Spanish Families with Cerebral Cavernous Angioma Do Not Bear 742C→T Hispanic American Mutation of the *KRIT1* Gene

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Truncating mutations in *CCM1*, encoding *KRIT1*, cause hereditary cavernous angiomas,[1] and 65% of non-Hispanic American families have been linked to *CCM1* with no founder effects.[2] The common mutation, 742C→T transition, recently described in 16 of 21 families,[3] supports the previously described strong founder effect in Hispanic Americans.[4] We have recently reported on Spanish families with cerebral cavernous angioma linked to *CCM1* locus, which do not match the Hispanic American *CCM1* haplotype,[5] although 2 of 9 families partially shared the Hispanic American haplotype of *CCM1*. We have studied the possibility of an ancestor chromosome in a cohort of Spanish cavernous angioma families by a single screening test that detects the 742C→T transition in exon VI of *KRIT1* gene.

The exon VI was analyzed in the probands of 39 nuclear Spanish families. A fragment of *KRIT1* gene containing exon VI was PCR amplified with primers forward (5′ TTGTTAGATTGTGATGTA) and reverse (5′ AACATAATAAAAACTTTC). Aliquots of the amplified DNA were initially screened by analysis of single-strand conformational polymorphism (SSCP). Polymerase chain reaction (PCR) products were electrophoresed in 10% acrylamide in the absence and in the presence of 10% glycerol as recently described.[1]

We analyzed the 742C→T transition by the formation of the sequence 5′ TTAA that is the target for the restriction endonuclease *Mse*I. The product of the PCR includes exon VI and an intronic *Mse*I restriction site that served as internal control of the endonuclease digestion. The undigested PCR product consists of a 238-bp fragment, which, following the digestion with *Mse*I, is divided in two fragments of 210 and 28 bp (Fig). The presence of the 742C→T transition should split the PCR product in three fragments of 141, 69, and 28 bp.

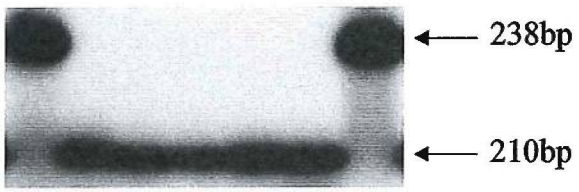

Fig. Analysis of the 742C→T transition in exon VI. Mse*I digested and undigested (U) polymerase chain reaction fragments of exon VI from different patients were separated under denaturing conditions in urea-containing 6% acrylamide gel. The image was obtained by autoradiography of the gel. The 28-bp fragment, located far below the indicated bands, was omitted in the photograph.*

DNA sequencing was carried out with 5′ ^{32}P-labeled primers and terminal dideoxynucleotides (*fmol* kit of Promega, Lyon, France). The forward primer was 5′ CGAATATA CAGAATGGATG, and the above-described oligonucleotide was the reverse primer.

The results showed that only 1 of 39 probands gave a significant SSCP, but both the sequencing of the exon VI and the lack of the *Mse*I restriction site demonstrated that the 742C→T transition was not present in the affected chromosome. Given the great diversity of mutations recently described in *KRIT1* gene,[1,3] the finding in the Spanish population of a single chromosome with the 742C→T transition could support the existence of an ancestor chromosome. We failed to find this mutation in 39 unrelated patients, however, further supporting that the strong founder effect described in Mexican American families[4] with cerebral cavernous angioma are specific for this population.

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Rapid Diagnosis of Peroxisome Biogenesis Disorders through Immunofluorescence Staining of Buccal Smears

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Peroxisome biogenesis disorders (PBDs), including Zellweger syndrome and neonatal adrenoleukodystrophy, are fatal autosomal recessive diseases with severe brain dysfunction and defective peroxisome biogenesis.[1] Diagnosis is usually made by findings such as an accumulation of very-long-chain fatty acids, deficiency of plasmalogen, and absence of peroxisomes in hepatocytes or cultured fibroblasts. However, liver biopsy is an invasive method, and culture of skin fibroblasts is time-consuming. Buccal smear analysis has been used as noninvasive materials for sex determination and DNA analysis of genetic diseases.[2] We reported the detection of peroxisomes in buccal smears.[3] Here we report the usefulness of immuno-

