

Genetics of familial colorectal cancer: the experience in Cyprus

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ABSTRACT

Familial adenomatous polyposis (FAP) and Hereditary Non Polyposis Syndrome (HNPCC) are the two commonest familial syndromes that predispose to colorectal cancer. FAP is caused by mutations in the Adenomatous Polyposis Coli (APC) tumour suppressor gene that has a high penetrance. The disease is characterized by the occurrence of hundreds to thousands of colorectal polyps, which if left untreated give rise to colorectal cancer. On the other hand HNPCC is characterized by development of colorectal cancer at an early age, in the absence of polyps. This syndrome is caused by germline mutations in the mismatch repair genes (hMLH1, hMSH2, hMSH6, hPMS1 and hMSH2).

In Cyprus there are no molecular data available as yet, on families with FAP or HNPCC. This work presents the results of APC analysis in our population for the first time. The APC gene was analysed in 33 DNA samples from 20 individuals belonging to 4 FAP families and 13 patients with sporadic polyposis. We identified 3 truncating mutations, 4 missense mutations and 11 polymorphisms. It is of interest that 2 of the 3 truncating mutations, 2307delA and Q1242X are novel which confirms the existence of a unique genetic pool in the Cypriot population. In the case of HNPCC one extended Cypriot pedigree has been identified and affected members of the family are currently being investigated at the molecular level. This ethnic molecular study in addition to highlighting population heterogeneity, also contributes to phenotype-genotype associations, that are essential for the clinical management of both FAP and HNPCC families in Cyprus.

Key words: Familial Adenomatous Polyposis (FAP), APC mutations, HNPCC, Cypriot patients

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal, dominantly inherited cancer predisposition syndrome, which accounts for 1% of colorectal cancer cases (Houlston et al., 1992). The characteristic clinical feature of FAP is the expression of hundreds to thousands of adenomatous colonic polyps at a young age. More than 90% of all individuals who are predisposed to FAP develop adenomas and if these are left untreated they lead 100% to the development of colorectal cancer (Bisgaard et al., 1994). FAP families may also frequently present with extra colonic manifestations that include Congenital Hypertrophy of the Retinal Pigment Epithelium (CHRPE) (Blair and Trempe, 1980) and less frequently epidermoid cysts, brain (Paraf et al., 1997), thyroid and liver tumours (Kingston et al., 1983) as well as benign fibromas (Eccles et al. 1996).

FAP is associated with mutations in the APC gene which is located on chromosome 5q21 (Groden et al., 1991; Kinzler et al., 1991). The APC gene consists of 15 exons, the largest of which is exon 15, which comprises more than 75% of the coding sequence of the gene. Exon 15 is the most common target for both germline and somatic mutations (Beroud and Soussi, 1996). The majority of mutations are small deletions or insertions and about 95% of these, lead to truncation of the APC protein with abnormal function. De novo mutations in the APC gene, occur at a frequency of 1 in 8.000 live births and are responsible for the disease in about one third of affected individuals (Bisgaard et al., 1994). The gene has a high penetrance which is close to 100% at the age of 40.

The APC protein consists of many functional domains, and plays an important role in key cellular processes, such as cell-cell adhesion, cell cycle regulation and apoptosis. The APC protein is an integral part of the wnt-signalling pathway (Fearnhead et al., 2001) and functions overall as a tumour suppressor gene. One of its most important roles is in regulating the

intracellular level of β -catenin which is an important mediator of cell adhesion (Munemitsu et al. 1995).

Hereditary Non Polyposis Colorectal Cancer (HNPCC) accounts for the majority of familial colorectal cancer and is caused by germline mutations in one of at least five DNA mismatch repair genes (Fishel et al., 1993; Bronnet et al., 1994). Like FAP is also an autosomal dominantly inherited syndrome (Lynch and de la Chapelle, 2003) and affected individuals have an increased risk of developing extra colonic cancer, such as cancer of the endometrium or stomach. Mutations in the mismatch repair genes cause the majority of HNPCC related cancers and in addition to mutation analysis, microsatellite instability (MIS) is also used to detect affected individuals (Wahlberg et al, 2002).

