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Risk estimation in familial adenomatous polyposis using DNA probes linked to the familial adenomatous polyposis gene

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Abstract

The familial adenomatous polyposis gene has recently been assigned to the long arm of chromosome five through linkage to several 5q DNA probes. These probes can now be used to trace inheritance of the disease gene in affected families. In this study, DNA samples from 152 members of 10 Australian familial adenomatous polyposis families have been examined for restriction fragment length polymorphisms detected by DNA probes C11P11, ECB27, and YN5.48. Linkage analysis confirmed linkage between the familial adenomatous polyposis gene and each probe with a maximum combined LOD score of 2·82 for C11P11, 2·90 for ECB27 and 5·49 for YN5.48 all at a recombination fraction of zero. Risk estimates were determined for the 51 at risk individuals in these families based on their restriction fragment length polymorphism data alone or in addition by including the effect of age dependent penetrance. Thirty two of those at risk (63%) could be assigned specific high (≥95%) or low (≤5%) risks of developing familial adenomatous polyposis on the basis of their probe results. When the effect of age dependent penetrance was included, 26 (51%) fell at the extremes of risk (≥99% or ≤1%). Such estimates provide a sound basis for planning sigmoidoscopic screening of at risk family members and will thus facilitate surveillance in familial adenomatous polyposis families.

Familial adenomatous polyposis is an autosomal dominant disorder affecting approximately one in 10 000 individuals. Until recently, regular sigmoidoscopic examination was the only means of presymptomatic diagnosis. Presymptomatic diagnosis is critical because more than 60% of symptomatic individuals have already developed cancer.[1]

Prompted by a report of a chromosome 5q deletion in a patient with Gardner's syndrome and mental retardation,[2] linkage studies in familial adenomatous polyposis families have identified several chromosome 5q DNA probes closely linked to the familial adenomatous polyposis gene.[3-7] These probes can be used to trace the familial adenomatous polyposis gene through suitable families and allow prediction of genotype in individuals at risk of inheriting the disease. The technique is limited, however, by the availability of family members, the informativeness of the probes and the possible confounding factors of recombination and non-paternity.

The aim of this study was to assess the value of three probes, C11P11,[3,4] ECB27[5] and YN5.48[6] in the assessment of family members at risk for familial adenomatous polyposis.

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Methods

SUBJECTS

Families were included in the study if the proband and his immediate family resided in the Sydney metropolitan region and agreed to participate. All relatives living within Australia were then contacted and asked to provide a blood sample and access to medical records. Of the 10 families selected, six were initially under the care of the A W Morrow Gastroenterology and Liver Centre and four were referred by surgical colleagues. The disease status of family members was confirmed by review of medical records. Ethical approval for the study was obtained from the institutional human ethics review committee.

Heparinised blood samples (20 ml) were collected from family members and buffy coats prepared. The latter were washed in ice cold saline and 1mM $MgCl_2$ and incubated overnight in lysing solution (10 mM Tris-HCl pH8, 10 mM NaCl, 10 mM EDTA, 0·5% sodium dodecyl sulphate (SDS), Proteinase K (Boehringer Mannheim) 5 mg/100 ml) at 37°C. Two extractions in phenol/chloroform/isoamyl alcohol (25:24:1) and two in chloroform/isoamyl alcohol (24:1) were carried out followed by precipitation of the DNA in 1:10 volume 4 M NaCl and two volumes ice cold ethanol. After drying, DNA was reconstituted in sterile water.

DNA (10 μg) were digested for six hours with 30 units of each restriction enzyme (Boehringer Mannheim) at 65°C (*Taq*I) or 37°C (*Bgl*II). The resulting fragments were separated according to size by electrophoresis in 0·8% agarose gels (18 hours, 30 volts). *Hin*dIII fragments of lambda DNA (Boehringer Mannheim) were run in parallel as size markers. Gels were stained in ethidium bromide and photographed under ultraviolet light to determine marker position. They were then denatured in 1 M NaOH for one hour and neutralised in 3 M NaCl, 1 M Tris-HCl pH 7·5 for three hours. DNA were transferred from gels to nylon filters (Hybond-N, Amersham) by Southern blotting.

