

The APC I1307K allele and cancer risk in a community-based study of Ashkenazi Jews

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Mutations in APC are classically associated with familial adenomatous polyposis (FAP), a highly penetrant autosomal dominant disorder characterized by multiple intestinal polyps and, without surgical intervention, the development of colorectal cancer¹ (CRC). APC is a tumour-suppressor gene, and somatic loss occurs in tumours. The germline T-to-A transversion responsible for the APC I1307K allele converts the wild-type sequence to a homopolymer tract (A_n) that is genetically unstable and prone to somatic mutation. The I1307K allele was found in 6.1% of unselected Ashkenazi Jews and higher proportions of Ashkenazim with family or personal histories of CRC (ref. 2). To evaluate the role of I1307K in cancer, we genotyped 5,081 Ashkenazi volunteers in a community survey. Risk of developing colorectal, breast and other cancers were compared between genotyped I1307K carriers and non-carriers and their first-degree relatives.

Identification of a founder *BRCA1* mutation in approximately 1% of Ashkenazim prompted the collection of DNA samples and personal and family cancer history information from 5,318 Ashkenazim from the Washington D.C. metropolitan area³. Genotyping anonymous samples from individuals who gave consent for studies of other genes allowed us to investigate cancer risks associated with I1307K in 5,098 Ashkenazim. Demographic characteristics of the subjects have been reported³. It was not possible to assign genotypes to 17 individuals (0.3%) because of repeated failure of PCR amplification, and these individuals were not included in subsequent analyses.

Of 5,081 subjects, 4,712 were wild type, 367 were heterozygous and two were homozygous for I1307K. Although the number of homozygotes was somewhat less than the number expected under conditions of Hardy-Weinberg equilibrium (χ^2 test, $P=0.06$), this result did not reach statistical significance. At 32 and 76 years of age, the I1307K homozygotes did not report personal histories of cancer or other notable phenotypic features. I1307K allele frequency was 0.037 (95% confidence interval (CI) 0.033–0.040) and carrier frequency was 7.3% (95% CI 6.6–8.0%). As some of the study volunteers were related, another estimate of the population carrier frequency, 7.0% (95% CI 6.3–7.8%) was derived from a set of 4,635 individuals with no volunteer relatives in the study and one randomly selected member from each family set. Carrier frequency was approximately equal across all age groups.

I1307K carrier frequencies in subjects with or without personal and family histories of CRC or breast cancer are shown (Table 1). Of 10 individuals with a positive personal and family history of CRC, a group that might be expected to have the highest carrier frequency, no carriers were found. Only 0.7 of these subjects would be expected to be carriers under conditions of independent assortment, however.

To assess the effect of I1307K on cancer risk, two groups were examined. The first was the set of 5,037 volunteers with known genotypes and personal cancer history information, and the second was the set of the volunteers' 26,467 first-degree relatives, both living and deceased.

A personal history of CRC was reported by I1307K carriers with an odds ratio (OR) of 1.9 (95% CI 0.84–4.2, $P=0.12$) compared with non-carriers. An OR 1.3 (95% CI 0.83–2.0, $P=0.26$) was found when comparing histories of breast cancer in carriers and non-carriers. ORs for these and all other cancers that occurred in two or more carrier probands are shown (Table 2). Although the above results did not reach statistical significance, I1307K carriers were more likely to have had any type of cancer (excluding non-melanoma skin cancer) than non-carriers, OR 1.5 (95% CI 1.1–2.0, $P=0.01$). These results were unchanged after adjusting for age and sex distributions in the sample.

