Germ-Line Mosaicism in Tuberous Sclerosis: How Common?

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Summary

Two-thirds of cases of tuberous sclerosis complex (TSC) are sporadic and usually are attributed to new mutations, but unaffected parents sometimes have more than one affected child. We sought to determine how many of these cases represent germ-line mosaicism, as has been reported for other genetic diseases. In our sample of 120 families with TSC, 7 families had two affected children and clinically unaffected parents. These families were tested for mutations in the TSC1 and TSC2 genes, by Southern blotting and by single-strand conformational analysis. Unique variants were detected in six families. Each variant was present and identical in both affected children of a family but was absent in both parents and the unaffected siblings. Sequencing of the variants yielded two frameshift mutations, one missense mutation, and two nonsense mutations in TSC2 and one nonsense mutation in TSC1. To determine which parent contributed the affected gametes, the families were analyzed for linkage to TSC1 and TSC2, by construction of haplotypes with markers flanking the two genes. Linkage analysis and loss-of-heterozygosity studies indicated maternal origin in three families, paternal origin in one family, and either being possible in two families. To evaluate the possibility of low-level somatic mosaicism for TSC, DNA from lymphocytes of members of the six families were tested by allele-specific PCR. In all the families, the mutant allele was detected only in the known affected individuals. We conclude that germ-line mosaicism was present in five families with mutations in the TSC2 gene and in one family with the causative mutation in the TSC1 gene. The results have implications for genetic counseling of families with seemingly sporadic TSC.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disease with a prevalence as high as 1/6,000 (Osborne et al. 1991). One-third of cases are familial; two-thirds of cases are sporadic and are attributed to new mutations (Sampson et al. 1989; Osborne et al. 1991). Two causative genes have been identified and cloned: TSC1 at 9q34.3 (MIM 191100) and TSC2 at 16p13.3 (MIM 191092) (The European Chromosome 16 Tuberous Sclerosis Consortium 1993; van Slegtenhorst et al. 1997). TSC has variable expressivity and is thought to be highly penetrant. Fifteen families with two or more affected children and apparently unaffected parents have been reported and are thought to illustrate examples of germ-line mosaicism (Lowry et al. 1979; Osborne et al. 1991; Northrup et al. 1993; Ruggieri et al. 1997). Yates et al. (1997) have proved germ-line mosaicism for TSC2 in one family, by molecular analysis. Germ-line mosaicism has been reported for many other genetic diseases, such as osteogenesis imperfecta type II (Byers et al. 1988) and Duchenne muscular dystrophy (Edwards 1986), as well as for disorders associated with malignant changes, such as retinoblastoma (Sippel et al. 1997), Von Hippel–Lindau disease (Murgia et al. 1997), and neurofibromatosis type I (Lazaro et al. 1994). In addition, somatic mosaicism has been reported for TSC (Verhoef et al. 1995; Sampson et al. 1997).

Unaffected parents who have had a child affected with TSC usually are given a low (1%) recurrence risk. Evidence that germ-line mosaicism is not an uncommon phenomenon would increase the estimated risk in cases of sporadic TSC and, thus, would have implications for genetic counseling. The goal of this study was to determine germ-line mosaicism in seven families with unaffected parents and two affected siblings, by linkage studies and mutation analysis. We also evaluated the parents for low-level somatic mosaicism in their lymphocytes.

Subjects and Methods

Family Studies

In our sample of 120 families with TSC, we categorized 82 as sporadic (one affected child and unaffected

Table 1

Epidemiological Data and Medical Evaluation of Parents

	Age (in Years) of Parents at Birth of		Results of[a]			
Family and Parent	1st Affected Child	2d Affected Child	Physical Examination	Brain Imaging	Renal Imaging	Ophthalmologic Examination
HOU-2:						
Mother	21	33	N	N	N	N
Father	21	33	N	N	N	N
HOU-21:						
Mother[b]	27	45	N	N	N	N
Father	27	45	N	N	N	N
HOU-23:						
Mother[b]	20	21	N	...	...	...
Father	27	28	N	...	...	...
HOU-28:						
Mother	27	31	N	...	...	...
Father	27	31	N	...	...	...
HOU-33:						
Mother	27	31	N	N	N	...
Father[b]	31	35	N	N	N	...
HOU-34:						
Mother	29	32	N	N	...	...
Father	32	35	N	N	...	...
HOU-37:						
Mother[b]	26	34	N	N	N	N