In Cyprus, colorectal cancer is the third commonest type of malignancy after breast and prostate cancer with an annual incidence of more than 200 cases. It occurs with equal frequency in men and women, as is the case with other European countries. Previous work from our group has shown the presence of novel mutations in the BRCA genes in patients with the familial breast ovarian cancer syndrome (Hadjisavvas et al., 2004), supporting the existence of a unique genetic pool within the Cypriot population. It was therefore of interest to characterize mutations in the APC gene in Cypriot families with FAP and this presents the first molecular report on the spectrum of APC mutations in our population.

PATIENTS AND METHODS

Patients with FAP and their families were referred, through the Gastroenterological Society of Cyprus as well as Surgeons and Oncologists, to the Cyprus Institute of Neurology and Genetics, for molecular testing. The present study is based on molecular analysis of the APC gene in 33

DNA samples that include both familial and sporadic cases. All patients received genetic counseling and gave their written consent before the blood sample was taken.

Of the 33 samples, 20 were from individuals who belong to 4 families with FAP, of which 10 were treated with colectomy; the remaining 13 samples were from patients with sporadic polyposis. A detailed family history was taken from the FAP families and histopathological findings were confirmed in the patients that were surgically treated. Eight other individuals had no family history but had undergone colectomy due to the presence of more than 100 intestinal polyps.

For HNPCC one large pedigree was identified with incidence of four colorectal cancers in first and second degree relatives. In addition to DNA sequencing, of mismatch repair genes DNA from tumours from the affected family members were also subjected to MSI. This family is still being investigated at the molecular level.

Mutation analysis

Mutation analysis was performed using polymerase chain reaction (PCR) and sequencing of all exons, as well as intron boundaries of the APC gene. The PCR products were sequenced using the same forward and reverse primers used for the PCR amplification. Sequencing was carried out using ABI PRISM di-Deoxy Terminator Cycle sequencing kit on an ABI 9700 thermal cycler and an ABI 310 Genetic Analyzer, (Applied Biosystems, Foster City, CA). In addition to the 33 DNA samples from polyposis patients, a pool of 50 DNA samples from healthy Cypriot individuals without a history of adenomatous polyposis were also used to verify and characterize the detected variants.

RESULTS

Analysis of the APC gene in the 33 DNA samples from patients with adenomatous polyposis revealed the presence of 3 truncating mutations, 4 missense mutations, 11 polymorphisms and two intronic variants (see table 1). All three truncating mutations were detected in exon 15 and these are: 2307delA, 3927delAAAGA and mutation Q1242X. The presence of the truncated mutations was confirmed by resequencing a second DNA sample from the patients. The first is a novel frameshift mutation 2307delA which introduces a STOP, 7 amino acids downstream. The second is a novel nonsense mutation of codon 1242, a glutamine to a STOP (Q1242X). The third is the most commonly reported frameshift mutation 3927del5, in the APC gene. Similarly the four missense mutations, E1317Q, V1822D, P1843L and G2502S were also detected in exon 15. Details of the 11 polymorphisms and the two intronic variants appear in table 1, which also displays the frequency of all identified variants in 50 control DNA samples from healthy individuals.

DISCUSSION

The incidence of colorectal cancer in Cyprus is approximately 30 cases per 100,000 population, including men and women of all ages, which is similar to other countries in Southern Europe (Parkin et al., 1999). This is the first molecular study in the APC gene in Cypriot patients with adenomatous polyposis and it is interesting that novel mutations have been identified in the Cypriot population.

