

**APC I1307K and the Risk of Prostate Cancer**

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**Abstract**

The kin-cohort design has been proposed as an alternative to traditional case-control and cohort measures to evaluate inherited susceptibility to cancer in population-based studies. Here, we used this design to evaluate inherited susceptibility to prostate cancer associated with APC I1307K using data from the Molecular Epidemiology of Colorectal Cancer study. Two techniques were used to compare the incidence of prostate cancer in APC I1307K carriers. First, we compared the incidence of prostate cancer in relatives of mutation carriers and noncarriers using standard techniques for survival analysis. Second, we used the marginal maximum likelihood method for kin-cohort analysis to infer the genotypes in the relatives. We also evaluated APC I1307K in 75 Ashkenazi Jewish individuals with prostate cancer from 27 families enrolled in the University of Michigan Prostate Cancer Genetic Study. We observed a slightly increased risk of prostate cancer in relatives of APC I1307K carriers, however, this difference was not statistically significant (hazard ratio, 1.6; 95% confidence intervals, 0.7-3.4). Similar conclusions were drawn using both techniques for kin-cohort analysis. APC I1307K was found in 7.4% of families genotyped, which is slightly higher than the allele prevalence reported in Ashkenazi Jews in the general population. In addition, we did not observe loss of heterozygosity at APC or a somatic mutation near APC I1307K using microdissected tumor DNA from mutation carriers enrolled in the Prostate Cancer Genetic Study. Overall, the evidence for an association between APC I1307K and prostate cancer is not compelling. APC I1307K is unlikely to play a clinically meaningful role in susceptibility to prostate cancer. (Cancer Epidemiol Biomarkers Prev 2006; 15(3):468–73)

**Introduction**

The low penetrance susceptibility allele, APC I1307K, is a well established risk factor for colorectal cancer (1-5). Several studies have also evaluated a potential role of APC I1307K in other cancers, including prostate cancer. Alterations in APC have been identified in both primary and metastatic prostate cancers, including both somatic alterations (6, 7) and promoter hypermethylation (8, 9). In addition to studies suggesting a role for APC in prostate cancer, two studies have looked specifically at APC I1307K and risk of prostate cancer. An increase in prostate cancer risk associated with APC I1307K was observed in a community-based study of Ashkenazi Jews (Washington Ashkenazi Study), although the confidence limits were wide due to the small number of prostate cancers reported (odds ratio, 2.0; 95% confidence intervals, 0.81-4.7; ref. 10). A second study using a case-only design was not able to directly estimate the risk conferred by the mutation, however, APC I1307K was reported to modify the association between body mass index and prostate cancer risk (11). The implications of this study are unclear because it seems unlikely that APC I1307K was considered a priori as an effect modifier of BMI and risk of prostate cancer. Nevertheless, APC I1307K remains an intriguing candidate allele for prostate cancer. The kin-cohort design was developed to permit estimation of penetrance associated with rare mutations that would be more generalizable to the overall population of mutation carriers than estimates from cancer families (12). If a comprehensive family history of cancer is collected from participants in a case-control study, this method also allows estimation of the risk of multiple types of cancer in a single study. To date, several methods for kin-cohort analysis have been developed. These methods include a simple incidence approach and the original kin-cohort design that both use Kaplan-Meier survival techniques (12), as well as several likelihood-based approaches (13-15). A preliminary technique that has often been used is a simple comparison of the incidence of cancer in relatives of carriers compared with the incidence of cancer in relatives of non-carriers (10, 12). This comparison is done using Kaplan-Meier survival curves and data collected for family members of study participants. If the proportional hazards assumption is met, Cox proportional hazards models can also be used to obtain risk estimates associated with a particular mutation or polymorphism. The kin-cohort design extends the incidence method by inferring the genotypes of the relatives using the genotype of the proband and the frequency of the mutation in the population. To address the conditional independence assumption that is required for kin-cohort analysis, Chatterjee and Wacholder developed a marginal likelihood approach that has the advantages of a full likelihood approach (14), yet is more robust in the presence of residual correlation between family members (13). Together, these techniques provide an opportunity for evaluating the potential role of APC I1307K in prostate cancer susceptibility using data from a population-based case-control study of colorectal cancer. Here, the simple incidence method and the marginal maximum likelihood method were implemented using data from the Molecular Epidemiology of Colorectal Cancer (MECC) study.