[a] Medical and diagnostic evaluation of unaffected parents of affected children. Both parents were examined in all families, except family HOU-37, in which only the mother of the two half siblings was evaluated. The parents of family HOU-23 declined further testing: at the time of this study, the mother was 57 years of age, and the father was 64 years of age; neither had any findings consistent with TSC. N = normal result. An ellipsis (...) indicates that the test was not performed.

[b] Parent who contributed the mutated gamete.

parents), 32 as multigenerational (multiple affected individuals in a family with autosomal dominant inheritance), and 7 as possibly representing examples of germ-line mosaicism (two affected children and unaffected parents). TSC diagnoses were reached in accordance with standard diagnostic criteria (Gomez 1991; Roach et al. 1992). The parents of all sporadic patients were examined carefully for TSC, with the exception of the father in family HOU-37, who is deceased. No findings consistent with TSC were detected. Some parents underwent additional clinical testing, including retinal examination, renal ultrasonography, and/or computed tomography or a magnetic-resonance–imaging scan of the brain. During the course of the study, one family initially categorized as sporadic, on the basis of lack of clinical findings in the parents, was reassigned into the category of families with germ-line mosaicism, after the birth of a second affected child. These families included six sets of full sibs and one set of half sibs. Family history was negative for any findings suggestive of TSC, for all seven families. Parental ages at the birth of the affected children and results of diagnostic studies are summarized in table 1. The study was approved by the institutional review board at the University of Texas Medical School–Houston.

Linkage Studies for Chromosomes 9 and 16

Five markers that flank TSC1 on 9q34.3 were tested: D9S149, D9S2127, D9S1199, D9S1198, and D9S150 (van Slegtenhorst et al. 1997). Four markers that flank TSC2 on 16p13.3 were tested: D16S525, TSC2EcoRV, KG8, and D16S665. TSC2EcoRV is an RFLP within the TSC2 gene, and KG8 is located at the 3′ end of TSC2 (Au et al. 1997b). All markers, except TSC2EcoRV, were tested by PCR amplification followed by electrophoresis on a sequencing gel. TSC2EcoRV was tested by PCR amplification, enzyme digestion, and electrophoresis on an agarose gel.

Southern Blotting, PCR, Single-Strand Conformational Analysis (SSCA), and Direct Sequencing

