loci on these or other chromosomes influence the expression of the TSC genes.

Clinical features in familial TSC mapping to chromosomes 9 and 16

One question which is currently being addressed is whether there are clinical differences in chromosome 9 linked TSC families versus chromosome 16 linked TSC families. Current indications are that there is considerable clinical heterogeneity in families mapping to chromosome 9 and in families mapping to 16. Indeed we have evidence that the degree of clinical severity differs even in two branches of one extended chromosome 16 linked TSC kindred who share a common affected ancestor. There is some evidence that periungual fibromas may occur more frequently in chromosome 9 linked TSC families . (Northrup et al.,1992; Winship et al.,1992). There is also a report which suggests that the confetti type of hypomelanotic freckling may occur more commonly in non 9 linked TSC families. (Winship et al., 1992). We have however noted that confetti type hypomelanotic freckling may occur in some affected members of a family while typical ashleaf spots occur in other family members and that hypomelanotic freckling and ashleaf spots may occur in the same individual.

Cytogenetic abnormalities in TSC

In the case of neurofibromatosis two patients were reported who had neurofibromatosis an unbalanced chromosomal translocation. These translocation breakpoints were in the neurofibromatosis 1 gene region on chromosome 17q and their location facilitated cloning of the neurofibromatosis gene. There have been several reports of chromosomal abnormalities in patients with tuberous sclerosis . One case was found to have an unbalanced 11/22 chromosomal translocation (Clark et al., 1991). Rubino et al., (1992) and Elia et al., (1992), have described patients who have Down's syndrome and TSC. Fahsold et al., (1991), described a case with TSC and an unbalanced 3/12 chromosomal translocation. It is possible that the co-occurrence of these three forms of cytogenetic abnormality and TSC in these cases represents a chance event.