It is noted that two of three truncating mutations namely 2307delA and Q1242X are novel. These two pathogenic mutations were detected in patients from four families with the typical phenotype of FAP and moreover, mutation 2307delA was detected in patients from 3 unrelated Cypriot families. This is an interesting finding since in addition to this being the most frequent mutation in Cypriot families with FAP, the possibility exists that it may represent a founder

mutation in our population. In this context, it is noted that a novel founder mutation in the BRCA2 gene of Cypriot families with the breast/ovarian cancer syndrome, has recently been characterized by our group (Hadjisavvas et al., 2004). The third novel pathogenic mutation namely 3927del5 was detected in a patient with florid polyposis, but with no family history, so this is likely to represent a de novo mutation, as observed by other investigators (Aretz et al., 2004). The clinical phenotype, of florid polyposis, seen in this patient, agrees with earlier observations that mutations around code 1309, lead to the most severe intestinal phenotype (Nagase et al., 1992).

In agreement with other population studies all three pathogenic mutations occur in exon 15 which contains the Mutation Cluster Region (MCR) of the APC gene (Fearhead et al., 2001). In our study, four missense mutations were also detected namely: E1317Q, V1822D, P1843L and G2502S. Of these P1843L represents a novel variant in our population which was not present in the control group, suggesting that this variant may be pathogenic. However, further work is needed in order to evaluate the functional significance of this variant and its effect on the APC protein.

In addition, 11 polymorphisms were detected in this molecular study 3 of which, (L10L, Q222Q and S775S) are novel in the Cypriot population. It is noted that two of these three polymorphisms, Q222Q and S775S, were not detected in the 50 DNAs from the control group and may represent rare polymorphisms in our population.

Previous work from other groups has revealed the presence of missense variants in the APC gene that increase the risk of developing multiple adenomas and colorectal cancer. The most notable example is the variant I1307K which is found in Ashkenazi Jews and carriers are at an increased risk of developing adenomas and colorectal carcinomas (Frayling et al., 1998).

Currently there is an increased effort to improve our understanding of the effect of missense variants since these may contribute to multifactorial disease inheritance, possibly with low penetrance (Bodmer, 1999).

In conclusion, the results of this molecular study have revealed the existence of novel pathogenic mutations in the APC gene in Cypriot families with FAP. In addition to enabling phenotype-genotype correlations to be performed, these results are currently being used in the clinical management of the affected family members.

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Table 1. Mutations detected in the APC gene in Cypriot patients

Exon	Nt mRNA	Codon	Base change	AA change	Designation	Mutation type	Mutation effect	Frequency of the control group (%)
Truncating Mutations								
15/2	2307	769	del A		2307delA	F	F	0
15.6	3724	1242	C to T	Gln to STOP	Q1242X	N	F	0
15.7	3927	1309	delAAAGA		3927Del5	F	F	0
Missense Mutations								
15/7	3949	1317	G to C	Glu to Gln	E1317Q	M	M	2
15/1 2	5465	1822	T to A	Val to Asp	V1822D	M	M	81
15/1 2	5528	1843	C to T	Pro to Leu	P1843L	M	M	0
15/2 0	7504	2502	G to A	Gly to Ser	G2502S	M	M	2
Polymorphisms								
1	30	10	A to G	Leu to Leu	L10L	P	P	6
6	666	222	G to A	Gln to Gln	Q222Q	P	M	0
11	1458	486	T to C	Tyr to Tyr	Y486Y	P	P	52
13	1635	545	G to A	Ala to Ala	A545A	P	P	50
15.1	2253	751	T to G	Ser to Ser	S775S	P	P	0
15/9	4479	1493	G to A	Thr to Thr	T1493T	P	P	52
15/1 0	5034	1678	G to A	Gly to Gly	G1679G	P	P	49
15/1 1	5265	1755	G to A	Ala to Ala	A1755A	P	M	2
15/1 1	5268	1756	T to G	Ser to Ser	S1756S	P	P	49
15/1 4	5880	1960	G to A	Pro to Pro	P1960P	P	P	50
15/1 8	7201	2401	C to T	Leu to Leu	L2401L	P	P	5
Intronic Variants								
5			C to T		IVS5+32C/T	P	P	15
13			T to A		IVS13+17T/A	UV	UV	0

F=Frameshift; N=nonsense; UV=unclassified variant; M=missense; P=polymorphism; Boldface= novel variants

Novel variants appear in bold.