Probes (C11P11 and ECB27 kindly donated by Sir Walter Bodmer, Imperial Cancer Research Fund, Potters Bar, UK; YN5.48 kindly donated by Dr Yusuke Nakamura, Howard Hughes Medical Institute, Salt Lake City, USA) were labelled with $\alpha-^{32}P$ by nick translation (Amersham). C11P11 and YN5.48 detect restriction fragment length polymorphisms with

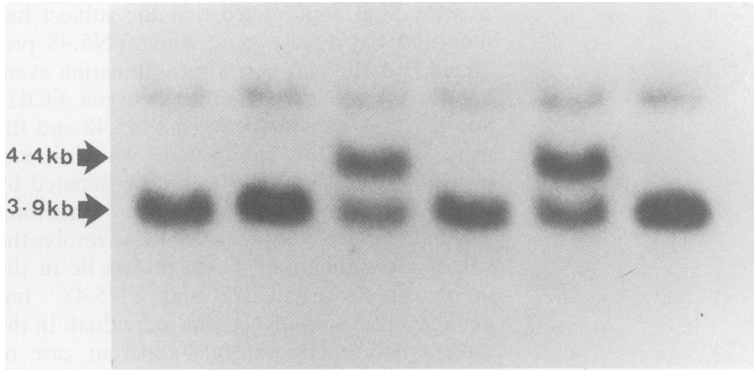
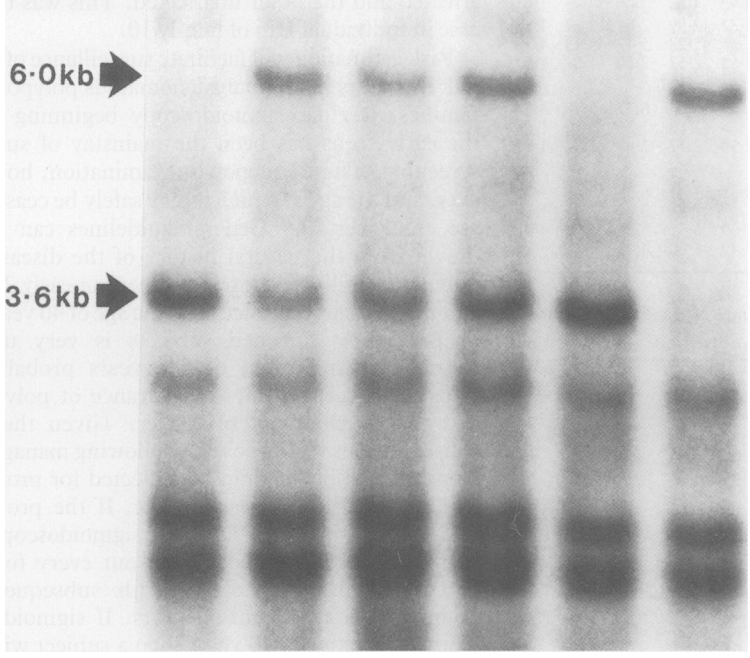


Figure 1: Autoradiograph showing the 4·4 and 3·9 kb alleles of the Taq*I restriction fragment length polymorphism detected by probe C11P11. A larger, faint, constant band is also present.*

*Taq*I[3 4 6] while ECB27 detects a restriction fragment length polymorphism with *Bgl*II.[5] Filters were prehybridised at 65°C in 6×SSC (1×SSC= 150 mM NaCl, 15 mM Na_3 Citrate), 5% dextran sulphate, 0·5% SDS, 5×Denhardt's solution containing 20 μg/ml salmon sperm DNA (C11P11 and ECB27) or human placental DNA (YN5.48). Hybridisation was overnight at 65°C in 1–2 ml of the same solution to which labelled probe was added. Probe YN5.48 was incubated for one hour at 65°C with 50 μg human placental DNA in 1 ml hybridisation solution before addition to the filters. Filters were washed to a final stringency of 0·1×SCC, 0·1% SDS at 65°C. They were then wrapped in plastic and exposed to radiographic film (Kodak) at −75°C for two to seven days.