**Materials and Methods**

**Study Population.** The MECC study is a population-based case-control study of incident colorectal cancer in northern Israel. Cases eligible for participation included all individuals...
diagnosed with colorectal cancer between May 31, 1998 and March 31, 2004 who lived in a geographically defined area of northern Israel. Controls were identified from the same source population using the Clalit Health Services (CHS) database. The Clalit Health Service is the largest health care provider in Israel and covers ~70% of the persons at least 60 years old. Controls were individually matched to cases by exact year of birth, gender, primary clinic location, and Jewish versus Arab ethnicity. Potential controls were excluded if they had a prior history of colorectal cancer. Participants provided written informed consent at the time of enrollment. Participants were interviewed to assess information on ethnicity, personal and family history of cancer, reproductive history, medication use, personal medical history, health habits, and nutritional habits using a validated food frequency questionnaire. Diagnoses of colorectal cancer, made in six different hospitals in the region, were confirmed by standardized review of pathology slides by one pathologist at the University of Michigan. The Institutional Review Boards at the Carmel Medical Center and the University of Michigan approved all procedures.

Study Subjects for Kin-Cohort Analysis. A complete three-generation pedigree and family history of cancer were collected from participants in the MECC study during a structured in-person interview. Data on history of any type of cancer including age at diagnosis as well as vital status and current age/age at death were collected for grandparents, parents, siblings, offspring, and aunts/uncles/cousins. This analysis was limited to first-degree relatives (parents, siblings, and offspring) of participants because reporting of cancer is likely to be more accurate in first-degree relatives (16). The ethnicity of each proband was evaluated by assessing religious affiliation, self-described ethnicity, and country of birth of parents and grandparents. Ashkenazi Jewish heritage was determined as previously described (17).

Relatives were excluded for probands who had a proxy interview in which the proxy was not a relative (42 male first-degree relatives of 12 probands). Relatives were considered to be at-risk for prostate cancer until censoring due to diagnosis with prostate cancer or death due to any cause. Competing risks due to diagnosis of colorectal cancer were not considered in the analysis. Ages were imputed for individuals with information on vital status using the median age at follow-up for the relative type and vital status. Analyses were repeated excluding individuals with imputed ages to ensure that the results were not influenced by imputation. Relatives with unknown vital status were excluded from the analyses. Participants were asked to report age at cancer diagnosis for each relative. When age at diagnosis was not provided, current age or age at death was used as a proxy for age at diagnosis (13 prostate cancers).

Prostate Cancer Samples. Genomic DNA was available for 75 Ashkenazi Jewish individuals from 27 families enrolled in a separate prostate cancer study. These individuals were participants in the University of Michigan Prostate Cancer Genetics (PCPG) Study which is a family-based study of prostate cancer susceptibility. The enrollment criteria for this study includes families with two or more living family members with prostate cancer and families with at least one individual with early-onset prostate cancer (age at diagnosis ≤55 years). PCPG participants provide DNA samples as well as access to medical records and pathologic specimens. All study consent forms and protocols have been reviewed and approved by the Institutional Review Board at the University of Michigan Medical School. Study participants were asked to describe their own ethnicity as well as the country or origin of their four grandparents. An entire pedigree was considered to be Ashkenazi if at least one participating family member described themselves to be Ashkenazi Jewish or if there was evidence of Eastern European Jewish heritage in grandparents of at least one affected family member.

Genotyping Methods. The APC I1307K mutation was identified as previously described (1, 18). Briefly, genomic DNA was extracted from blood using the Puregene kit (Gentria Systems Inc., Minneapolis, MN). Twenty nanograms of genomic DNA were amplified using the following primers: forward 5′ TCC ACA CCT TCA TCT AAT GCC, and reverse 5′ TAA ACT AGA ACC CTG CAG TCT GC. PCR amplification was done in a 20 μL reaction containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L MgCl2, 0.2 mmol/L of each deoxynucleotide triphosphate, 0.1 μmol/L of each primer, and 1 unit of AmpliTaq (Applied Biosystems, Foster City, CA). Following PCR amplification, allele-specific oligonucleotide hybridization was then done using 32P end-labeled probes specific for the wild-type (5′ CTG TTC TTT TAT TTC TGC) and mutant (5′CTT TTC TTT TTT TTC TGC) alleles. Results were double-scored and double-entered to ensure data quality.