Cancer risks associated with I1307K (Table 2) may have been affected by volunteer or survival bias. Reports of cancer in family members should be less biased with respect to survival. ORs for common malignancies in first-degree relatives of carrier and non-carrier probands (using family sets as the reporting unit) are shown (Table 3). The OR for CRC in first-degree relatives of carriers compared with those of non-carriers was 1.3 (95% CI 0.92–1.8, $P=0.13$). An excess of breast cancer in first-degree relatives, OR 1.4 (95% CI 1.1–1.8, $P=0.01$), was reported in family sets of proband I1307K carriers. Relatives of I1307K carriers were also more likely to report having had any type of cancer than were relatives of non-carriers, OR 1.2 (95% CI 1.0–1.6, $P=0.01$).

Table 1 • APC I1307K carrier rates and personal and family history of CRC and breast cancer

Group	Carriers	Total
<i>No prior history of CRC</i>		
No family history of CRC	310 (7.0%)	4,452
Positive family history	45 (9.1%)	496
<i>CRC survivors</i>		
No family history of CRC	7 (15.6%)	45
Positive family history	0 (0%)	10
Total	362 (7.2%)	5,003
<i>No prior history of breast cancer</i>		
No family history of breast cancer	266 (6.9%)	3,837
Positive family history	71 (8.0%)	887
<i>Breast cancer survivors</i>		
No family history of breast cancer	18 (8.9%)	203
Positive family history	7 (9.2%)	76
Total	362 (7.2%)	5,003

Probands contributing data overlapping with that from related study participants excluded. Numbers do not sum to 5,081 because of missing information.

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Table 2 • Cancer in APC I1307K carrier and non-carrier probands

Cancer type	Carriers	Non-carriers	Odds ratio (95% CI)	P-value
Breast	25	254	1.3 (0.83–2.0)	0.26
Colorectal	7	48	1.9 (0.84–4.2)	0.12
Prostate	6	41	2.0 (0.81–4.7)	0.14
Melanoma	5	33	2.0 (0.76–5.0)	0.17
Thyroid	3	35	1.1 (0.34–3.6)	0.87
Bladder	3	21	1.8 (0.55–6.2)	0.33
Lymphoma (Non-Hodgkin's)	3	17	2.3 (0.66–7.8)	0.19
Ovary	2	15	1.7 (0.39–7.5)	0.48
Any cancer	56	516	1.5 (1.1–2.0)	0.01
Total	365	4,672		

ORs for breast, prostate and ovarian cancers were calculated for female (3,275 wild-type, 257 carrier) and male (1397 wild-type and 108 carrier) probands as appropriate.

Cumulative risks of CRC and breast cancer in relatives of carriers and non-carriers (Fig. 1a,c, respectively), were compared by the Wilcoxon and log-rank survival function tests. The Wilcoxon test is more sensitive to differences in hazard ratios elevated at early ages and the log-rank test to differences occurring at later ages. For CRC, the *P*-value for a difference between relatives of carriers and non-carriers was 0.03 with the Wilcoxon test and 0.08 for the log-rank test. Kaplan-Meier curves showing estimated risks of breast cancer in relatives of carriers and non-carriers were superimposable until 65 years of age (Fig. 1c). As described above, an overall excess of breast cancer was noted in first-degree relatives of I1307K carriers, but these curves were not found to be significantly different by the Wilcoxon and log-rank survival function tests, with *P*-values of 0.48 and 0.26, respectively. This may reflect assumptions underlying these tests that are dependent on the age distributions of carriers and non-carriers.

The cancer risk attributed to I1307K in first-degree relatives is diluted approximately 50% because only approximately half of these relatives should carry I1307K. The kin-cohort approach⁴ allows us to account for this effect by inferring genotypes of the first-degree relatives of the genotyped carrier and non-carrier probands. Confidence intervals surrounding the derived estimates for cancer risk in these subjects were determined by a bootstrap procedure. Graphs showing the results of this analysis for CRC and breast cancer are shown (Fig. 1b,d, respectively). As was the case for the curves (Fig. 1a,c), data points beyond age 80 are based on a limited number of I1307K carriers.

