SHORT COMMUNICATIONS

The use of DNA from paraffin wax preserved tissue for predictive diagnosis in familial adenomatous polyposis

D G Morton, F Macdonald, M B Cachon-Gonzales, P M Rindl, J P Neoptolemos, M R B Keighley, J D A Delhanty, C M McKeown, M Kilpatrick, M A Hultén

Familial adenomatous polyposis (FAP) is a dominantly inherited condition which predisposes family members to the development of colorectal carcinoma, usually in their thirties. Carcinoma is preceded by the development of widespread adenomatous polyps in the large bowel, and this feature has been used as a diagnostic marker for over 50 years. In 1987 the causative gene (the APC gene) was localised to a small area on the long arm of chromosome 5 (5q21-22).^{1,2} Since that time it has been possible to identify gene carriers using polymorphic DNA probes closely linked to the APC gene on chromosome 5. With the development of flanking markers diagnostic accuracy can be greater than 99%.³ Unfortunately, such analysis needs complete family pedigrees and, as a consequence of early death from colorectal cancer, is not possible in many FAP families. In the West Midlands Region, of 47 families studied only 17 (36%) had suitable pedigrees for analysis. Similar findings have been reported from other centres.⁴ Fifteen of the families in our region (32%) were unsuitable because of the early death of key affected family members. For this reason we have investigated the analysis of DNA extracted from paraffin wax embedded tissue from dead family members in order to try to extend informativity in these families. Such tissue is widely available because most dead affected relatives have undergone surgery for colorectal cancer or its complications.

In 1990 a known FAP family from Sheffield was referred for genetic counselling (figure). This was prompted by the death of the proband (III-6) from advanced colorectal carcinoma at the age of 32. She left two children who were at risk of developing the disease (IV-1 and IV-2). Neither had evidence of colonic polyps at the time of bowel screening (15 and 18 years of age respectively), but at these ages such findings do not dramatically alter their future risk of developing the condition.⁵ Neither offspring had any evidence of congenital hypertrophy of the retinal pigment epithelium, but this was of limited prognostic significance because diagnostic lesions were not seen in their two affected cousins. It was not possible to establish phase for the two children primarily because the maternal DNA was unavailable for analysis.

However, paraffin wax embedded tissue had been stored from her operation in 1987.

Ideally a 0.4 g piece of histologically normal bowel mucosa is used for DNA extraction. In this case, however, only omental tissue (including tumour) was available. Tumour material is not ideal for analysis as deletion of the APC gene can also result in deletion of the DNA region containing the polymorphism. However, in this case there was no evidence of allele loss for C11p11 (figure) so the DNA results could be used. The sectioned tissue was treated with xylene to dissolve the wax, then washed in ethanol and water, and recovered by centrifugation. DNA extraction was performed by resuspending the tissue in 5 ml of lysis buffer (Tris 0.1 mol/l, EDTA 0.01 mol/l, pH 9,⁶ to which were added 0.5 ml 10% SDS and 250 µl proteinase K (20 mg/ml). The DNA was purified with phenol/chloroform and precipitated in two volumes of 100% ethanol overnight at -20°C. DNA was resuspended in 200 µl of TE buffer.

Two primers for the 4 base pair deletion in C11p11 were used to amplify the genomic DNA fragment from each family member.⁷ The amplified products were run on a 12% polyacrylamide gel at 110 V overnight. The alleles (66 and 70 bp) were identified by staining with ethidium bromide. The alleles associated with each tested family member are shown in the figure. Similarly, two PCR primers across a CA repeat region in ECB27 were used to amplify a second polymorphic region (fragment size 115 and 117 base fragments). These fragments were separated on an 8% denaturing polyacrylamide gel run at 40 W for three to four hours. The bands were identified by inclusion of ³²P radiolabelled dCTP in the PCR reaction mixture and subsequent exposure to x ray film. A discussion of the results is given in the legend to the figure.

Discussion

We have shown that DNA extracted from the preserved tissue of dead relatives can be used to extend informativeness in FAP families. Colorectal cancer remains the commonest cause of early death in subjects affected by

Department of Surgery, University of Birmingham, Birmingham.
D G Morton
J P Neoptolemos
M R B Keighley

West Midlands Regional Genetics Service, East Birmingham Hospital, Birmingham.
F Macdonald
P M Rindl
C M McKeown
M A Hultén

Department of Clinical Genetics, University of Birmingham, Birmingham.
M Kilpatrick
M R B Keighley

Department of Genetics and Biometry, Galton Laboratories, University College London.
M B Cachon-Gonzales
J D A Delhanty

Correspondence to Mr Morton FRCS, Department of Clinical Genetics, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST.