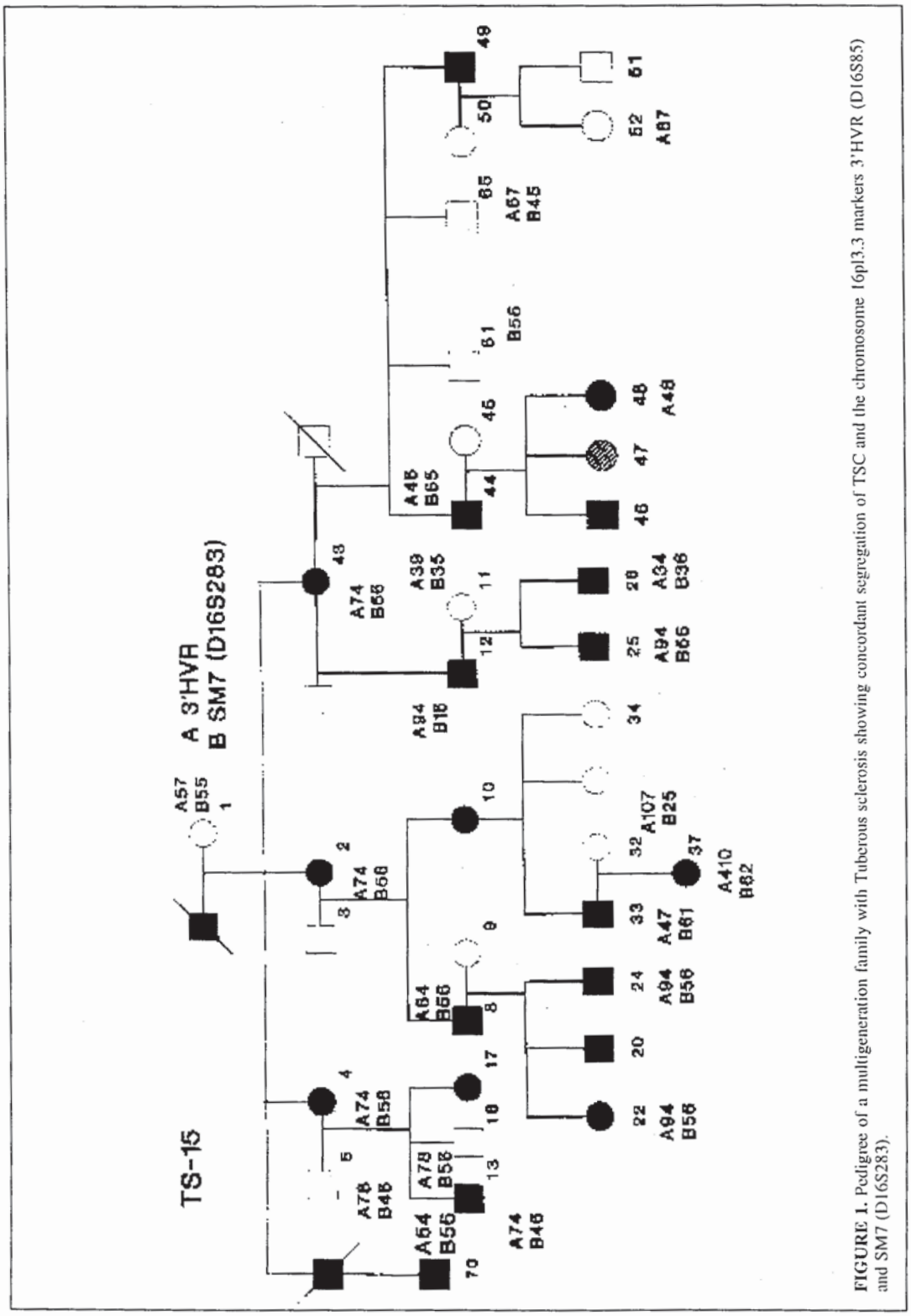

FIGURE 1. Pedigree of a multigeneration family with Tuberous sclerosis showing concordant segregation of TSC and the chromosome 16pl3.3 markers 3'HVR (D16S85) and SM7 (D16S283).

POSITION OF THE CHROMOSOME 9Q34 TSC GENE

Results of analysis of multiple markers in chromosome 9 linked TSC families indicate that the TSC1 gene is located in the region between D9S125 (see Povey et al., 1992) and D9S114 (Northrup , 1993). The genetic distance between these markers is approximately 4 centimorgans. The physical distance between the markers has not been clearly defined. Markers mapped between D9S125 and D9S114, include ABO (bloodgroup), DBH (dopamine beta hydroxylase) and D9S10, and these three markers are known to map on a single 550kb NRUl fragment (Handa et al., 1992). Additional pulsed field gel electrophoresis experiments are required to determine the physical distance between ABO-DBH-D9S10 and D9S125, and between ABO -DBH-D9S10 and D9S114.

POSITION OF THE CHROMOSOME 16 TSC GENE

Current information from the studies of Kandt et al., (1992) and Povey et al., (1993) indicate that the chromosome 16 TSC gene maps between 3'HVR (D16S85) and D9S291, i.e. within a 5 centimorgan distance, this distance is approximately 2 megabases according the physical mapping information generated by Harris et al., 1990 and Somlo et al., 1992.

APPROACHES TO POSITIONAL CLONING OF THE TSC GENES

Strategies to further refine mapping using genetic linkage analysis

To further refine the genetic mapping of the chromosome 9q34 TSC gene it is important that additional markers which map in the interval between D9S125 and D9S114 be typed to more precisely locate the position of the cross over event in key recombinant individuals. In addition to using available markers it will be necessary to develop new markers in this interval. Before initiating experiments to clone the TSC gene in this region it would be advantageous to accumulate information from a number of recombinant individuals in families in whom TSC clearly map to chromosome 9, so that one does not base the entire cloning strategy on a single recombination event.

In the case of the chromosome 16p13.3 TSC locus additional probes which map between D16S85 and D16S291, need to be analyzed. There are at least seven highly polymorphic markers currently available which map in this interval. Development of new probes may also be necessary however since there are two areas within this 5 megabase region where highly polymorphic probes are relatively sparse. It would be advantageous to identify additional recombinant individuals.

There are a number of difficulties in considering analysis of additional TSC families to identify additional key recombinant individuals. The map distance between the currently identified flanking markers, in the case of the chromosome 9 TSC and the chromosome 16 TSC gene is 5 centimorgans. Therefore one could expect to analyze an additional 20 informative phase known meioses to detect a recombinant. Furthermore, given the genetic heterogeneity in TSC, it is important that any family used in linkage analysis be sufficiently large and informative, so that the family can be unequivocally assigned to chromosome 9 or 16.

In the case of TSC it may therefore be important to transition relatively soon from linkage mapping strategies to physical mapping strategies.

Physical mapping strategies

We plan to search for size difference in DNA from affected and unaffected individuals, using pulsed field gel electrophoresis followed by Southern blotting and analysis with a set of DNA probes mapping within the regions of interest. The affected individuals to be studied will include not only individuals with familial TSC but cases of TSC who represent apparent new mutations. In such cases it will be important to compare DNA fragment sizes obtained in analysis of patient and parental DNA samples.

We plan to examine existing probes and any newly developed probes, for the occurrence of

di-trinucleotide repeat elements. Probes containing these elements will be used not only for polymorphism analysis but also to determine if there are size difference in these repetitive elements, in affected individuals versus unaffected individuals.