Although measurements of the effect of I1307K are imprecise, it is possible to provide age-specific estimates of the lower and upper bounds of these risks in carriers and non-carriers. Thus, by age 70, 5.1% (95% CI 1.5–9.0%) of carriers developed CRC (that is, penetrance of the I1307K allele is less than 10% in this sample) compared with 3.1% (95% CI 2.6–3.5%) of non-carriers (Fig. 1b). Cumulative breast cancer risk by age 70 is estimated to be 17.4% (95% CI 10.3–24.1%) in carriers and 13.8% (95% CI 12.8–14.8%) in non-carriers (Fig. 1d).

Study participants were more likely to report personal and family histories of breast cancer than the general population³. To a lesser degree, a volunteer effect is also seen for CRC. The estimated risk in Europeans for developing breast cancer by age 70 is approximately 7.7% (refs 5,6). Comparable figures are not available for Ashkenazim; however, non-carrier kin here had an estimated breast cancer risk of 13.8% by age 70. Corresponding figures for CRC are 2.2% for Europeans and 3.1% for non-carrier Ashkenazim. To examine the possibility that personal cancer history of volunteers affected cancer risk estimates, kin-cohort

analysis was performed excluding first-degree relatives of probands with either breast cancer or CRC. Estimated cancer risks for breast cancer and CRC were essentially unchanged (data not shown). The study volunteers had elevated rates of cancer in their families, but they could not have been aware of their I1307K status. Although this volunteer bias will inflate estimates of cumulative risks in carriers and non-carriers, it is unlikely to introduce biases in the comparison of estimated cancer risks between the two groups.

All subjects in this study had previously been genotyped for common Ashkenazi founder *BRCA1* and *BRCA2* mutations³. Six of one-hundred-eighteen (5.1%) *BRCA1* and *BRCA2* mutation carriers were also positive for I1307K. This number was too small to detect any deviation from the expected number of double heterozygotes, should one be present. One subject was diagnosed with breast cancer at age 44; the other five double heterozygotes did not have cancer. It has been demonstrated in this cohort that whereas *BRCA1* and *BRCA2* mutations are associated with breast, ovarian and prostate cancer, they do not modify CRC risk³. With *BRCA1* and *BRCA2* carriers excluded, the ORs for personal and family history of breast cancer and other cancers in I1307K carriers were almost identical to results obtained when they were included.

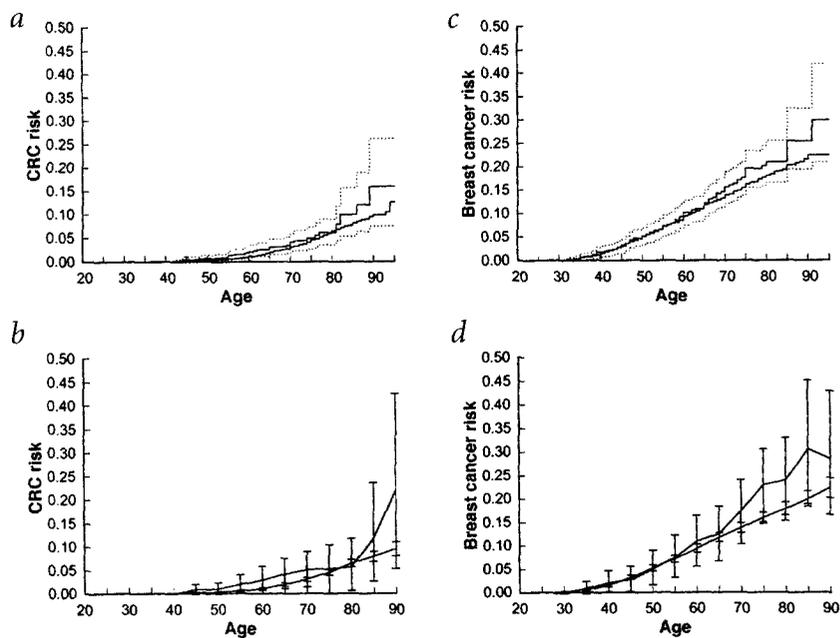
Although detection of such modest effects was at the limits of detection of the large community-based study described here, we observed an association between I1307K and CRC risk. The estimated cumulative CRC risk of first-degree relatives of I1307K carriers was greater than that of relatives of non-carriers. Although it did not reach statistical significance, the OR in proband I1307K carriers was 1.9, consistent with results using pooled sets of 211 CRC cases². In a larger case-series from Toronto, 48 of 476 Ashkenazi CRC cases were I1307K carriers (R. Gryfe and M. Redston, manuscript submitted). This 10% carrier rate was more frequent than the 7.3% carrier frequency found in our sample (*P*=0.02). In a study of 158 breast-ovarian cancer kindreds, twelve I1307K carriers were found. In five kindreds with histories of CRC, no carriers were found, but the sample was too small to rule out even a large effect of I1307K on CRC risk⁷.

