

LETTER TO THE EDITOR

Low Level Mosaicism Detectable by DHPLC But Not by Direct Sequencing

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Direct sequence analysis of PCR amplification products is widely regarded as the benchmark against which alternative strategies for mutation detection should be measured. However, the method does not have absolute sensitivity or specificity for the detection of point mutations. We have recently formatted denaturing high performance liquid chromatography (DHPLC) for mutation analysis of the *TSC1* (MIM# 191100) and *TSC2* (MIM# 191092) genes that are mutated in the autosomal dominant disorder tuberous sclerosis (TSC). In three cases we observed aberrant DHPLC elution profiles that were not associated with any sequence variation detectable on direct manual or automated sequence analysis of the PCR products.

The first case was the father of a TSC patient (169) who had been shown to carry the *TSC1* splicing mutation 2724-1G>C. Analysis of DNA extracted from a venous blood sample from the father revealed a DHPLC elution profile in which the mutant signal was present, but reduced relative to that in his son [Jones et al., 2000]. Direct manual and automated PCR product sequencing in both forward and reverse directions failed to detect the mutation. The amplicon was therefore cloned into pGEMT-Easy (Promega, Southampton, UK) and sequenced, revealing that only 8/124 clones contained the 2724-1G>C mutant allele (Fig. 1a). Clinical and radiological assessment revealed only minor skin signs of TSC that had never brought the father to clinical attention.

In the second case, DHPLC analysis of DNA extracted from venous blood from a mildly affected TSC patient (395) revealed an altered elution profile for exon 14 of *TSC2*. Automated and manual

direct sequencing of the amplicon failed to identify a sequence variant, but cloning and sequencing revealed 18/104 clones containing the mutation 1462-28del42 (Fig. 1b).

In the third case (22715) who was again only mildly affected by TSC, DHPLC analysis of a peripheral venous blood DNA sample identified an altered profile for exon 16 of *TSC2*. Manual and automated sequencing of PCR products again failed to identify any corresponding sequence variant. Cloning and sequencing revealed that 7/93 clones contained the mutation 1772-1774del4 (Fig. 1c).

Somatic mosaicism is a frequent phenomenon in disorders exhibiting a high mutation rate. For example approximately 10% of sporadic TSC cases are thought to be somatic mosaics for *TSC1* or *TSC2* mutations [Verhoef et al., 1999]. This could result in the failure of molecular genetic diagnosis, due to an inability to detect mutant alleles present at low frequency [Kwiatkowska et al., 1999]. Using DHPLC we were able to detect mutant alleles present at low level that were not detectable by manual or automated direct sequencing. Our findings also have implications for the identification of somatic genetic alterations in tumors, where mutant alleles present at low level due to clonal expansion of sub-populations of cells and contamination with normal tissue are the norm.

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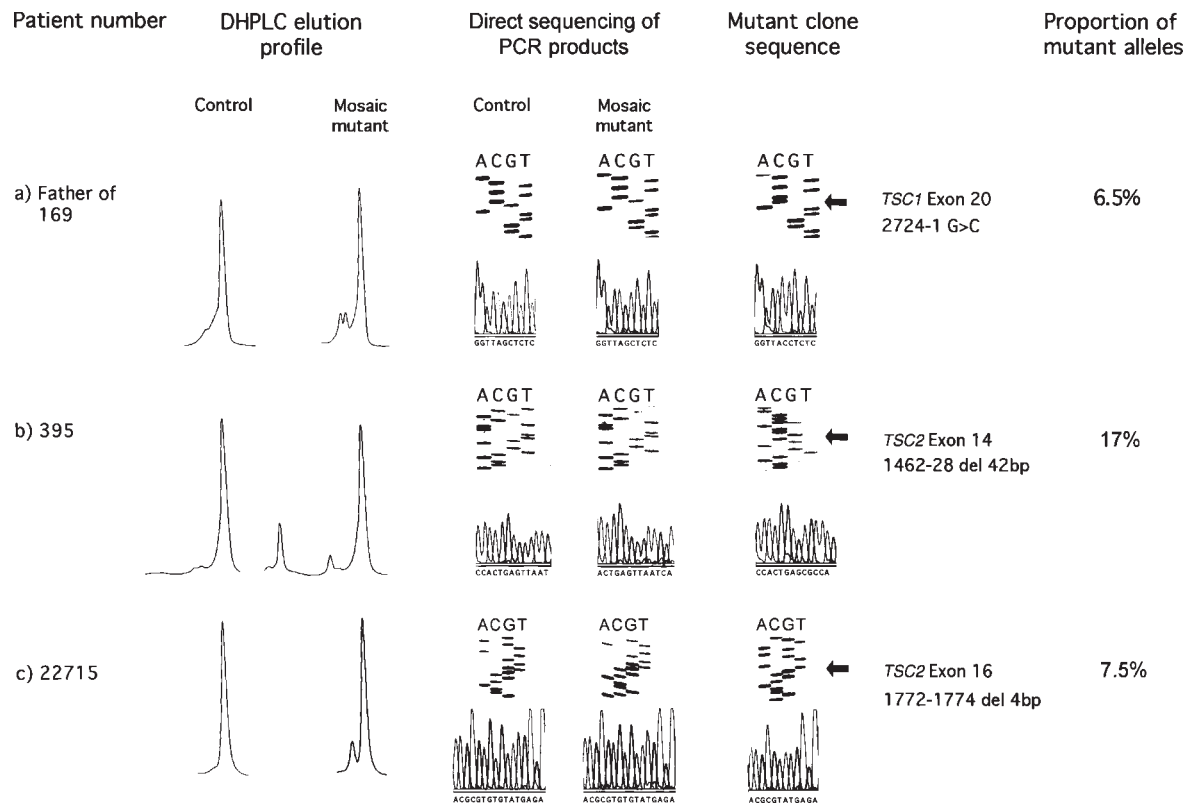


FIGURE 1. Mosaicism detected by DHPLC but not by direct sequencing of PCR products. Primer sequences, PCR and DHPLC conditions have been described previously [Jones et al., 2000]. PCR products were purified using the Qiagen (Crawley, UK) PCR purification kit. Manual sequencing was performed using the Thermo Sequenase cycle sequencing kit (Amersham-Pharmacia Biotech, Amersham, UK) and automated sequencing was carried using the Big-Dye Terminator cycle sequencing kit (P.E. Biosystems, Warrington, UK) with products analyzed on an ABI-377. Both forward and reverse PCR primers were used for manual and automated sequencing. All sequences except those in **b** are shown in the forward direction. (a) Father of case 169: despite the mutant DHPLC elution profile observed for exon 20 of *TSC1*, direct manually and automatically generated sequence of the PCR product was indistinguishable from the control and the mutation 2724-1G>C was only demonstrated after cloning (8/124 clones). (b) Patient 395: the aberrant DHPLC profile for exon 14 of *TSC2* corresponded to the mutation 1462-28del 42bp that was only revealed following cloning (18/104 clones). (c) Patient 22715: the aberrant DHPLC profile for exon 16 of *TSC2* corresponded to the mutation 1772-1774 del 4bp that was only revealed following cloning (7/93 clones). Arrows indicate the positions of mutations in the sequence.

Apparent "DHPLC false positives" that were not associated with demonstrable sequence changes were the focus of much discussion at the recent DNA 2000 International Symposium on the State-of-the-Art in Genetic Analysis in Boston, Massachusetts [Bray and Paalman, 2000] (www.casss.org/dna2000). We suggest at least a proportion of these cases result from low level mosaicism for sequence variation that has been detected due to the exquisite sensitivity of DHPLC.

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