THE PRESYMPTOMATIC MOLECULAR DIAGNOSIS OF FAMILIAL ADENOMATOUS POLYPOSIS IN SINGAPORE

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ABSTRACT

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder which predisposes to the development of colorectal cancer. The adenomatous polyposis coli (APC) gene, mutation of which is responsible for FAP, has been localised to chromosome 5q21. Linkage studies using DNA markers have proven useful for presymptomatic diagnosis of at-risk individuals.

We have examined 8 FAP families from the Singapore Polyposis Registry by using 4 linked and 2 intragenic DNA markers. Presymptomatic diagnosis could be made in 84% (37 of 44) of at-risk individuals. Among these presymptomatically diagnosed cases, positive prediction was made in 32% (12 of 37) whereas negative prediction was possible in 68% (25 of 37).

As the accuracy of genetic diagnosis is high and the test reliable in most cases, the major impact of these tests will be the reduction of unnecessary anxiety and a significant reduction in the frequency of screening for at-risk individuals who are not carrying the affected gene.

Keywords: *presymptomatic, familial adenomatous polyposis (FAP), adenomatous polyposis coli (APC), DNA markers, diagnosis.*

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INTRODUCTION

Familial adenomatous polyposis (FAP), an inherited colon cancer syndrome, is an autosomal dominant condition. It accounts for approximately 1% of all colorectal cancer cases and has a prevalence of 1 in 100,000 individuals[1-3]. The condition is characterised by the presence of hundreds of polyps in the colon and rectum, some of which, if not removed, invariably progress to carcinomas[1,2]. Other features such as polyps in the upper gastrointestinal tract, osteomas, epidermoid cysts, desmoid tumours and congenital hypertrophy of retinal pigment epithelium (CHRPE) may also accompany the lower bowel polyps[1,2,4]. Gardner's syndrome which includes extraintestinal features such as benign bone and soft-tissue tumours is a clinical variant of FAP[2,4].

Offspring of an affected individual have a 50% risk of inheriting the condition. As the age of onset of colonic polyps is variable[1], usual screening management for those at-risk individuals consists of an annual endoscopic examinations of the colon beginning at around puberty. The objective of screening is the early detection of polyps so that prophylactic colectomy can be performed.

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Germ-line adenomatous polyposis coli (APC) gene mutations have been identified in FAP patients as well as in FAP carriers[5,6]. The APC gene has been localised to chromosome 5, specifically at 5q21, and recently cloned and sequenced[7-11]. It is a large gene containing an 8538-base pair open reading frame in 15 exons[9], and three intragenic polymorphisms have been reported in exons 11, 13 and 15[9]. The gene encodes a relatively large protein composed of 2844 amino acids[9] and the majority of the reported mutations give rise to truncated APC proteins[6]. Since mutations are scattered all over the transcript[6], direct analysis of APC mutations is very labour intensive and is only practicable for presymptomatic diagnosis where the site of mutation has been previously established in a family member.

Although the exact function of APC protein has not been elucidated, there is some evidence to indicate the APC gene functions as a tumour suppressor gene[12]. In sporadic colorectal cancers and adenomas, somatic APC mutations have been reported in nearly 60% of cases[13]. Recently, it has been reported that the inactivation of both alleles of the APC gene may be essential for the development of early-stage adenomas as well as for the development of desmoid tumours in FAP patients[14,15].

The localisation of the APC gene to chromosome 5q21 has led to the first clinical application of molecular biology techniques in the presymptomatic diagnosis of colorectal cancer. Linked markers have been used with a high degree of accuracy to diagnose presymptomatic FAP cases[16-20]. The identification of intragenic polymorphisms of the APC gene has also led to the possibility of using these intragenic markers in linkage studies of FAP families. The benefits of such genetic testing are a reduction of uncertainty for at-risk individuals and a modification of the routine screening procedure for negatively predicted cases. In the present study we have evaluated the usefulness of four linked markers and two intragenic markers for presymptomatic diagnosis of FAP among FAP families in Singapore.

MATERIAL AND METHODS

Subjects

A total of 95 family members from 8 FAP families registered with the Singapore Polyposis Registry were studied. Forty-four individuals with 50% risk of inheriting the disease were identified. There were 22 males and 22 females with an age

range of 8 to 55 years (average age 18.9 year).

DNA isolation

Five to 10 ml of whole blood were collected in ethylenediaminetetra-acetic acid (EDTA) treated tubes and stored at -80°C until required. DNA was extracted according to the method of Ausubel et al[21] with minor modifications. Briefly, white blood cell lysate was digested with proteinase K at 55°C overnight. The resulting solution was extracted twice with phenol/chloroform/ iso-amyl alcohol, followed by two extractions with chloroform/ iso-amyl alcohol and the DNA precipitated with sodium acetate and ethanol.