Southern blotting, PCR, and SSCA were performed as described elsewhere (Au et al. 1997b). Allele-specific PCR was performed as described by Kaltenbock and Schneider (1998), with the following modifications: annealing temperatures were 10°C lower than the calculated melting temperatures of the allele-specific primers, and 21 thermal cycles were used for amplification. Direct sequencing was performed by the Molecular Genetics Core Facility, Department of Microbiology and Molec-
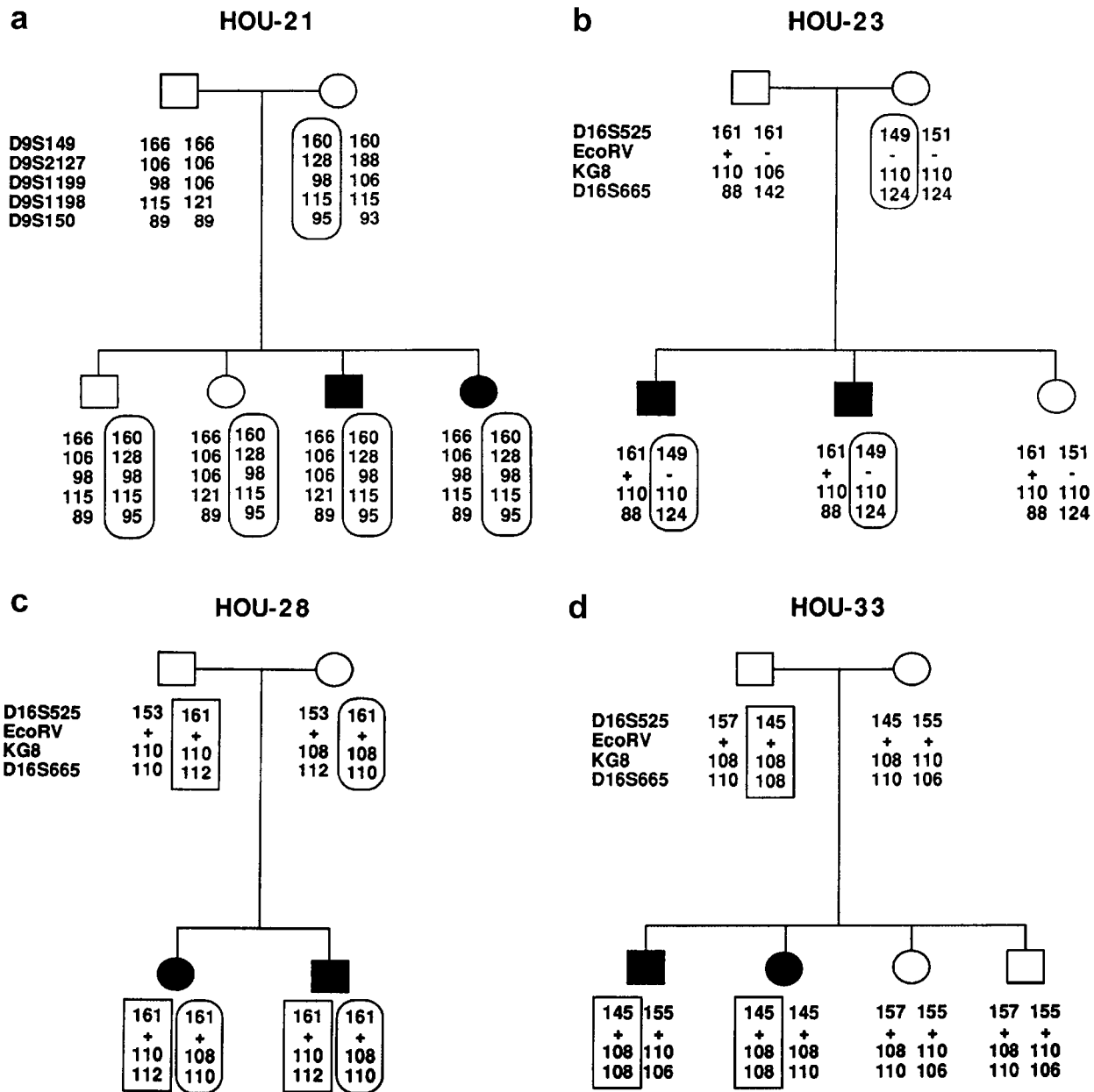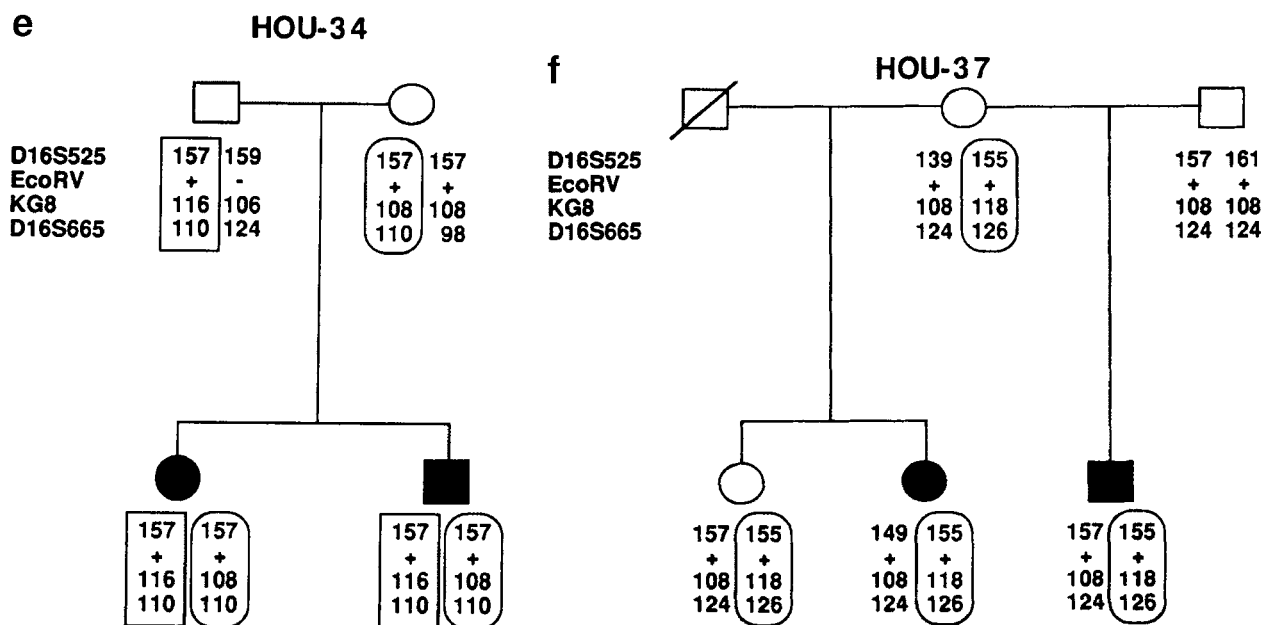
Figure 1 Haplotype analysis of pedigrees. Paternal haplotypes are boxed, and maternal haplotypes are within ovals. *a*, HOU-21. Mosaicism in the father was excluded because the two affected siblings have inherited different paternal alleles. The mother gave the same chromosome to all the children, which is consistent with her having germ-line mosaicism. *b*, HOU-23. On the basis of the haplotype analysis, either parent could have germ-line mosaicism; however, LOH studies indicated that the mother is the parent with mosaicism. *c*, HOU-28. Either parent could have germ-line mosaicism. *d*, HOU-33. The father gave the same chromosome 16 to both affected children but not to the unaffected children, indicating that he is the parent with mosaicism. The mother was excluded because she gave opposite haplotypes to the affected children. *e*, HOU-34. Either parent could have germ-line mosaicism. *f*, HOU-37. The mother has germ-line mosaicism.

