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SHORT REPORT

A cautionary note: false homozygosity for *BRCA2* 6174delT mutation resulting from a single nucleotide polymorphism masking the wt allele

Angela R Solano*[,1,2], Ricardo J Dourisboure[1], Jeffrey Weitzel[3] and Ernesto J Podesta[2]

[1]*Laboratory ACDM-Instituto Alexander Fleming, Buenos Aires, Argentina;* [2]*Laboratory HRDC-Department of Biochemistry, Faculty of Medicine, University of Buenos Aires, Argentina;* [3]*Department of Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, California, USA*

Sequencing an amplification product of the terminal segment of *BRCA2* exon 11 showed apparent homozygosity for the 6174delT mutation in two healthy sisters. Subsequent sequencing of an alternate overlapping amplicon revealed the presence of the 5972C > T polymorphism, which is within the standard upstream amplification primer. This mismatch was responsible for the failure to amplify the normal (5972T) allele in both sisters who were heterozygous for the 6174delT mutation. Though the unexpected finding of apparent homozygosity for the 6174delT mutation prompted re-evaluation of the assay, the potential for false negative results due to masking of a mutation-bearing allele by such a circumstance should be a cautionary note for the testing and also in the interpretation of the results published under such assay conditions.
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Genetic testing for germline mutation is one of the benefits of the genetic revolution of the last decade, allowing the detection of individuals at high risk for developing a disease. This information is important in the clinical management of the patient and the family in many instances.[1–5]

One of the most common approaches for the detection of a mutation in the germline is the amplification of the genomic DNA in the region flanking the site to be tested, and direct sequencing of that product. As a rule, both alleles are supposed to be amplified and analysable by sequencing or by other methods.[6,7]

*Correspondence: Dr AR Solano; Departamento de Bioquímica (Lab. HRDC), Facultad de Medicina (UBA), Paraguay 2155 5to. piso, (1121) Buenos Aires, Argentina. Tel/fax: 54 – 11- 45552967; E-mail: asolano@ciudad.com.ar
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Using standard published primer sequences to test for the *BRCA2* 6174delT Ashkenazi Jewish founder mutation,[7] we studied a healthy 54-year-old woman with a family history of cancer on both the maternal and paternal sides of the pedigree (Figure 1). Initial analysis by sequencing an amplification product indicated apparent homozygosity for the 6174delT mutation (Figure 1). Her sister, 56 years old and also healthy, showed the same result. These findings were confirmed by bi-directional sequencing of the amplification products in both cases (not shown).

The presence of polymorphic sites in the genome region where the amplification primers anneal might cause a base pair mismatch and, as a result, a lack of amplification of the allele carrying the polymorphism. The genomic sequences of the regions corresponding to both amplification primers contain a few polymorphic sites as follows: 5965T > C and, as mentioned, 5972C > T for TDSFB and 6283T > C and 6286G > A for CGORF-RH. Therefore we searched for the

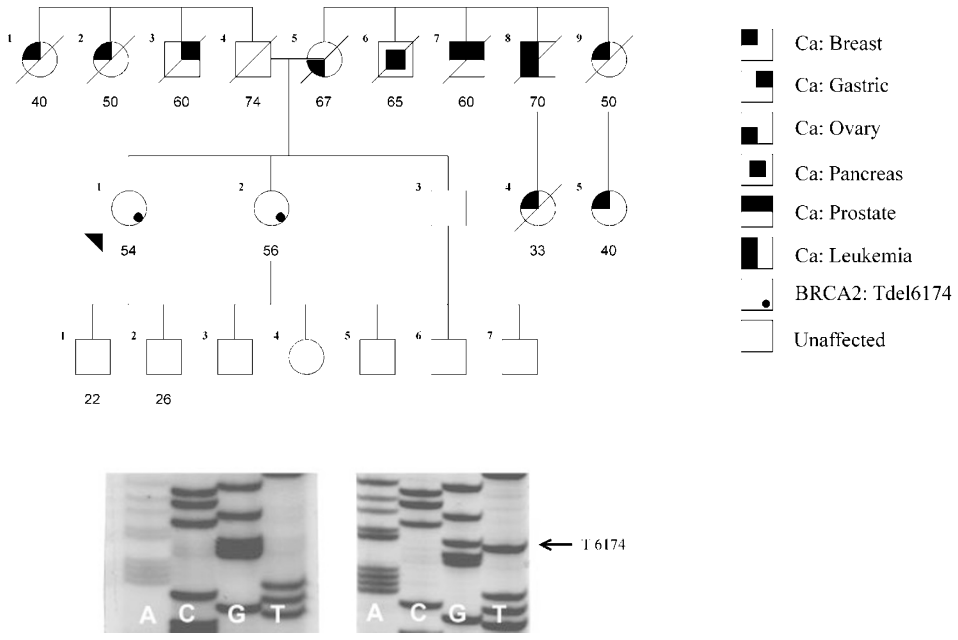

Figure 1 Family kindred with strong history of cancer: the proband is marked with the arrow. Number under the symbol is the age. Gel sequences: compatible with homozygosity for the *BRCA2* 6174delT mutation (left) and normal sequence with the 6174T marked by the arrow (right)

presence of these polymorphisms in the genomes of both sisters. Primers flanking the original amplified segment were designed to amplify and sequence the region where the former primers anneal. The results showed heterozygosity for the 5972C>T polymorphism and also for the *BRCA2* 6174delT mutation.

The recognition of this technical problem is important. As was discovered here, a false report of homozygosity could be described for the mutated allele using the standard assay. Had the situation been the reverse, the mutation would have been missed, and consequently underestimated their cancer risk. The exceptional nature of this result made possible this report. Similar implications were described in a report of a human BRCA1 knockout caused by mispriming during polymerase chain reaction.[8]

An ancillary observation from this case is the fact that both mutation carriers in this report are healthy in their fifties, prompting speculation about possible epistatic interaction modifying the expression of the abnormal *BRCA2* allele. It is unknown whether there is any effect of association of the 5972T polymorphism and *BRCA2* 6174delT in an individual and the absence of disease. We do not know the status of the 5972T polymorphism or 6174delT mutation in the sole living relative that has already developed breast cancer because she lives in another country. Double heterozygotes for *BRCA1* plus *BRCA2* mutations have been described,[9] but the phenotype is apparently not worse than for either mutation alone.

The described method[7] was used to estimate the carrier frequency of the *BRCA2* 6174delT mutation among Ashkenazy Jewish individuals[10] and also in many other citations,[11–13] these valuable initial findings may be different depending on the association in the same allele of the 5972T polymorphism and 6174delT mutation.

In summary, unless heterozygosity is detected, genetic testing results should be carefully reviewed for the potential that one of the alleles is masked. The key point is ensuring the analysis of both alleles to avoid false negative reports. Overlapping amplicons that contain the same mutation may allow the detection of both alleles, as shown in this report. The primers design must take into account for the presence of polymorphic sites in the genome sequence, and still be alert of a difficult problem to circumvent as long as a complete catalogue of the genetic variation in the gene under study is lacking. These concepts should also be taken into account, when population studies are conducted using a unique amplification product as a substrate for sequencing or other techniques like ASO (allele specific oligomerization).

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