Loss of Heterozygosity Assays. Loss of heterozygosity (LOH) at APC was assessed using the microsatellite marker, D5S5346. LOH assays were done as follows using microdissected DNA from paraffin-embedded normal and tumor samples. One microliter of microdissected DNA was amplified using the following primers: forward 5′ ACT CAC TCT AGT GAT AAA TCG, and reverse 5′ AGC AGA TAA GAC GAT ATT ACT AGT T. The forward primer was end-labeled with 32P and PCR amplification was done with an annealing temperature of 57°C in a 20 μL reaction containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L MgCl2, 0.2 mmol/L of each deoxynucleotide triphosphate, 0.2 μmol/L of each primer, and 1 unit of AmpliTaq Gold (Applied Biosystems). PCR product was run on a 6% polyacrylamide gel at 65 W. Following electrophoresis, gels were exposed to film at ~80°C for 8 to 16 hours. LOH was determined using the standard criteria of a 50% reduction in one of the alleles in the tumor DNA as compared with the normal DNA. Films were double-scored and double-entered to minimize errors and ensure data quality.

Somatic Mutation Analysis. We evaluated the presence of somatic mutations in the sequence surrounding APC I1307K as previously described (1). A 371 bp PCR fragment surrounding APC I1307K was amplified in microdissected tumor DNA from mutation carriers and mutations were identified by direct sequencing. The primers and reaction conditions described above (APC I1307K genotyping) were used. PCR product was purified using the Roche High Pure PCR Purification Kit (Roche Diagnostics Corporation, Indianapolis, IN). Sequencing was done on an ABI 3100 sequencer. Mutations were detected using Mutation Surveyor software (Softgenetics, Inc., State College, PA), and confirmed by visual inspection by two readers (J.N. Poynter and L.P. Tomsho).

Statistical Methods

Assumptions. Although the methods for estimation vary between the different techniques for kin-cohort analyses, similar assumptions are required for the overall class of methods. These assumptions include conditional independence of the relatives’ phenotypes given their genotypes at the locus of interest, specified mode of inheritance of susceptibility, homogeneity of risk, constant frequency of the mutant allele, and accurate reporting of cancer history by probands (12, 19). The conditional independence assumption is likely to have the most serious effect. Gail et al. investigated the effect of residual familial correlation using simulations and concluded that violation of the conditional independence assumption seems to increase the estimates for penetrance in mutation carriers and decrease the penetrance estimates for noncarriers (19).
For these analyses, an autosomal dominant mode of inheritance was assumed for APC I1307K. This is likely to be an appropriate mode of inheritance for this mutation because molecular evidence shows that heterozygous mutation carriers exhibit an increased frequency of somatic mutations (1). Data from the controls enrolled in the MECC study were used to estimate the mutation frequency.

The analysis for prostate cancer was conducted using all male first-degree relatives. Analyses were not done separately for relatives of cases and relatives of controls due to limited sample size. This requires the assumption that the mutation of interest is statistically independent of any unknown risk factors and that there are no multiplicative interactions with these unmeasured risk factors (20).

**Incidence Method.** Kaplan-Meier analyses for the incidence method were done using SAS v8.2 for Windows (SAS Institute, Cary, NC). Person years of risk for the cancer of interest were obtained from either the age at diagnosis, age at death, or current age for each relative. Standard Kaplan-Meier curves using the product limit method were used to estimate the incidence of cancer in the relatives of carriers and noncarriers. A graphical check of the proportional hazards assumption was done by plotting the log of the negative log of the survival function against log time. If the lines were parallel, the hazards were assumed to be proportional and Cox proportional hazards models were used to estimate a summary hazard ratio.

**Marginal Maximum Likelihood Method.** To address the conditional independence assumption, Chatterjee and Wacholder developed a marginal likelihood approach (13), that has the advantages of a full likelihood approach (14), yet is more robust in the presence of residual correlation between family members.