Linkage analysis was performed using the computer program LIPED with an age at diagnosis correction derived from all affected individuals in the 10 families.[8 9] Risk estimates were calculated using the MLINK program of LINKAGE.[10] This program allows at risk individuals to be assigned to liability classes based on age dependent penetrance. For this analysis, estimates of age dependent penetrance were made from the age at diagnosis of those affected family members who had undergone regular sigmoidoscopic surveillance before diagnosis. At risk family members were assigned to a specific liability class according to their age at last normal sigmoidoscopy. Unscreened individuals (children and those adults who had refused sigmoidoscopic screening) were assigned to liability class one with an associated age dependent penetrance of zero. Allele frequencies were estimated from the results for each restriction fragment length polymorphism in 30 unrelated Australian subjects. A disease gene frequency of 1:10 000[1] and a chromosome order of C11P11-ECB27-FAP-YN5.48[11] were assumed. Two risk estimates were calculated for each at risk individual: the 'probe derived risk' was based on that individual's restriction fragment length polymorphism results alone while the 'overall risk', incorporated, in addition, the impact of the individual's liability class assignment.

Figure 2: Autoradiograph showing the 6·0 and 3·6 kb alleles of the Taq*I restriction fragment length polymorphism detected by probe YN5.48. Several constant bands are also evident.*



Results

Ten unrelated Australian familial adenomatous polyposis families were studied. Blood samples were collected from 152 family members while four (two adults, two children) refused blood collection. There were 44 affected individuals, 57 unaffected (including spouses) and 51 who were at risk. All family members who may have inherited the disease gene and were less than 50 years old were included in the 'at risk' category with the exception that those who had had a normal sigmoidoscopy or barium enema after the age of 40 were regarded as unaffected.

C11P11 detects a two allele restriction fragment length polymorphism after *Taq*I digestion with bands of 4·4 kb and 3·9 kb (Fig 1) while YN5.48 detects bands at 6·0 kb and 3·6 kb (Fig 2). ECB27 detects a two allele *Bgl*II restriction fragment length polymorphism with bands of 11·9 kb and 10·5 kb (Fig 3). Allele frequencies for the Australian population were estimated from results in 30 unrelated subjects: C11P11 4·4 kb allele 0·08, 3·9 kb allele 0·92; ECB27 – 11·9 kb allele 0·38, 10·5 kb allele 0·62; YN5.48 – 6·0 kb allele 0·50, 3·6 kb allele 0·50. These frequencies are similar to those reported from Europe and North America.[3-6]

For the purposes of linkage analysis using LIPED, penetrances were determined from the age at diagnosis in all affected members of the 10 families: 2% for 0–11 years; 37% for 12–21 years; 75% for 22–33 years, 98% for 34–45 years; and, 100% for greater than 45 years. Linkage analysis confirmed linkage between the familial adenomatous polyposis disease locus and each probe with a maximum combined LOD score of 2·82 for C11P11, 2·90 for ECB27 and 5·49 for YN5.48 all at a recombination fraction (θ) of zero. For risk estimation values of θ were selected for each probe based on our own results and those reported in the literature (Table I): for C11P11 we chose a recombination fraction of 0·05; for ECB27 0·03; and 0·02 for YN5·48. Seven liability classes were adopted (Table II). Nineteen individuals were assigned to liability class one: 15 had not yet commenced sigmoidoscopic screening and four (aged 16, 34, 35 and 42 years) had previously refused such screening.