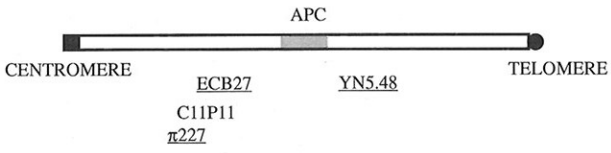
DNA studies by linked markers

Restriction enzyme digestion, gel electrophoresis, southern blotting, hybridisation with radioactive probes and autoradiography were performed as previously described[22]. Briefly, 5-7 micrograms of DNA was digested to completion with the appropriate enzymes and separated by agarose gel electrophoresis. The DNA fragments were transferred to nylon filters by capillary action. After hybridisation with P^{32} labeled DNA probes, namely π227[23], C11P11[7], ECB27[24] and YN5.48[25], filters were exposed to Fuji RX film at -80° C for 3-7 days. DNA probes and appropriate enzymes used are shown in Table I. These DNA probes were chosen for our study as they were shown by linkage analysis to be closely linked to the APC locus and, moreover, they flank the locus on both sides. The relative positions of the probes to the APC gene are indicated in Fig 1.

Table I - DNA probes and enzymes used in linkage studies

Probe (Locus)	Enzyme	Allele Size (Kb)		
		No 1.	No 2.	No 3.
π227 (D5S37)	Bcl I	3.0	1.8	1.2
	BstX I	2.7	2.3	
C11P11 (D5S71)	Taq I	6.5	4.0	
ECB27(D5S98)	Bgl II	11.9	10.5	
YN5.48 (D5S81)	Msp I	13.0	11.0	8.2

Fig 1 – Schematic diagram of chromosome 5q21 showing the relative positions of probes used. The APC gene is represented by the shaded area



DNA studies by intragenic markers

A PCR and enzymatic digestion method was used to study the intragenic polymorphisms in the APC gene at nucleotide positions 1458 (exon 11) and 5037 (exon 15). Conditions of PCR reaction, primers and restriction enzymes were essentially the same as those used by Kraus and Ballhausen[26] except that Bsi HKA I was used in place of Hgi A I. A 3% agarose gel was used instead of a polyacrylamide gel to fractionate the digestion products of half to three quarters of the PCR reaction. Depending on the presence or absence of Rsa I (exon 11) and Bsi HKA I (exon 15) cutting sites, different sizes of DNA fragment could be observed in the gel. Digestion of a 215 base pair (bp) PCR product (exon 11) with Rsa I will produce two fragments (130 bp and 85 bp) if enzyme recognition site is present in the amplified fragments. For Bsi HKA I digestion, two fragments (123bp and 43bp) will be produced from a 166 bp PCR product (exon 15). Unlike linked markers which are separable from the APC locus by recombination during meiosis, intragenic markers provide a more accurate assessment of transmission of the diseased allele.

RESULTS

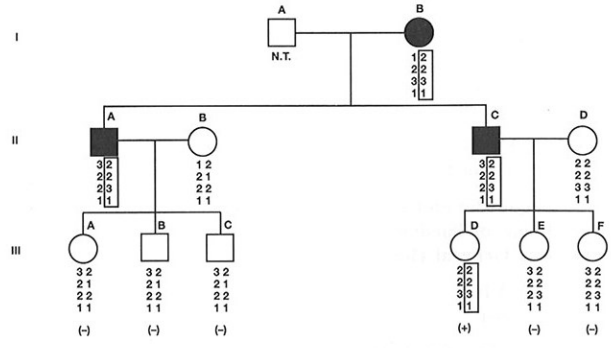
Linked markers

Polymorphic fragments detected were as reported for Caucasians except for YN 5.48. Using YN5.48 to probe Msp I digest, the polymorphic bands were of 13.0, 11.00 and 8.2 Kb in size (Table I) in contrast to the 7.0 and 6.5 Kb reported in the Caucasian population[16]. Among the 4 linked markers, YN 5.48 was the most informative, as it was at least partially informative in 7 families. ECB27 and π 227 were informative in 6 and 5 families respectively while C11P11 was not informative in any of the families. A combination of 3 markers was at least partially informative for all but one family. Presymptomatic diagnosis could be made in 25 (59%) of 42 at-risk individuals. Of these, 9 (36%) were positive for FAP and 16 (64%) were negative (Table II). A typical pedigree in which presymptomatic diagnosis could be made with the use of linked markers is shown in Fig 2.

Table II - Preclinical diagnosis of FAP with DNA markers

Category	Markers		
	Linked	Intragenic	Combination
No. of families examined	8	8	8
No. of informative families	7	5	7
No. of members examined	94	92	95
No. of at-risk members	42	42	44
No. of at-risk for whom presymptomatic diagnosis can be made	25 (59%)	18 (43%)	37 (84%)
No. of positive predictions	9 (36%)	5 (28%)	12 (32%)
No. of negative predictions	16 (64%)	13 (72%)	25 (68%)

Fig 2 - Part of pedigree (FAP family 12) showing polymorphisms for four linked markers. Alleles for YN5.48 (Msp I), ECB27 (Bgl II), π227 (Bcl I) and C11P11 (TaqI), are shown in descending order under each person tested.