In addition to searching for evidence of DNA amplifications or deletions in lymphoblastoid cell lines established from TSC individuals, we will carry out experiments where the lesions (tumors) from TSC individuals are analyzed and where host DNA is compared with tumor DNA. Such studies may provide insight into the somatic event which leads to TSC.

Identification of expressed sequences

We plan to commence the search for cDNA clones when the TSC mapping interval (i.e. the distance between flanking markers), is 500-lOOOkb. If a particular genomic clone detects a size difference in affected versus unaffected individuals, this clone or DNA fragments of pulsed field gels which hybridize to this clone, will be used for isolation of cDNA clones. A number of groups have described the successful isolation of cDNA clones using immobilized genomic DNA followed by hybridization with DNA from a cDNA library, (Parimoo et al., 1991, Vetrie et al., 1993).

If we fail to identify causative deletions or duplications in the TSC regions it may be necessary to examine cDNA's mapping to these region to search for point mutation. For this purpose we techniques such as PCR (polymerase chain reaction) followed by SSCP (single stranded conformational polymorphism analysis (Orita et al., 1989), could be used.

SUMMARY

Tuberous sclerosis is a dominantly inherited genetic disorder, with a high frequency of new mutations, which has been shown through genetic linkage studies to be genetically heterogeneous. In this paper we summarize recent progress in linkage studies which indicate that there is one TSC gene on chromosome 9q34 which accounts for approximately 40% of cases and another on chromosome 16pl3.3 which accounts for approximately 50% of cases. We discuss the map position of these TSC genes on chromosome 9q34 and 16pl3.3. We discuss physical mapping data in these two regions. We postulate on the pathogenesis of TSC lesions and on the cause of the high frequency of new mutations. We present a strategy to progress from the mapping of the TSC genes to isolation of these genes.

RESUMEN

Esclerosis Tuberosa es un desorden dominantemente genético heredado, con alta frecuencia de nuevas mutaciones, el cual ha sido mostrado mediante estudios de eslabón genético ser geneticamente heterogéneo. En esta disertación resumimos progresos recientes en estudios de conexiones las cuales señalan que existe un TSC gene sobre cromosoma 9q34 el cual cuenta por aproximadamente 40% de casos y otro sobre cromosoma 16p13.3 el cual corresponde aproximadamente a 50% de los casos.

Discutimos la posición en el mapa de estos TSC genes sobre cromosoma 9q34 y 16p13.3 . Discutimos datos de mapeo físico en estas dos regiones. Postulamos sobre la nueva patogenia de estas TSC lesiones y sobre la causa de la alta frecuencia de nuevas mutaciones, Presentamos una estrategia para el progreso desde el mapeo de los TSC genes al aislamiento de estos genes.

RÉSUMÉ

La sclerose tubereuse est trouble à dominance génétique et héréditaire, avec de mutations fréquantes: ce trouble à été prouvé à travers l'étude des chaines génétiquement hetérogenes. Nous résumons ici les progrès les plus récents sur l'étude des conections qui

signalent qu'il éxiste un gène Tsc sur le chromosome 9q34 dans le 40% des cas et un autre sur le chromosome 16p13.3 dans le 50% des cas nous avons dicuté leur position sur des cartes des gènes Tsc des chromosoms 16p13.3 et 9q34. Nous avons discuté les données de la cartographie de ces 2 régions.

On propose pour la pathogenie de ces Tsc des lésions et la cause de la haute frequance de nouvelles mutations.

Nous présentons une strategie pour le progres de cartographie des Tsc genes et l'individualisation de ces genes.

ZUSAMMENFASSUNG

Die Sclerosis Tuberosa ist eine erbliche genetische Krankheit, und dominant. Sie hat haenfige Mutationen bei der man durch Untersuchungen der genetischen. Bindungen nachweisen konnte, dass sie genetisch heterogenisch. In diesem Vortrag fassen wir die neuesten Fortschritte ueber die Untersuchungen ueber die Verbindungen zusammen, die zeigen dass in 40% der Faelle ein Gen TSC auf dem Chromosom 16p13.3 und eines auf den Chr. 9q34 in nahezu 50% der Faelle ist. Wir besprachen die Daten ueber die physische Topographie und in beiden Regionen. Wir stellten Vermutungen an ueber die Pathogenesen dieser TSC Lesionen und ueber die Ursache der haeufigen nenen Mutationen.Wir aeusserten uns ueber die Strategie fuer die Fortschritte, sowohl bei der Topologierug der TSC Gene als auch bei der Isolierung dieser Gene.

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