Of the 51 at risk individuals in these families 32 (63%) were assigned probe derived risks of

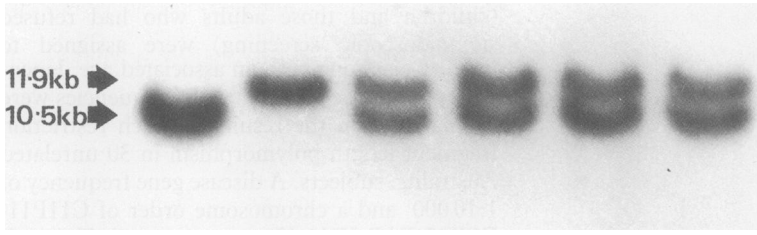


Figure 3: Autoradiograph showing the 11·9 and 10·5 kb alleles of the BglII *restriction fragment length polymorphism detected by probe ECB27.*

≤5% or ≥95% while 34 (67%) had overall risks in these categories. Twenty six of 51 (51%) had overall risks ≤1% or ≥99% (Fig 4). Fifteen individuals were informative for bridging probes (probes lying on either side of the familial adenomatous polyposis gene): in nine cases ECB27 and YN5.48 were both informative, in four cases C11P11 and YN5.48 were both informative and in two cases all three probes were informative.

In 19 cases (37%) the probe derived risk fell in the intermediate range between 5% and 95%. The two main reasons for this were a deceased parent whose restriction fragment length polymorphism data could not be fully predicted (10 individuals at risk) and parents who were uninformative for all three probes (six individuals at risk). In one family, the proband appeared to represent a new mutation so that diagnosis in either of his two at risk children will not be possible unless one is found to be clinically affected. In one case, informative for bridging probes, ECB27 predicted that the subject had inherited the disease gene while YN5.48 predicted that she had not: a recombination event has therefore occurred either between ECB27 and the FAP gene or between YN5.48 and the gene. Study of this family with two additional probes, L5.62 and EF5.44 (kindly donated by Dr Yusuke Nakamura, Cancer Institute, Toshima-ku, Tokyo, Japan), failed to resolve the site of recombination. These probes lie in the interval between ECB27 and YN5.48[11] but neither was informative in this individual. In the same family there was one apparent case of undisclosed non-paternity although in this instance it did not prevent prediction of the child's disease status (Fig 5).

TABLE I *Linkage between the familial adenomatous polyposis locus and probes C11P11, ECB27 and YN5.48*

			θ				
Probe	*Z max*	*θ*	*0·00*	*0·05*	*0·10*	*0·15*	*0·20*
FAP/C11P11							
Bodmer et al[4]	3·26	0·00	3·26		2·56		1·88
Leppert *et al*[4]	3·37	0·00	3·37	3·04	2·69		1·94
Nakamura *et al*[6]	3·32	0·00	3·32	2·99	2·64	2·27	1·88
Dunlop *et al*[11]	5·45	0·09	....	5·23	5·43	5·13	4·59
This study	2·82	0·00	2·82	2·52	2·20	1·87	1·53
FAP/ECB27							
Varesco *et al*[5]	6·94	0·03	....	6·84	6·27	5·48	4·59
Dunlop *et al*[11]	1·89	0·06	....	1·88	1·76	1·50	1·20
This study	2·90	0·00	2·90	2·72	2·49	2·22	1·93
FAP/YN5.48							
Nakamura *et al*[6]	8·25	0·00	8·25	7·41	6·55	5·67	4·75
Tops *et al*[12]	2·86	0·05	....	2·86	2·63	2·27	1·85
Dunlop *et al*[11]	7·00	0·00	7·00	6·31	5·56	4·79	4·01
This study	5·49	0·00	5·49	5·00	4·45	3·86	3·23

TABLE II *Age dependent penetrance in familial adenomatous polyposis*

Liability class	*Age (yr)*	*Penetrance*	*Individuals at risk**
1	0–10	0·00	19
2	11–15	0·12	3
3	16–20	0·54	5
4	21–25	0·81	7
5	26–30	0·88	9
6	31–40	0·96	8
7	>40	1·00	

*At risk individuals have been assigned to a liability class according to their age at last sigmoidoscopy. Those who have not previously undergone sigmoidoscopic screening have been assigned to class 1.

Discussion

This study confirms linkage between three chromosome 5q probes and the familial adenomatous polyposis gene in 10 Australian familial adenomatous polyposis kindreds and shows the application of such probes to risk estimation. Sixty three per cent of at risk individuals were assigned probe derived risks of ≤5% or ≥95% and, when the impact of age dependent penetrance was included, 51% had overall risks ≤1% or ≥99%.