ular Genetics, at the University of Texas Medical School–Houston. The families were tested for mutations in the TSC1 and TSC2 genes. TSC2 was evaluated by Southern blotting. TSC1 and TSC2 were screened exon by exon, by use of SSCA. To determine which parent contributed the affected gametes, we analyzed the fam-

ilies for linkage to TSC1 and TSC2 by constructing haplotypes, using markers flanking the two genes (fig. 1).

Results

No gross deletions or rearrangements in TSC2 were detected by Southern blotting. Unique variants were de-

tected, by SSCA, in six families. Each variant was present and identical in both affected children of a family but was absent in both parents and the unaffected siblings (fig. 2). Sequencing of variants yielded two frameshift mutations, one missense mutation, and two nonsense mutations in TSC2 and one nonsense mutation in TSC1 (table 2). With the exception of the missense mutation, these mutations are predicted to cause premature termination of the gene products (tuberin and hamartin).

Linkage analysis indicated maternal origin in two families, paternal origin in one family, and either being possible in three families. In family HOU-23, angiomyolipoma tissue was available from the two affected individuals. The results of loss-of-heterozygosity (LOH) studies of the tumors were positive for five of eight tumors (Au et al. 1997a). The results of LOH and linkage studies indicated that the paternal allele was lost (data not shown). We therefore concluded that the maternal allele harbored the mutation.