In the marginal likelihood method, the \( n_i \) relatives of the \( i \)th participant are treated separately, so a family of \( (n_i + 1) \) members with one participant and \( n_i \) relatives is broken into \( n_i \) pseudofamilies. The probability of the genotype of the probands conditional on phenotype is a function of the probability mass function of the phenotype and the carrier frequency of the mutation of interest. The marginal maximum likelihood method was implemented using functions written for MatLab v. 7.0.1 for Windows (MathWorks, Natick, MA; functions were written by Dr. Nilanjan Chatterjee, National Cancer Institute). The program uses piecewise exponential hazards models to obtain cumulative survival and hazard estimates. Data were loaded into MatLab using a series of column vectors for the person-years at risk, an indicator variable for disease status, mutation status of the probands, and an indicator variable for relative type (1 = parent/offspring, 2 = sibling) for each relative reported. Other input variables included the mutation frequency in the population as well as the knots for the piecewise constant hazard function. For a fixed value of the mutation frequency, the marginal likelihood was maximized using an expectation-maximization algorithm. Cumulative survival estimates are provided for each knot for both carriers and noncarriers. A separate MatLab function was used to compute bootstrap estimates for the hazard estimates. Bootstrap resampling was done 1,000 times, and the estimates were then used to generate confidence intervals and SEs.

**Standardized Incidence Ratios.** Accurate reporting of family history is an important element in kin-cohort analysis. Because validating family histories for all relatives of MECC participants was not feasible, we used standardized incidence ratios as a crude estimate to evaluate potential underreporting of family history. The expected number of cases of each type of cancer in the relatives was estimated using the age-standardized population rates with the number of relatives in each age category as the weight. The standardized incidence ratio was then estimated by comparing the observed and expected number of cases. Determining the appropriate rates to be used as the reference is challenging for kin-cohort analysis because affected individuals are from three different generations and will typically have been diagnosed during different time periods. This is especially challenging in Israel with its profound history of recent immigration from many different countries. For these analyses, standardized incidence ratios were calculated using both the 1982 and 1986 (21) and the 2000 Israeli population age-standardized rates (22).

**Results**

Of the 4254 participants in the MECC study (2,152 cases and 2,102 controls), 24 did not provide family history data for any first-degree relatives, 4 were adopted and did not provide family history for any biological relatives, 191 did not have sufficient DNA for I1307K testing, and 3 did not report any first-degree male relatives. Interview data were excluded for an additional 11 participants in which the interview was completed by a proxy who was not a relative. Thus, family history data from 4,021 individuals were available for this analysis. Ages were missing for 1,813 first-degree relatives. Ages were imputed for 1,592 of these individuals as described previously and the remaining 221 with unknown vital status were excluded. After excluding relatives for whom no information on vital status or age was available, 16,023 first-degree male relatives were available for this analysis. The number of relatives available for analyses of APC I1307K by relative type and mutation status is shown in Table 1.

Eighty-two cases of prostate cancer were reported in first-degree male relatives of MECC participants. One family was