Although loss of heterozygosity involving APC has been reported in sporadic primary breast tumours^{8–10}, breast cancer has not been found at increased frequency in women with FAP. Until

Table 3 • APC I1307K carrier and non-carrier probands having first-degree relatives with specific cancers

Cancer type in first-degree relatives	Carriers	Non-carriers	Odds ratio (95% CI)	P-value
Breast	88	867	1.4 (1.1–1.8)	0.01
Colorectal	46	461	1.3 (0.92–1.8)	0.13
Prostate	32	336	1.2 (0.83–1.8)	0.32
Lung/trachea	27	306	1.1 (0.74–1.7)	0.62
Pancreas	17	136	1.6 (0.95–2.7)	0.08
Melanoma	14	121	1.5 (0.83–2.6)	0.19
Lymphoma (Non-Hodgkin's)	13	133	1.2 (0.69–2.2)	0.48
Ovary	11	114	1.2 (0.65–2.3)	0.55
Stomach	11	110	1.1 (0.59–2.2)	0.72
Bladder	10	90	1.4 (0.72–2.7)	0.32
Leukaemia	9	149	0.8 (0.38–1.5)	0.41
Uterus	7	93	0.94 (0.43–2.0)	0.88
Kidney	6	86	0.87 (0.38–2.0)	0.74
Oral	6	45	1.7 (0.71–3.9)	0.24
Hodgkin's disease	5	39	1.6 (0.63–4.1)	0.32
Any cancer	216	2,467	1.2 (1.0–1.6)	0.01
Total	344	4,291		

Fig. 1 Graphs showing: (a) cumulative risks of CRC in first-degree relatives of carriers (red) and non-carriers (blue), (b) estimated risks of CRC in I1307K carriers (red) and non-carriers (blue), (c) cumulative risks of breast cancer in first-degree relatives of carriers (red) and non-carriers (blue) and (d) estimated risks of breast cancer in I1307K carriers (red) and non-carriers (blue). The 95% CI surrounding cancer risk estimates in (a) and (c) are indicated for relatives of carriers with interrupted lines. For clarity, the narrower 95% CI around the curves showing cancer rates in first-degree relatives of non-carriers carriers are omitted. Error bars indicate 95% confidence limits around risk estimates in (b) and (d).



recently however, premature mortality from CRC may have meant that only a limited number of women with FAP survived long enough to be at a substantial risk of breast or other extra-colonic cancers¹¹. In a mouse FAP model, mice with an *Apc* mutation (*Apc^{Min}*) develop mammary tumours only on genetic backgrounds in which lethality of intestinal tumours is delayed¹². In this study, we found that I1307K carriers were more likely to report having first-degree relatives who had had breast cancer than were non-carriers. A large case series of Ashkenazi women with breast cancer also found a similar increase in risk for APC I1307K carriers¹³.