Received 16 December 1991.
Revised version accepted 7 February 1992.

Pedigree and allele status of family members. The diagram shows the family pedigree, including disease status and age at death for dead affected family members. For those subjects analysed, the alleles identified for each of the two polymorphisms are written below each symbol (C11p11 deletion alleles given above those for the ECB27 CA repeat). The corresponding band fragments are shown in the accompanying photographs. Using the deletion polymorphism in C11p11, the affected gene was found to be carried with allele 1 (III-1, III-3, III-6). Thus the first offspring (IV-1) had inherited allele 2 from her mother and was therefore at low risk. Unfortunately, as the father (III-7) of the second child (IV-2) was heterozygous for this polymorphism, analysis was not informative for this child. Using the CA repeat polymorphism in ECB27 the affected gene was shown to be inherited with allele 2 (115 base pair band). The pedigree shows that both offspring have inherited allele 1 from their mother and carry the low risk haplotype. These results independently reduce the future risk of developing FAP for these two offspring to less than 5%.

FAP, accounting for 28 of 35 (80%) deaths in the West Midlands Region. The mean age at death was 38 years. By its nature, advanced colorectal cancer necessitates surgery for the majority of these cases, providing tissue for histology and subsequent DNA extraction.

The use of preserved tissue DNA has the potential to double the number of FAP families who could benefit from this form of predictive diagnosis. DNA extracted from more than 25 separate preserved tissue samples, which had been stored for up to 20 years,

has successfully amplified with both the sets of primers used in this study.

The main diagnostic limitation was the absence of flanking markers. However, since the APC gene has been sequenced⁸⁹ new CA repeat polymorphisms have been identified.¹⁰ These will greatly increase the accuracy of predictive diagnosis using preserved tissue from dead family members.

Those FAP families that remain uninformative for RFLP analysis are predominantly isolated cases. Predictive diagnosis is now a

possibility even in these families by the use of direct mutation analysis.

Note added in proof

The details of the CA repeat polymorphism in ECB27 have been published (Cachon-Gonzales MB, Wolfe J, Delhanty JDA. Dinucleotide repeat polymorphism at the D5S98 locus. *Nucleic Acids Res* 1992;20:924).

We would like to acknowledge the contribution of Dr C E Blank, Howard Hughes, and John Quick of the North Trent Regional Genetics Services who compiled the clinical information and collected the blood samples for this study.

- 1 Bodmer WF, Bailey CJ, Bodmer J, *et al.* Localisation of the gene for familial adenomatous polyposis within a small region of chromosome 5. *Nature* 1987;328:614-6.

- 2 Leppert M, Dobbs M, Scambler P, *et al.* The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 1987;238:1411-3.
- 3 Dunlop MG, Wyllie AH, Steel CM, Piris J, Evans HJ. Linked DNA markers for presymptomatic diagnosis of familial adenomatous polyposis. *Lancet* 1991;337:313-6.
- 4 Houlston R, Slack J, Murday V. Risk estimates for screening adenomatous polyposis coli (letter). *Lancet* 1990;335:484.
- 5 Murday V, Slack J. Inherited disorders associated with colorectal cancer. *Cancer Surv* 1989;8:139-57.
- 6 Goelz SE, Hamilton SR, Vogelstein B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem Biophys Res* 1985;130:118-26.
- 7 Tops CMJ, Breukel C, Klift HM, *et al.* A new deletion polymorphism at D5S71 raises the linkage information on adenomatous polyposis coli: implications for presymptomatic diagnosis. *Hum Genet* 1991;86:365-8.
- 8 Kinzler KW, Nilbert MC, Su LK, *et al.* Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
- 9 Groden J, Thliveris A, Samowitz W, *et al.* Identification and characterisation of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
- 10 Wijnen J, Tops C, Breukel C, *et al.* CA repeat polymorphism from YAC W25 at the D5S318 locus, distal to adenomatous polyposis coli. *Nucleic Acids Res* 1991;19:6965.