<table>
<thead>
<tr>
<th>Relative type</th>
<th>Total</th>
<th>Alive</th>
<th>Dead</th>
<th>Person-years</th>
<th>Prostate cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>All MECC participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>3,961</td>
<td>201 (5.1)</td>
<td>3,760 (94.9)</td>
<td>269,313</td>
<td>43 (1.1)</td>
</tr>
<tr>
<td>Brother</td>
<td>6,240</td>
<td>3700 (59.3)</td>
<td>2,540 (40.7)</td>
<td>364,785</td>
<td>34 (0.5)</td>
</tr>
<tr>
<td>Son</td>
<td>5,822</td>
<td>5570 (95.7)</td>
<td>252 (4.3)</td>
<td>228,109</td>
<td>1 (0.02)</td>
</tr>
<tr>
<td>Total</td>
<td>16,023</td>
<td>9471 (59.1)</td>
<td>6552 (40.9)</td>
<td>802,007</td>
<td>78 (0.5)</td>
</tr>
<tr>
<td>APC I1307K Carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>260</td>
<td>10 (3.9)</td>
<td>250 (96.1)</td>
<td>17,722</td>
<td>5 (1.9)</td>
</tr>
<tr>
<td>Brother</td>
<td>287</td>
<td>159 (55.4)</td>
<td>128 (44.6)</td>
<td>16,731</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Son</td>
<td>320</td>
<td>302 (94.4)</td>
<td>18 (5.6)</td>
<td>12,989</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>867</td>
<td>471 (53.4)</td>
<td>396 (45.7)</td>
<td>47,442</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Noncarriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>3,701</td>
<td>191 (5.2)</td>
<td>3,510 (94.8)</td>
<td>251,591</td>
<td>38 (1.0)</td>
</tr>
<tr>
<td>Brother</td>
<td>5,953</td>
<td>3,541 (59.5)</td>
<td>2,412 (40.5)</td>
<td>348,054</td>
<td>32 (0.5)</td>
</tr>
<tr>
<td>Son</td>
<td>5,502</td>
<td>5,268 (95.7)</td>
<td>234 (4.3)</td>
<td>215,120</td>
<td>1 (0.02)</td>
</tr>
<tr>
<td>Total</td>
<td>15,156</td>
<td>9,000 (59.2)</td>
<td>6,156 (40.8)</td>
<td>814,765</td>
<td>71 (0.4)</td>
</tr>
</tbody>
</table>
excluded from the analysis because prostate cancer was reported in all male first-degree relatives. A diagnosis of prostate cancer could not be confirmed in the one medical record that could be traced in this family (benign prostatic hypertrophy, not cancer), and this family was removed from the analysis due to confirmed inaccuracy of the family history portion of the interview. After excluding this family, a total of 78 prostate cancer cases were available for this analysis, including 7 cases in relatives of APC I1307K carriers and 71 cases in relatives of noncarriers.

Most of the participants who reported prostate cancer in a relative had only one affected relative, whereas four noncarriers reported two affected relatives (Table 2). The incidence of prostate cancer is slightly higher in the relatives of carriers between the ages of 60 and 80, and then is markedly higher after the age of 80 (Fig. 1A; Table 3). A summary hazard ratio was computed for the incidence data, and we observed a nonsignificant increase in risk of prostate cancer (hazard ratio, 1.6; 95% confidence intervals, 0.7-3.4). We also observed a slightly increased incidence of prostate cancer in the I1307K carriers using the marginal maximum likelihood method up to age 80 (Fig. 1B); however, the confidence intervals overlap the incidence curve for noncarriers.

The number of cases of prostate cancer reported in the study is much lower than expected. Rates of prostate cancer in the Israeli population were used to determine the expected number of cases for our population. Both 1982 and 1986 rates of prostate cancer as well as the 2000 rates were used as the standard population because fathers of probands would have been diagnosed with prostate cancer during a different time period than brothers or sons of probands. Using the 1982 and 1986 rates of prostate cancer in the Israeli population, the standardized incidence ratio for relatives of all probands was 0.54. Standardized incidence ratios were also calculated by relative type under the assumption that fathers of probands would have been diagnosed ~20 years before brothers of probands. Using the 1982 and 1986 rates as a standard, the standardized incidence ratio for the fathers of probands was 0.49. Using the 2000 rates as a standard, the standardized incidence ratio for the brothers of probands was 0.20, whereas the standardized incidence ratio for sons of probands was 0.27.

Participants were unaware of their mutation status, so we would be unlikely to observe differential reporting by carrier status. In fact, we looked at standardized incidence ratios by carrier status and we did not detect significant differences (data not shown). These calculations are obviously limited by the lack of an appropriate reference population; however, they do suggest that there is a significant amount of underreporting of prostate cancer by participants in the MECC study assuming the rates from the Israeli Cancer Registry are representative of rates of cancer in the relatives.