An association between I1307K and cancers other than CRC theoretically could reflect a more widespread biologic effect, subtle biases caused by unknown effects on cancer survival or study participation, or a chance finding. Although the pattern of uncommon extra-colonic tumours described in FAP kindreds^{14,15} was not seen in this study, slightly elevated ORs were noted for carriers and first-degree relatives for melanoma, bladder cancer and non-Hodgkin's lymphoma and in first-degree relatives for pancreatic cancer. Somatic *APC* mutations, as occur at increased frequency at the unstable homopolymer tract found in I1307K carriers, are frequently early events in the pathogenesis of CRC (ref. 16), and β -catenin, a protein whose role in signal transduction is modulated by interaction with APC, is mutant in CRC and melanoma^{17,18}. These findings are consistent with impaired function of the ubiquitously expressed APC protein leading to deregulation of transcriptional control of a variety of downstream targets and a generalized signalling defect resulting in uncontrolled cellular proliferation.

The combination of the results from our large community-based study, the case series examining CRC and breast cancer risk that have been described and the elucidation of the pathogenetic mechanism by which the I1307K allele leads to loss of *APC* gene function are all consistent with APC I1307K carriers having a modestly elevated risk for developing cancer. Nevertheless, the data presented here are not sufficient to determine the appropriateness, possible use and spectrum of genetic and clinical screening and intervention programs for I1307K. It is important to emphasize that the large majority of I1307K carriers will not develop colon or breast cancer, and that only a small proportion of Jewish individuals who develop these cancers will be carriers.

The prevalence and level of cancer risk conferred by I1307K are more similar to those of metabolic gene polymorphisms, such as NAT2, GSTM1 and CYP1A1, than to those associated with variant alleles of other genes more usually associated with inherited cancer syndromes^{19,20}. As is likely to be the case for other complex diseases, interactions with modifying genes and environmental influences may alter the chance that I1307K carriers will develop cancer. Identification of such genetic and environmental factors could offer substantial opportunities in efforts to better understand and control the development of malignancy.

Methods

Sample collection. Study subjects were initially recruited to determine the risk of breast and ovarian cancers associated with the *BRCA1* 185delAG mutation among Ashkenazi Jews³. Samples from 5,098 individuals who gave consent at the time of recruitment for studies of other genes were available for this study (approved by the NIH Office of Human Subjects Research). Blood droplets were collected onto Isocode paper collection cards (Schleicher and Schuell) and genomic DNA prepared as described and stored at -20°C . Subjects also completed a self-administered questionnaire that included items regarding cancer history for themselves and their first-degree relatives. Validation of medical diagnoses was not possible in this study. Following classification of any related volunteers into family sets, samples were made anonymous.

Genotype assignment. To obtain signals that were consistently strong enough for reliable analysis using small quantities of the impure DNA samples that were available, it was necessary to make modifications to the I1307K detection procedures². DNA amplification was made more robust and reproducible by the addition of T3 and T7 tails to the published APC sequences forming the following primers: APC-T3F, 5'-ATTAACCCTCACTAAAGG-GATGAAATAGGATGTAATCAGACG-3' and APC-T7R, 5'-TAATACGAC-TCACTATAGGGACTTCGCTCACAGGATCTTCAGC-3'. Amplification of genomic DNA samples was performed in a 96-well format containing positive and negative controls. Replicate membranes were made by spotting aliquots of PCR product (2.5 μl) onto positively charged nylon membranes (Schleicher and Schuell) using a Hydra 96 robotic sample dispenser (Robbins Scientific). Membranes were denatured with 0.5 M NaOH/1.5 M NaCl and then neutralized with 0.5 M Tris (pH 7.5)/1.5 M NaCl for 5 min. Inclusion of cresol red (2 mM) in the PCR amplification buffer aided visual inspection of deposition, denaturation and neutralization of the samples on the nylon membranes. DNA was covalently crosslinked to the nylon membrane by exposure to 120 mJ UV light (254 nm).