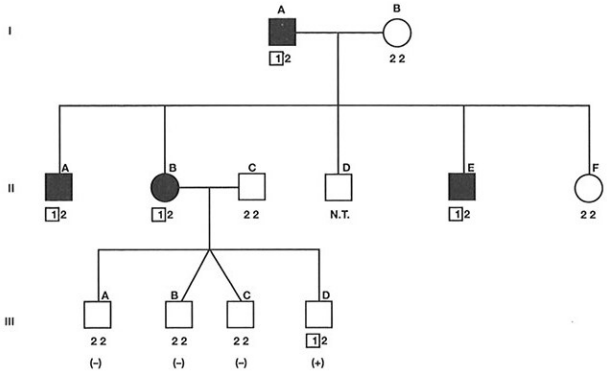
Analysis of the pattern of inheritance of various alleles shows that the mutant FAP allele is on the chromosome carrying the haplotype indicated by the box.

□○ – unaffected
■● – polyposis
N.T. – not tested
(+) – positive presymptomatic diagnosis
(-) – negative presymptomatic diagnosis

Intragenic markers

Exon 11 as well as exon 15 intragenic markers were at least partially informative in 5 out of 8 families. Preclinical diagnosis was possible in 18 (43%) of 42 at-risk members. Of these, 5 (28%) were positive and 13 (72%) were negative (Table II). Fig 3 shows a part of a FAP family where presymptomatic diagnosis was made using the exon 15 intragenic marker.

Fig 3 - Part of pedigree (FAP family 5) showing exon 15 (Bsi HKA I) polymorphism. Alleles are indicated under each person tested. Allele [1] is the mutant allele which is responsible for the disease transmission.



□○ – unaffected
■● – polyposis
N.T. – not tested
(+) – positive presymptomatic diagnosis
(-) – negative presymptomatic diagnosis

Combining linked and intragenic markers

If both linked and intragenic markers were considered together, 7 out of 8 families were informative for either one or both types of marker. A combination of the 2 types of marker improved the predictability of mutant allele carrier status. Presymptomatic diagnosis was possible in 37 (84%) of the 44 at-risk individuals studied. Positive and negative predictions could be given to 12 (32%) and 25 (68%) of these at-risk individuals respectively (Table II).

DISCUSSION

Our results show that presymptomatic diagnosis of FAP could be obtained in approximately 8 out of 10 at-risk members from 8 FAP families with the use of 4 linked and 2 intragenic markers. Many studies have shown that the accuracy of prediction can be as high as 99% if the results from closely linked flanking markers are consistent[16,17]. We have used 4 closely linked markers which flank the APC gene in the following order[19]: centromere, π227, C11P11, ECB27, APC, YN 5.48 (Fig 1). As these markers have low recombination rates with the APC gene, our predictions have an accuracy ranging from 89% to 99%[16]. For intragenic markers, recombination is essentially nil and the accuracy is almost 100%.

The combined use of linked and intragenic markers increased the number of cases for whom presymptomatic diagnosis was possible. By using only one type of marker, prediction was made for 57% of at-risk persons with linked markers and 43% with intragenic markers. When the two types of markers were used in combination, this figure increased to 84%. Most of the cases where definite prediction could not be made were from families with less than optimal pedigree structure or where one of the affected members presented with atypical clinical features (eg less than 100 polyps) or where blood from key family members was not available. Since clinically diagnosed cases were excluded from at-risk group, the number of negatively predicted at-risk individuals were higher than the number of positively predicted at-risk individuals in our study (68% vs 32%).

The benefits of presymptomatic diagnosis for at-risk cases are substantial. For those with a positive prediction, family members as well as the person himself will have more time to prepare psychologically and financially for the future changes. However no change in the screening regimen is usually recommended for such cases. For individuals with a negative prediction, a reduction in frequency of colon screening is usually advisable, for example at the ages of 18, 25 and 35 year, and thus an increase in screening compliance and effective surveillance without repeated sigmoidoscopy of colonoscopy can be expected.

Presymptomatic diagnosis using linkage analysis is relatively fast, inexpensive and accurate if appropriate markers are used. Furthermore, recent reports have shown that the informativeness can be increased to nearly 100% by using intragenic and highly variable microsatellite markers which are linked to the APC gene[27-30]. After the cloning of the APC gene, specific APC mutations in FAP cases as well as in at-risk individuals have been reported[11,31]. In those families in which linkage studies are not informative, identification of the specific mutation of the APC gene is necessary for presymptomatic diagnosis of at-risk individuals[31]. However a common mutation has not been identified in the majority of patients and these mutation studies are expensive and labour intensive. Therefore linkage studies are important at this stage of technology for the early diagnosis and management of FAP at-risk cases.

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