Fifteen individuals were informative for bridging probes: in this situation the probe data will correctly identify affected and unaffected individuals with an accuracy approaching 100%. Twelve of our 15 had probe derived risks ≤0·4% or ≥99·8% while two had a higher risk (4%) because their mother was herself at risk rather than affected. The computer derived estimate of risk reflects the small probability of two recombination events occurring, one between each probe and the familial adenomatous polyposis gene. In reality this probably never occurs because chiasma interference prevents two simultaneous recombinations over such a short distance.[12] With informative bridging probes, a single recombination is immediately apparent because one probe predicts that the subject will be affected and the other unaffected. This was the case in individual III5 of family 10.

Risk estimation will facilitate surveillance of at risk members in familial adenomatous polyposis families. Regular sigmoidoscopy beginning in the early teens has been the mainstay of such screening. The frequency of examination, however, and the age at which it may safely be ceased are controversial.[13] General guidelines can be drawn from the natural history of the disease: polyps generally develop in the teens or early 20s and their first appearance after the age of 40 years in previously screened subjects is very uncommon; a minimum of five years probably elapses between the first appearance of polyps and the development of cancer.[1] Given these considerations we propose the following management plan. Blood should be collected for probe studies at about 12 years of age. If the probe derived risk is low (≤5%), sigmoidoscopic examinations should be carried out every four years until the age of 30 with subsequent examinations at 35 and 40 years. If sigmoidoscopy is negative at 40 years then a subject with

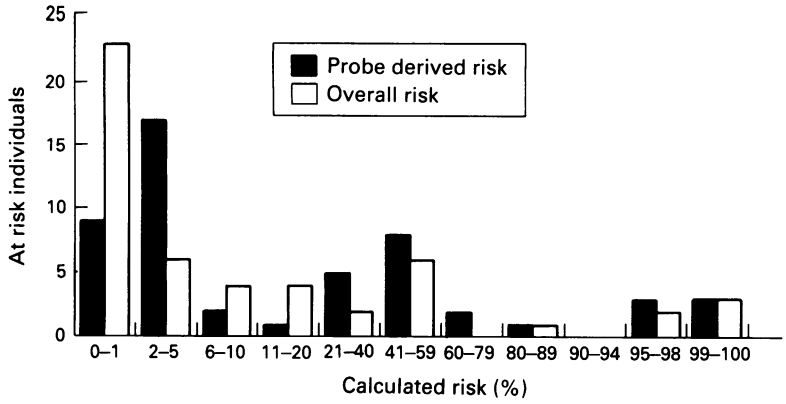


Figure 4: Calculated risk of developing familial adenomatous polyposis in 51 at risk individuals. Probe derived risks were calculated on the basis of the individual's restriction fragment length polymorphism data. Overall risks incorporate, in addition, the impact of the individual's liability class assignment.

an initial probe derived risk of 5% will have an overall risk of approximately 0·1% and could confidently be discharged from screening. If the probe derived risk is high (≥95%) sigmoidoscopy should be done every one to two years until 40 years of age and every five years thereafter. Such patients should probably remain under review throughout their lives despite their falling overall risk. By so doing, the rare case of late onset polyposis will not be inappropriately discharged from follow up.

Nineteen of our subjects had probe derived risks in the intermediate range (between 5% and 95%). Management of this group remains partly arbitrary. We recommend sigmoidoscopy every two to three years to 40 years of age. The proportion of individuals with intermediate risk estimates can be reduced by the use of additional linked probes.[11] Clinical markers of the disease gene may also aid in assessment of risk. Any of the extracolonic manifestations of familial adenomatous polyposis appearing before the development of polyps can suggest inheritance of the disease gene. Congenital hypertrophy of the retinal pigment epithelium has received the greatest recent attention.[14–16] When multiple and bilateral, congenital hypertrophy of the retinal pigment epithelium lesions predict inheritance of the disease gene and their absence in an at risk member of a family manifesting the trait implies a substantially reduced risk.