Nylon membranes were preincubated at 44 °C in hybridization solution (7% SDS, 0.25 M Na₃HPO₄, 1 mM EDTA and 10 mg/ml BSA) for 1 h before adding oligonucleotide probe (20 pmol) end-labelled with 20 µCi [γ -³²P]ATP. Preliminary experiments showed that the published I1307K ASO oligonucleotides produced lower intensity hybridization signals than those obtained with probes for other loci. An alternative pair of oligonucleotide probes complementary to the APC coding strand (APC-I1307K-wt, 5'-CTTTTCTTTTATTCTGC-3' and APC-I1307K-mut, 5'-CTTTTCTTTTCTGC-3') were found to give signals over 10-fold stronger than those obtained with the original probes complementary to the T-rich non-coding strand. Hybridization proceeded for 1–2 h in the presence of radiolabelled wild-type or mutant probe, also at 44 °C. Membranes were then rinsed briefly with 2×SSC/0.1% SDS at RT and washed in the same solution at 44 °C for 20 min. Signals were detected by autoradiography for up to 16 h.

Genotypes were assigned by comparing intensities of signal produced by wild-type and mutant allele-specific probes for each template. Each locus was scored independently by two investigators. PCR amplification and ASO hybridizations were repeated for all samples which on initial analysis were PCR failures, otherwise ambiguous, or scored as heterozygous or potentially homozygous for I1307K. Repeat tests were also performed for a random selection of 6% of wild-type samples. Discrepancies between scores obtained on the first and second round hybridizations were resolved by re-extracting DNA samples from archived blood spots and repeating PCR amplification and ASO hybridization. Genotypes for each of the two subjects scored as homozygous for the I1307K allele and a selection of samples including 23 heterozygotes and 11 wild-type individuals were confirmed by dideoxynucleotide sequencing.

Statistical analysis. Risks associated with I1307K were estimated from two separate groups of individuals. The first consisted of the 5,057 genotyped volunteers with information regarding their personal histories of cancer. The second group was the set of 26,467 first-degree relatives of the genotyped probands. Cancer risks in first-degree relatives were calculated based on family sets classified as either carrier, if they contained volunteer probands that were positive for I1307K, or non-carrier, if none of the geno-

typed family members had the allele. Survival analysis was performed using a set of non-overlapping first-degree relatives classified by the status of their genotyped kin. When a relative was related to more than one genotyped volunteer, they were assigned to carrier or non-carrier groups by an algorithm that maximized available information, analogous to a previous approach³.

Odds ratios, 95% CI and *P*-values for these ratios were calculated to assess the association between I1307K and malignancy in the volunteers and in the first-degree relatives of the volunteers. Because of their small number, I1307K homozygotes were grouped together for analysis with heterozygotes and treated as carriers for analysis.

Time to CRC and breast cancer in first-degree relatives of carriers and non-carriers were compared by the log-rank and Wilcoxon survival function tests; the log-rank test is more sensitive to departures from the null hypothesis at early ages, whereas the Wilcoxon test is more sensitive to departures at older ages. The kin-cohort method^{3,4} was used to estimate penetrance for CRC and breast cancer in inferred carriers and non-carriers among the set of first-degree relatives of genotyped probands. In the kin-cohort method, the cumulative risks of cancer in first degree relatives of carriers and non-carriers are seen as weighted averages of the risks in the carriers and non-carriers themselves, with the weights determined by mendelian rules of inheritance and the allele frequency; thus, more than 50% of first-degree relatives of carriers are carriers themselves, whereas a much lower percentage of first-degree relatives of non-carriers are carriers. Solving two equations in two unknowns allows estimation of genotype-specific cumulative risk by specific ages. Ninety-five percent confidence intervals for the cumulative risk were obtained using bootstrap methods.

Acknowledgements

We are grateful to B. Vogelstein, K. Kinzler and M. Redston for communication of unpublished results. F. Collins, M. Gail, R. Hoover, K. Offit, W. Foulkes, B. Vogelstein and G. Petersen all made helpful suggestions regarding the manuscript.

Received 23 April; accepted 4 August, 1998.

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