Although the parents had none of the findings of TSC, on physical examination or by radiological testing, we performed additional testing to evaluate the possibility of somatic mosaicism for TSC. DNA from lymphocytes of all members of the six nuclear families in which a mutation was detected was tested for low-level mosaicism, by allele-specific PCR. In all families, the mutant allele was detected only in the known affected individuals. Although the parents may have somatic mosaicism, its absence in lymphocytic DNA in these families ex-

cludes that tissue as an easy source for diagnostic purposes.

A mutation has not been identified in one family. Exon-by-exon screening of TSC1 and TSC2 was negative. We have not exhausted all possibilities for the detection of mutation in this family, because SSCA is predicted to detect only ~70% of variants. Furthermore, we did not test the promoters and the intronic sequences, which also could harbor mutations.

Discussion

Germ-line mosaicism has been demonstrated in both common and uncommon genetic disorders (Hall 1988). Because most germ-line mutations are likely to be mitotic in origin and because the mutation rate multiplied by the number of mitoses necessary to form the gametes is >1 (van der Meulen et al. 1995), germ-line mosaicism would be expected to occur to some degree in all genetic disorders. Empiric recurrence risks of specific diseases can be estimated, but risks for individual families depend on the percentage of affected gametes in the germ line of the parent with mosaicism.

We suspected, on the basis of clinical findings, that germ-line mosaicism was present in 6% (7/120) of the families in our study. Five of the seven families have more than two children. In three of these five families, if the parents had chosen to limit the family size to two children, we would never have suspected germ-line mosai-
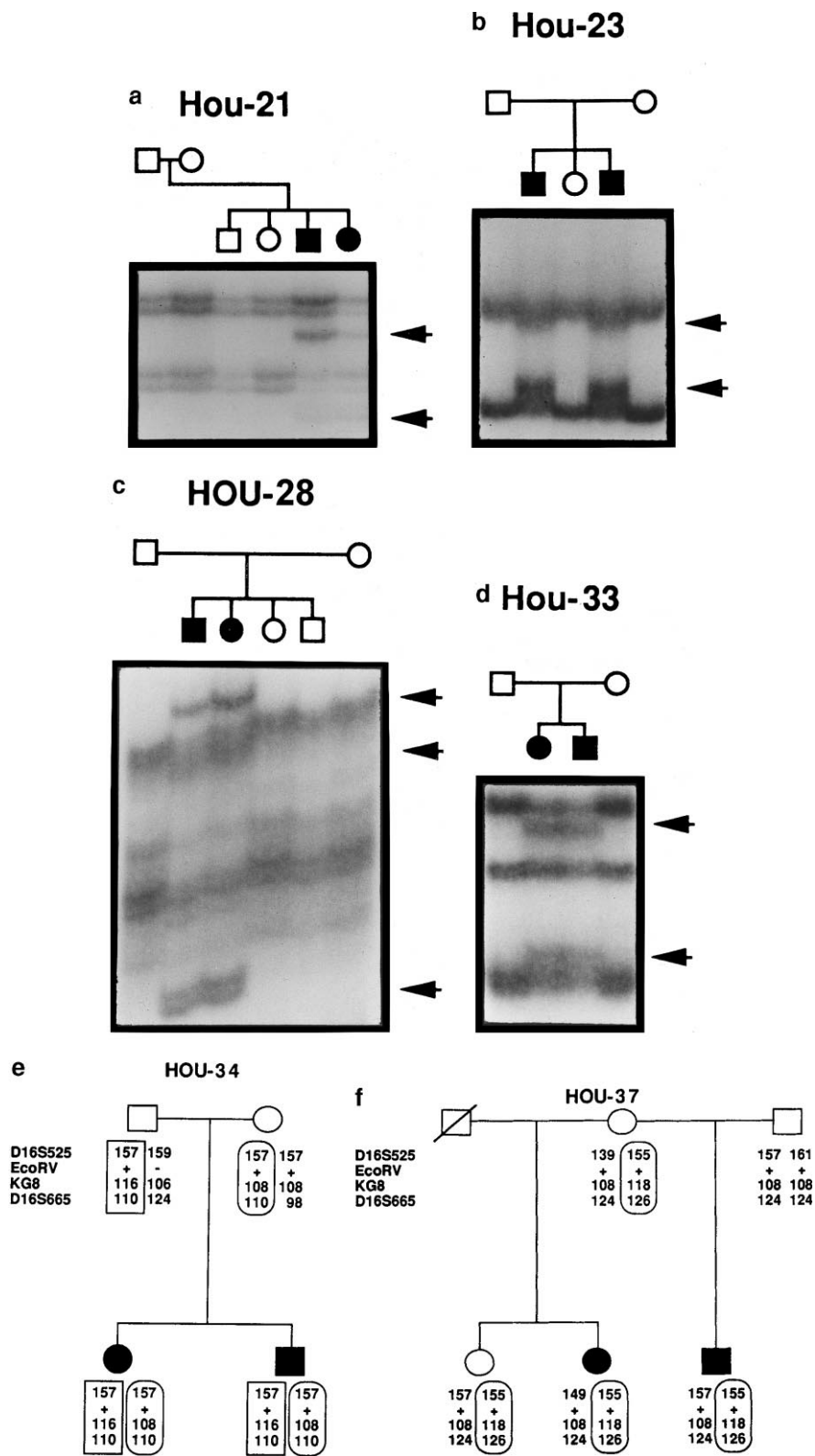
Figure 2   Detection of variant bands (*arrows*) in affected children. Variant bands were not seen in the parents or the unaffected siblings. HOU-21 (*a*) had variant bands in exon 8 of TSC1, HOU-23 (*b*) in exon 24 of TSC2, HOU-28 (*c*) in exon 33b of TSC2, HOU-33 (*d*) in exon 22 of TSC2, HOU-34 (*e*) in exon 13 of TSC2, and HOU-37 (*f*) in exon 16 of TSC2.

