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Analysis of Ha-ras 1 allele frequencies in hereditary non-polyposis colorectal cancer

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Abstract

Restriction enzyme digests of genomic DNA show multiple alleles of common, intermediate, and rare frequencies at the minisatellite locus of Ha-ras. It has been suggested that a higher frequency of rare alleles is associated with the presence of colorectal and other types of cancer. This study investigated the distribution of Ha-ras alleles in 40 members of hereditary non-polyposis colorectal carcinoma (HNPCC) families and in 34 cancer free subjects (spouses). There was no difference in rare allele frequency between the cancer group and cancer free group (χ^2=0·25, not significant).

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Hereditary non-polyposis colorectal carcinoma (HNPCC), accounts for about 5% of all colorectal cancers, and is characterised by autosomal dominant inheritance, early age of onset, a higher frequency of cancer in the proximal colon, and the presence of particular extracolonic malignancies.[1] The development of colorectal cancers in these families follows the adenoma-carcinoma sequence,[2] with an accompanying accumulation of specific genetic changes within the tumour. These include point mutations of the K-ras, p53, and APC genes.[5]

Recent insights linking HNPCC with a family of DNA mismatch repair genes on chromosome 2p16,[6 7] 3p21–23[8] and elsewhere do not detract from attempts to show additional hereditary factors. There is evidence that the HNPCC genes do not initiate adenoma development but rather accelerate adenoma evolution.[6] On the other hand, genetic factors may be responsible for adenoma proneness.[9] The absence of such factors could account for late onset malignancies or non-penetrance of the HNPCC gene. An example of genetic background influencing expression of an inherited cancer syndrome is seen in the mouse model for human familial adenomatous polyposis. Genetic studies of Min-induced intestinal neoplasia in the mouse have identified a major modifier locus Mom-1 that strongly modifies tumour number.[10]

The human Ha-ras-1 gene contains a polymorphic 3′ flanking 28 bp variable tandem repeat.[11] Variability in the number of tandem repeats in the variable tandem repeat region has been shown to give rise to a large number of alleles showing codominant Mendelian inheritance.[12] This variable tandem repeat has been shown to have transcriptional enhancer activity, which may change ras expression and play a part in tumorigenesis.[13] Higher frequencies of rare Ha-ras alleles have been correlated with several different types of cancer,[12 14] including lung,[15] breast,[16] and colorectal cancer[17] suggesting that these alleles may be a marker of predisposition to malignant transformation. Other studies have shown normal Ha-ras allele frequencies in patients with colorectal cancer,[18] myelodysplasia,[19] urothelial cancer,[20] and melanoma.[21] Recently an extensive case control study and a meta-analysis of all published studies to date found significant associations with the risk of cancer of the breast, colon, bladder, and acute leukaemia.[22]

It is conceivable that the association between particular Ha-ras allele frequencies and colorectal cancer could relate to a specific subgroup with a strong hereditary predisposition to large bowel cancer. Accordingly, the aim of this study was to analyse the distribution of Ha-ras alleles in affected members of HNPCC families, using non-affected spouses as the control population.

Methods

Peripheral blood samples were collected from 40 white cancer patients who were members of 15 different HNPCC families as defined by the Amsterdam criteria.[23] Neoplasms detected in these patients included colon cancers (32), adenomas (5), and cancers of the endometrium (6), ovary (6), breast (2), cervix (1), brain (1), small bowel (1), and skin (3). Twelve patients had multiple cancers. The control group consisted of 34 unaffected white subjects who were spouses of HNPCC family members. All subjects gave written informed consent in accordance with the guidelines of the Auckland Hospital ethics committee.

High molecular weight DNA was prepared from peripheral blood samples by standard methods of sodium dodecylsulphate-proteinase K digestion followed by phenol chloroform extraction and ethanol precipitation.[24] Three to 5 μg of DNA was digested with 20–30 units of either MspI or BamHI under conditions recommended by the manufacturer. DNA fragments were separated by electrophoresis on 1·2% gels for the MspI digests and 0·7% gels for the BamHI digests and transferred to Gene Screen Plus nylon membranes after denaturation and neutralisation. Filters were hybridised to a 6·4 kb genomic c-Ha-ras 1 probe obtained from the American Type Culture Collection.[11 25]

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TABLE I *Ha-ras allele frequencies in HNPCC*