In our study the major factor leading to intermediate risk estimates was the premature death of a key family member who was thus not available for probe studies. In Australia, members of earlier generations have generally not had access to effective screening programmes for familial adenomatous polyposis and therefore often presented with colon cancer. In addition, approximately 20% of presenting cases with familial adenomatous polyposis represent new mutations[17] as was the case in one of our families. In these families no predictions can be made until an affected child is diagnosed allowing determination of the restriction fragment length polymorphism alleles linked to the disease gene. In the future, characterisation of the familial adenomatous polyposis gene may eliminate this difficulty by allowing the new gene mutation to be delineated in the parent and then sought in the children. A candidate gene has recently been reported on chromosome 5[18] but further studies are necessary before its role in familial adenomatous polyposis is clarified. In view of the above, the banking of DNA samples from members of familial adenomatous polyposis families should be considered.[19] Such stored samples may prove vital to future diagnosis.

Despite these limitations, risk estimation using DNA probes linked to the familial adenomatous polyposis gene will have an increasing role in the management of familial adenomatous polyposis. In informative families it allows safe modification of the current, partly empiric, screening regimens for at risk individuals. The frequency and duration of sigmoidoscopic surveillance can be reduced in those unlikely to develop the disease and likewise the cost and potential morbidity of screening. Among those at high risk, vigilant follow up is required and, hopefully, improved compliance will result from the knowledge of an increased personal risk. Prenatal diagnosis also becomes a possibility although the improving outlook for at risk individuals in terms of surveillance and management of colonic and small bowel disease[13] may reduce the demand for such a service.

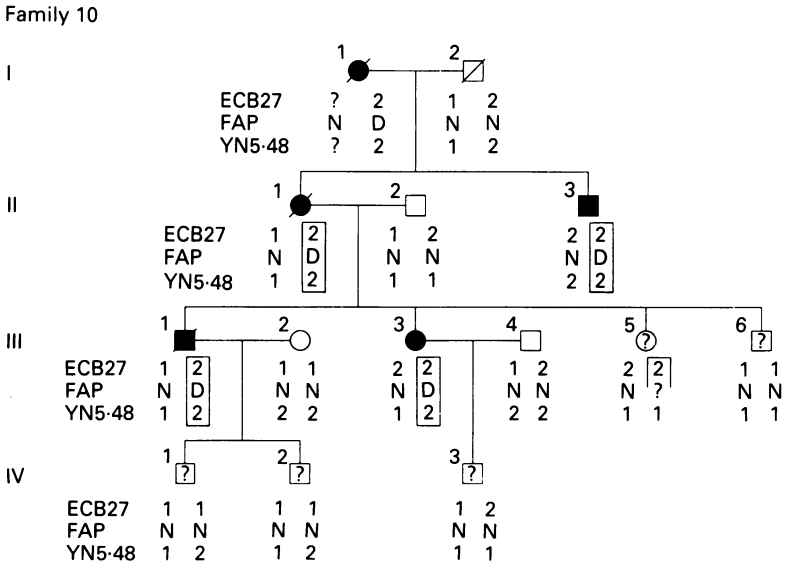


Figure 5: Partial pedigree of family 10 showing haplotypes for the restriction fragment length polymorphisms detected by ECB27 and YN5·48. C11P11 was uniformative in this family. For each probe, 1 denotes the larger allele and 2 the smaller. N represents the normal familial adenomatous polyposis gene and D the disease gene. The genotype of deceased family members has been inferred. Shading indicates clinically confirmed familial adenomatous polyposis and ? an at risk family member. Individuals III6, IV1, IV2 and IV3 are predicted to be unaffected with probe derived risks of 0·2%, 0·4%, 0·4%, and 2·0% respectively. The paternity of IV3 is in doubt. Individual III5 has a probe derived risk of 40%: a recombination event has occurred between ECB27 and YN5.48 on the maternal chromosome. Her probe derived risk reflects the greater likelihood of recombination between ECB27 and the familial adenomatous polyposis gene than between YN5.48 and the familial adenomatous polyposis gene.

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