We also genotyped 75 individuals from 27 U.S. Ashkenazi families enrolled in a hereditary prostate cancer study to evaluate the frequency of the APC I1307K mutation. The average number of confirmed affected individuals per pedigree was 2.6 (range 1-6). Fifty-two of the 75 individuals genotyped had been diagnosed with prostate cancer and the average age of prostate cancer diagnosis was 62.0 ± 10.2 years. The APC I1307K mutation was observed in two men with prostate cancer (diagnosed at ages 50 and 57) from two different families. In one of the families, an affected brother was also tested and was shown not to carry the APC I1307K mutation. Overall, we did not observe a substantial increased frequency of the mutation compared with what we would expect in the Ashkenazi Jewish population (7.4% of families carried the mutation compared with 5-8% reported in the control groups from previous studies; refs. 1-5). Additionally, our limited investigation of the molecular profile of selected familial prostate cancers does not provide evidence for a functional consequence of APC I1307K in prostate cancer. LOH was not observed at D5S346 in the two prostate tumors from individuals who carried APC I1307K (data not shown). Similarly, we did not observe a somatic mutation in the one tumor in which DNA was adequate to permit sequencing (data not shown). DNA from the second prostate tumor with APC I1307K failed to amplify, therefore, somatic mutation results are not available for this tumor.

### Table 2. Aggregation of prostate cancer in male relatives of MECC participants

<table>
<thead>
<tr>
<th>First-degree relatives with prostate cancer</th>
<th>APC I1307K carriers</th>
<th>Noncarriers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>256 (97.3)</td>
<td>3,690 (98.2)</td>
<td>3,946</td>
</tr>
<tr>
<td>1</td>
<td>7 (2.7)</td>
<td>63 (1.7)</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>4 (0.1)</td>
<td>4</td>
</tr>
</tbody>
</table>

### Discussion

Overall, these data do not provide compelling evidence to support an association between prostate cancer and APC I1307K, although the small number of cases limits our ability to
Table 3. Incidence of prostate cancer by APC I1307K status

<table>
<thead>
<tr>
<th>Age 60</th>
<th>Age 70</th>
<th>Age 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarriers (incidence)</td>
<td>0.1% (0.0-0.2)</td>
<td>0.7% (0.4-0.9)</td>
</tr>
<tr>
<td>Noncarriers (MMLE)</td>
<td>0%</td>
<td>2.1% (0.2-4.9)</td>
</tr>
<tr>
<td>Carriers (incidence)</td>
<td>0.3% (0.0-0.7)</td>
<td>1.2% (0.0-2.3)</td>
</tr>
<tr>
<td>Carriers (MMLE)</td>
<td>0%</td>
<td>2.1% (0.2-4.9)</td>
</tr>
</tbody>
</table>

draw definitive conclusions. The age-specific incidence of prostate cancer was slightly higher in the mutation carriers for the comparison of incidence in the relatives as well as for the kin-cohort technique, but the confidence limits are quite wide due to the small number of events. The only community-based study to date that has evaluated the association between APC I1307K and prostate cancer suggested an increased risk of prostate cancer (odds ratio, 2.0; 95% confidence intervals, 0.84-4.2; ref. 10). However, this article also reported the odds ratio using data from first-degree relatives of the study participants, with evidence for a much weaker association (odds ratio, 1.2; 95% confidence intervals, 0.83-1.8). Using traditional case-control measures for the MECC data, participants who carry the APC I1307K mutation were somewhat more likely to report at least one relative with prostate cancer (odds ratio, 1.5; 95% confidence intervals, 0.68-3.3). Data from the community-based study and from the MECC study do not support a substantial risk of prostate cancer in individuals with APC I1307K. In addition, we were able to evaluate the APC I1307K mutation in 27 Ashkenazi families with familial prostate cancer, with no evidence to support an increased frequency of APC I1307K. Limited molecular analyses also do not provide evidence for a functional consequence of APC I1307K in prostate tumors, although we did not have the power to exclude a functional role.

Although the APC I1307K mutation does not seem to strongly increase susceptibility to prostate cancer, several studies have suggested a potential role for APC in prostate cancer. In a study evaluating known or suspected tumor suppressor genes in prostate cancer samples, LOH was observed at APC in three out of seven informative cases (7). Similarly, a second study also found LOH at APC in 3 out of 15 prostate cancers, and all three tumors were metastatic (6). In addition to somatic alterations in APC, promoter hypermethylation at APC has also been described in prostate tumors. Hypermethylation at APC has been reported in prostate cancers with frequencies ranging from 56.8% to 90% (9, 23, 24). In addition, hypermethylation at APC, in addition to hypermethylation at GSTP1 and PTGS2 is correlated with prognostic factors for prostate cancer including Gleason score (23). These data suggest that although the APC I1307K polymorphism does not seem to play an important role in inherited susceptibility to prostate cancer, it is possible that APC may play some role in prostate tumorigenesis. Due to limited number of tumor tissue samples, we were unable to evaluate promoter hypermethylation of APC in the prostate tumor samples, so we are unable to rule out the possible inactivation of APC through this mechanism.