		Number (%) of alleles		
*Allele**		*Controls*	*Cancer*	*Probands‡*
Common:	a1	41 (60·3)	47 (58·8)	18 (60)
	a2	3 (4·4)	2 (2·5)	0 (0)
	a3	5 (7·4)	4 (5·0)	1 (3·3)
	a4	4 (5·9)	9 (11·3)	3 (10)
Total common alleles		53 (78·0)	62 (77·5)	22 (73·3)
Rare†:	a0·1	1 (1·5)	0 (0)	0 (0)
	a1·1	3 (4·4)	1 (1·3)	0 (0)
	a1·2	0 (0)	2 (2·5)	2 (6·7)
	a1·3	1 (1·5)	1 (1·3)	1 (3·3)
	a1·35	1 (1·5)	0 (0)	0 (0)
	a1·4	3 (4·4)	5 (6·3)	3 (10)
	a2·01	0 (0)	1 (1·3)	0 (0)
	a2·4	3 (4·4)	5 (6·3)	1 (6·7)
	a3·1	1 (1·5)	1 (1·3)	1 (6·7)
	a3·4	0 (0)	1 (1·3)	0 (0)
	a3·5	1 (1·5)	0 (0)	0 (0)
	a4·1	1 (1·5)	1 (1·3)	0 (0)
Total rare alleles		15 (22·0)	18 (22·5)	8 (26·7)
Total alleles		68	80	30

*Nomenclature of alleles according to Krontiris *et al*[22]; †includes rare and intermediate frequency alleles; ‡probands of 15 HNPCC families (subset of cancer group).

Probes were labelled with ^{32}-P by random primer extension, and hybridisation conditions were as previously described.[15] Autoradiography was performed at −70°C for one to three days using intensifying screens. Allele sizes were calculated from ethidium-bromide stained EcoR1 digested bacteriophage SPP1 DNA, and ^{32}P-labelled 1 kb DNA ladder. Control samples consisting of alleles a1, a2, and a3 were run as standards on all gels.

Results

After digestion with MspI, the four common progenitor alleles a1, a2, a3, and a4, were found in similar frequencies in both the cancer and control groups. Differences as small as 20 bp could be distinguished with MspI digests, and these were used to identify the rare alleles. BamHI digests did not permit accurate size determinations and were used only to confirm homozygosity or heterozygosity of MspI alleles. An additional 12 alleles of intermediate and rare frequencies were identified.

The data for the cancer group were collected from members of 15 different HNPCC families, with one to 12 patients from each family. As related members are likely to share alleles, allele frequencies within families are not independent. To obtain a set of independent findings we identified a subset of the cancer group consisting of the proband from each family. Table I summarises these results. Alleles were named according to the nomenclature of Krontiris *et al.*[22] The frequency of rare alleles did not seem to be higher in the cancer proband group than in the control group (χ^2=0·25, not significant).

Data from all the cancer patients were also analysed by taking allele frequencies within each family and weighting them by the inverse of family size, so that their contribution summed to one. This gave an effective total of 30 alleles or 15 genotypes, with no difference in the allele frequencies found between the control group and cancer group (χ^2=0·26, not significant).

In the analysis of genotypes, shown in Table II, a distinction was made between subjects having two common alleles, and those having one or two intermediate or rare alleles. The only notable difference between the cancer and control groups was the a1/a4 frequency, which was one of 34 (2·9%) in the control group and eight of 40 (20%) in the cancer group. Six of eight a1/a4 genotypes in the cancer group were members of the same pedigree while six other members of this family had different genotypes. There was no difference in the frequencies of common or rare genotypes between the control group and the cancer proband group (χ^2=0·76, not significant).

TABLE II *Ha-ras genotypes in HNPCC*

		Number (%) of genotypes		
Genotype		*Controls*	*Cancer*	*Probands†*
Common:	a1/a1	14 (41)	13 (32·5)	6 (40)
	a1/a2	2 (6)	2 (5)	0 (0)
	a1/a3	1 (3)	4 (10)	1 (6·7)
	a1/a4	1 (3)	8 (20)	3 (20)
	a3/a4	1 (3)	0 (0)	0 (0)
Rare*:		15 (44)	13 (32·5)	5 (33·3)
Total		34	40	15

*Includes genotypes with one or two rare alleles; †probands of 15 HNPCC families (subset of cancer group).

Discussion

Studies on Ha-ras alleles in colorectal cancer have produced contradictory results. Klingel *et al*[17] found an increased frequency of rare alleles in a study population of unrelated colorectal carcinoma patients. Our results are more in agreement with those of Wyllie *et al*[18] who found no significant difference in rare allele frequency between their cancer and control groups, although it was not specifically stated whether the cancer group consisted of sporadic or familial colorectal cancers.

It is of interest that Wyllie *et al* found the combined frequency of alleles a3 and a4 to be twice as high in the cancer group. In our study the a1/a4 genotype is present in 20% of the cancer group, but in only 2·9% of the control group. Six of eight a1/a4 genotypes are from family 3, however, the largest family used in this study, while the two remaining a1/a4 genotypes were from two different families. The high frequency of this genotype could simply result from the large contribution from a single family, particularly in view of the comparatively small numbers in this study.

The case control study and meta-analysis of Krontiris *et al*[22] suggest that Ha-ras rare alleles are indicative of an increased risk of cancer. Previous studies have all concerned unrelated cancer patients. This study differs in that it looked at rare allele frequencies in familial cancer. Clinical features including age at onset of cancer and multiple cancers were not associated with Ha-ras allele status. Thus it is possible that the mechanisms of pathogenesis are different in sporadic and familial cancer, and rare Ha-ras alleles may not play a part in the pathogenesis of HNPCC.

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