The Asp1822Val variant of the APC gene is a common polymorphism without clinical implications

Clara Ruiz-Ponte, Ana Vega, Roberto Conde, Francisco Barros, Angel Carracedo

Editor–Germline mutations of the adenomatous polyposis coli (APC) tumour suppressor gene result in the hereditary colorectal cancer syndrome familial adenomatous polyposis (FAP). Almost all APC mutations that have been identified are single nucleotide alterations, small insertions, or deletions that would cause a truncated APC protein.

Mutations causing amino acid changes in the APC protein have also been reported, but the association of these APC variants with the FAP phenotype is sometimes unclear.

In a recent issue of this journal, a homozygous single base substitution involving an A to T transversion at codon 1822 of the APC gene was reported as probably pathological in one FAP patient and two non-FAP patients.

This double substitution causes an amino acid change (aspartic acid to valine) in the middle of the β-catenin down regulation domain of the APC protein and was the only genetic alteration found in the three aforementioned patients. A whole APC allele deletion was not proven in the samples, but dismissed by the authors as the patients did not exhibit the classical FAP phenotype that would be expected in patients with whole deletions. In an assay carried out on 45 normal subjects, Wallis et al. did not observe any homozygous changes at codon 1822.

We have found the heterozygous genotype Asp1822Val in the affected members of four FAP families out of 17 analysed in Spain and the homozygous nucleotide substitution was found in one of them. Surprisingly, the affected members of one of these five families carried another germline mutation in the APC gene (Ser2621Cys).

Observations suggest that the common Asp1822Val alteration could be a lower penetrant allele that increases the risk of developing colorectal cancer.

To assess the risk of this APC allelic variant in colorectal carcinogenesis, we screened 158 chromosomes from a random white population from north western Spain and provide evidence against a disease causing effect of the Asp1822Val variant.

DNA was extracted from peripheral blood leukocytes of 79 healthy, unrelated subjects from Galicia (north western Spain), having first obtained informed consent for its use in research.

The identification of the Asp1822Val missense variant was carried out by real time fluorescent PCR in the LightCycler (Roche Molecular Biochemicals). This instrument is designed for mutation detection by melting point analysis with fluorescent hybridisation probes using the fluorescence energy transfer (FRET) principle. A 280 bp fragment including codon 1822 of the APC gene was amplified by real time fluorescent PCR using the forward primer 5’ GTCGTCTTTCTGCACCCCACAA and the reverse primer 5’ AGCCGTGTAAATGTAGGGGTGAATC.

The probes hybridise to the codon 1822 site internal to the APC primer pair. The sequence of the sensor fluorescein labelled probe was 5’ TAATTCCAGGACTTCAATGATAAGC and that of the anchor LC-Red 640 labelled probe was 5’ CCCCATAATGAGATAAGTGATCGAGGTAATC (Tib MolBiol). The adjacent probes emit fluorescence only while they are hybridised to their complementary strand. Amplification was performed using the LightCycler DNA Master Hybridization Probes kit (Roche Molecular Biochemicals) in a standard PCR containing 0.5 µmol/l of each primer and 0.1 µmol/l of each probe in 20 µl final volume with 2 µl of sample. A negative control without DNA sample was included in all assays. The reaction mixture was denatured at 95°C for two minutes, followed by 35 cycles of denaturation (95°C for 0 seconds, ramp rate 20°C/second), annealing (58°C for 10 seconds, ramp rate 20°C/second), and extension (72°C, five seconds, ramp rate 20°C/second).

After amplification of the APC gene fragment, the melting curve was determined by holding the reaction at 50°C for 10 seconds and then heating slowly to 94°C with a linear rate of 0.2°C/second while the fluorescence emitted was measured. Plotting fluorescence (F) versus temperature (T) generated melting curves. Discriminated melting peaks were produced by the LightCycler software.

We have genotyped codon 1822 of the APC gene in 158 chromosomes from a healthy random population. We have studied the mutation site (A to T) that corresponds to the second
position of codon 1822 by real time fluorescent PCR. Specifically, the sensor probe was designed complementary to the codon 1822 common allele (A). Thus, the A allele is expected to yield the highest melting temperature. The analysis of the melting curves showed that the A allele has a Tm of 63°C and the T allele a Tm of 59°C (fig 1). We found a total of 36 (23%) T alleles and 122 (77%) A alleles. The sample studied is in Hardy-Weinberg equilibrium (exact test $p=0.35$). In accordance with the Hardy-Weinberg law, the frequency of heterozygotes was 32 out of 79 subjects tested (40.5%) and that of the homozygotes (T/T) was two out of 79 (2.5%).

In our 17 unrelated FAP families we had detected the T allele in affected members from five families. In four families, the subjects were heterozygous and only one family had a member homozygous for the T allele. The number of samples studied from the colonic cancer population was low. However, we observed that the frequency of the T allele was no higher than in the healthy control population (17% v 23%), as would be expected with an allele that increases cancer risk. Moreover, we found one homozygously affected mother in a FAP family whereas her affected son was carrying both alleles. This observation led us to suggest that the homozygous substitution at codon 1822 of the APC gene was not the genetic cause of the disease in this family. Unfortunately, we were unable to rule out cosegregation of Asp1822Val with disease in the FAP families with this alteration as we could not analyse sufficient numbers of unaffected members from these families.

The data reported indicate that the Asp1822Val missense variant is a common polymorphism with no clinical significance. Special caution is needed concerning the interpretation of missense variants in genetic diagnosis and counselling of FAP families. In cases where the segregation of the mutation with the disease is not clear, a population study of the variant should be performed to rule out or accept the association of the genetic variant with the disease. New technologies such as real time fluorescent PCR permit a rapid genotyping of samples. The analysis takes 20 minutes and is performed in a single capillary tube, avoiding cross contamination and post-PCR manipulation. This makes the system suitable for the genotyping of large populations.

We failed to identify the genetic cause of the FAP phenotype in four of the five FAP families where the Asp1822Val polymorphism was detected. As other authors have reported, whole deletions of the APC gene, mutations affecting the promoter region of the APC gene, or other genes giving an identical phenotype could be responsible for the disease.**

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Reply

Fiona Macdonald, Yvonne Wallis

Dr Ruiz-Ponte and colleagues have suggested in their letter that the presence of a homozygous Asp1822Val variant in the APC gene is without clinical effect. We found the variant in the heterozygous and homozygous states in controls and patients in a similar frequency to that found by Ruiz-Ponte and colleagues. In both our study and theirs, the patients with the homozygous change had no other detectable mutation although we did add that we could not rule out the possibility of a large gene deletion. We obviously cannot be certain about the pathological nature of the mutation but as the phenotype of the patients with the homozygous change was not that of classical FAP we cannot rule out the possibility that it may be of some significance. The control population in the study of Ruiz-Ponte et al did include two subjects homozygous for the T allele. However, the details of the control population were given only as healthy but with no details of ages or other factors of relevance. Given that colorectal cancer is so common, the possibility of a role
for this allele as a low penetrance allele cannot therefore be ruled out completely. However, we would certainly agree that missense changes have to be treated with caution and we would never consider using them in a diagnostic setting. In the meantime we are pleased to see other studies such as this one looking further at these missense changes which may yet prove to have a role in determining the colorectal phenotype. Larger studies may yet be required, together with functional assays, to try to confirm or disprove the role of missense changes in predisposition to cancer.

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