Table 2

Mutations in TSC1 and TSC2 in Families with Germ-Line Mosaism

Family	Gene	Exon	Sequence Change	Codon Change
HOU-21	TSC1	8	C682T	R228X
HOU-23	TSC2	24	2779insC	P928 FS→X939
HOU-28	TSC2	33b	C4186T	Q1396X
HOU-33	TSC2	22	2638delA Ag+	K880 FS→X909
HOU-34	TSC2	13	C1372T	R458X
HOU-37	TSC2	16	C1831T	R611W

NOTE.—One mutation in TSC1 and five mutations in TSC2 were found in six families with mosaicism. Three families (HOU-21, HOU-28, and HOU-34) had nonsense mutations, two families (HOU-23 and HOU-33) had frameshift mutations, and one family (HOU-37) had a missense mutation.

cism in one of the parents. Many parents chose not to have additional children after the birth of a child affected with TSC, because of the large burden of the seizures (≥80% of patients) and mental retardation (≥50% of patients) caused by the disease. Owing to the small size of the families in this study, coupled with the general trend toward smaller family size in developed countries, the frequency of germ-line mosaicism for mutations in the TSC genes is difficult to predict.

With the availability of the TSC1 and TSC2 genes, we were able to determine the molecular basis for germ-line mosaicism in six of the seven suspected families. We have shown that germ-line mosaicism can result from mutations in either TSC1 or TSC2 and in either parent of a family. We conclude that germ-line mosaicism that results in TSC is not rare and that a percentage of cases currently categorized as sporadic may actually represent germ-line mosaicism. Unfortunately, despite our best efforts to determine recurrence risks by all clinical means available, we cannot predict which families have a parent with germ-line mosaicism. Molecular diagnosis does not solve the problem. We recommend counseling a conservative recurrence risk of 2%–3% for families with apparently sporadic TSC.

Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for TSC1 [MIM 191100] and TSC2 [191092])

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