A key element in the kin-cohort study is accurate reporting of family history by the probands. Several studies have attempted to quantify how accurately individuals report family history of cancer. A study of families referred to a genetic counseling clinic indicated that individuals report history of cancer relatively accurately in first-degree relatives (83% of primary cancer sites identified; ref. 16); however, these individuals may be more aware of family cancer history because they have a stronger family history than the general population. In a population-based study, high sensitivities of reporting for breast (83%), colorectal (73%), and prostate (70%) cancers were observed, with no significant difference between reporting in cases versus controls (25). Another methodologic study within a case-control study reported a lower sensitivity in reporting of colorectal cancer in first-degree relatives (0.566 and 0.529 in cases and controls, respectively), and again observed no difference in reporting between cases and controls (26). Low accuracy in reporting of family cancer history would be a serious drawback for kin-cohort analysis, although any resulting bias would most likely be nondifferential because the probands were unaware of their mutation status. Although we were not able to validate family history information in the MECC study, standardized incidence ratio provide evidence that we are likely to have significant underreporting of prostate cancer. If the underreporting is truly nondifferential, estimates of association between APC I1307K and prostate cancer would be attenuated.

There are two main methodologic limitations of the kin-cohort design. The first limitation is lack of precision due to the small number of cancers reported in relatives and the lack of direct measurement of carrier status in the relatives. The second major limitation is misclassification of cancer status and age at diagnosis in the relatives by the participants in the case-control study. The first limitation could potentially be addressed by acquiring genotype information for some of the relatives of the participants in the study. Gail et al. have described this as a potential way to reduce the sample size requirement in the kin-cohort design (14). In order to increase the number of events, the study could be done in a population with a higher incidence of the disease of interest, although this would limit the generalizability of the results. In order to obtain more accurate family history information, cancer history could be validated for relatives of the study participants. These options are likely to be cost-prohibitive and would eliminate one of the key advantages of the kin-cohort design, which is the ability to perform the analyses in the context of a previously existing study without the cost of collecting additional data.

Although the potential for estimating penetrance for a rare mutation using a population-based design makes the kin-cohort technique a potentially attractive study design, the measurement error inherent in this design may limit its utility. Accurate reporting of family history is unlikely to differ by mutation status, which means that observed estimates would be attenuated toward the null. In the case of a low-penetrance susceptibility allele, this may result in the inability to identify true associations. Although clinic-based designs have the potential to overestimate the penetrance due to selection of families with multiple affected individuals, a simulation-based study indicated that whereas other shared genetic and environmental risk factors had the potential to bias risk estimates upward, the bias was small compared with the SE in the estimates (27). In this same study, SEs were approximately twice as large using population-based designs as compared with family-based studies.

The present study has several limitations including lack of details regarding the prostate cancer diagnosis in the relatives. We are unable to determine if prostate cancers were diagnosed due to routine screening or due to symptoms. However, because routine prostate-specific antigen screening is not done in Israel and all individuals have health care coverage, our results are unlikely to be biased due to inadequate access to healthcare resources. In addition, the history of prostate cancer in relatives was ascertained through an open-ended question which may partly explain why the number of prostate cancers reported by MECC participants was lower than expected based on the population rates.

Despite these limitations, the population-based results presented here do not provide compelling evidence for the role of APC I1307K in the development of prostate cancer. In addition, data from a set of Ashkenazi Jewish families with prostate cancer do not support an increased frequency of APC I1307K in prostate cancer cases or somatic alterations that...
would suggest a biological role for their pathogenesis. Kin-cohort analyses using family history data provided by participants in the MECC study and data from prostate cancer families do not support a clinically meaningful increase in risk of prostate cancer in APC I1307K carriers.

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