The *APC* E1317Q Variant in Adenomatous Polyps and Colorectal Cancers[1]

D. Hahnloser, G. M. Petersen, K. Rabe, K. Snow, N. M. Lindor, L. Boardman, B. Koch, D. Doescher, L. Wang, K. Steenblock, and S. N. Thibodeau[2]

Division of Colon and Rectal Surgery [D. H.] and Departments of Health Science Research [G. M. P., K. R.], Medical Genetics [K. S., N. M. L.], Gastroenterology [L. B.], and Laboratory Medicine and Pathology [D. H., B. K., D. D., L. W., K. S., S. N. T.], Mayo Clinic, Rochester, Minnesota 55905

Abstract

Genetic susceptibility may play a role in many colorectal cancers (CRCs). Known syndromes such as familial adenomatous polyposis and hereditary nonpolyposis CRC account for <5% of CRCs. The germ-line missense variant of the *APC* gene, E1317Q, has been proposed to confer a risk for colonic adenomatous polyps (adenomas), but not for CRCs in the general population. These findings are contradictory and controversial. In the present study, 608 cases (377 patients with CRC, 145 patients with 4–100 lifetime adenomas, and 86 with ≤3 lifetime adenomas), and 679 controls (362 spouses and 317 patients with normal colonoscopy) were screened for the *APC* E1317Q variant. The frequency of heterozygotes for E1317Q among patients with CRC (2.4%), patients with 4–100 adenomas (1.4%), and ≤3 adenomas (3.5%) did not differ from spouse controls (2.8%). When CRC patients were examined by DNA mismatch repair status, age at onset (≤age 50 *versus* >50), or family history of CRC, no differences in the frequency of E1317Q were found. The *APC* variant E1317Q does not appear to be associated with increased risk for colorectal neoplasia in the general population. However, when we used normal colonoscopy controls (E1317Q carrier frequency = 0.3%), the prevalence of E1317Q was significantly increased in CRC patients, in patients with ≤3 adenomas, and in CRC patients with intact mismatch repair status, suggesting a possible role for E1317Q in colorectal tumorigenesis. These results underscore the importance of carefully defining the controls to be used in comparisons of allele frequencies.

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[2] To whom requests for reprints should be addressed, at Department of Laboratory Medicine and Pathology, Mayo Clinic, Hilton 920, 200 First Street SW, Rochester, MN 55905. Phone: (507) 284-9185; Fax: (507) 284-0670; E-mail: sthibodeau@mayo.edu.

Introduction

Approximately 15–20% of CRC[3] exhibits familial aggregation (1, 2). The two well-described autosomal dominant syndromes of hereditary nonpolyposis colorectal cancer and FAP account for ~5% and 1% of the overall CRC burden (2–5). The spectrum of FAP disease activity ranges from attenuated FAP with <100 recurrent colonic adenomatous polyps (adenomas) and a later age of onset for colon cancer (6, 7) to classical FAP with hundreds to thousands of adenomas and a nearly 100% risk for colon cancer by the age of 40. FAP is caused by germ-line truncating mutation in the *APC* tumor suppressor gene located on chromosome 5 (8–11).

In addition to highly penetrant mutations, potential low-penetrance missense variants of *APC* have been reported. *APC* N1822V was suggested as a mutation that might affect the colorectal phenotype (12), but a subsequent study suggests it is a common polymorphism (13). In contrast, *APC* I1307K has been associated with an increased risk for colon cancer and adenomas in people of Ashkenazi descent (14–16). The I1307K variant was reported to be carried by 6% of Ashkenazi controls, 10% of Ashkenazi CRC cases, and 28% of Ashkenazi CRC cases who had a family history of CRC (14).

The *APC* variant, E1317Q, was first described in 1996 in a family with late onset CRC (17). Frayling *et al.* (15) reported the E1317Q variant in 2 of 134 (1.4%) patients with multiple adenomas (≥3 adenomas without the classical phenotype of FAP) and in 2 of 30 (6.7%) CRC patients, but in none of 80 controls. By combining the data on these 80 controls with the data of White *et al.* (Ref. 17; $n = 133$), the finding that E1317Q confers increased risk in individuals for CRC or adenomas (4 of 164; 2.4%) reached statistical significance ($P = 0.035$). In another study, Lamlum *et al.* (18) found the E1317Q variant in 7 of 164 (4.3%) patients with multiple (3–100) colorectal adenomas compared with 2 of 503 (0.4%) population-based controls [which included the 133 controls used previously by White *et al.* (17) and 80 controls used by Frayling *et al.* (15)]. In that study, there was a significant difference in E1317Q frequencies between cases and controls ($P < 0.001$). These two studies concluded that the E1317Q allele contributes to a predisposition to colorectal adenomas and carcinoma, but with low and variable penetrance. In contrast, several other recently published analyses (19–21) did not find that E1317Q was associated with significant increased risk of CRC. Popat *et al.* (19) found that 2 of 364 (0.6%) CRC patients (age <55 years) and 2 of 290 (0.7%) healthy spouse controls carry the E1317Q variant ($P > 0.1$), whereas none of 194 Swedish CRC patients demonstrated the E1317Q polymorphism (20). In a study reported by Figer *et al.* (21), no differences in E1317Q frequen-

[3] The abbreviations used are: CRC, colorectal cancer; FAP, familial adenomatous polyposis; MMR, mismatch repair; APC, adenomatous polyposis coli.

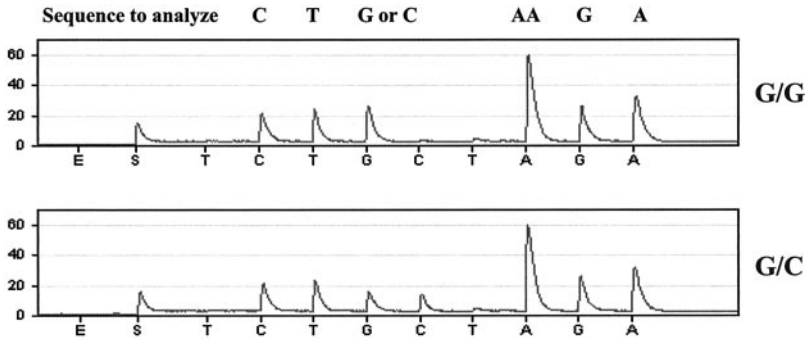

Fig. 1. Pyrogram result from pyrosequencing. The two genotypes (G/G or G/C) are represented.

cies were found, after comparing CRC patients (4 of 85; 4.7%), polyp patients (1 of 21; 4.8%), and controls (2 of 148; 2.7%).

These conflicting reports appear to suggest that E1317Q is a colorectal adenoma predisposition variant, but apparently does not confer a risk for CRC in the general population. That a genetic predisposition for adenomas would not result in an increased risk for CRC is inconsistent with our current understanding of the adenoma-carcinoma process that results in the majority of CRCs (22, 23). Therefore, the aim of this study was to determine the prevalence of the *APC* E1317Q variant in patients with CRC, patients with 4–100 lifetime adenomas, and in patients with 1–3 lifetime adenomas. We used samples of two types of controls: spouses of CRC patients and patients who were confirmed not to have CRC by a colonoscopy screening examination.

Materials and Methods

This study was reviewed and approved by the Mayo Clinic Institutional Review Board, which is responsible for oversight of human subjects research.

Patient Population. Clinical data, family history of CRC, and peripheral venous blood samples were prospectively obtained from five groups of unrelated Caucasian subjects through Mayo Clinic, as follows.

Cases: Group 1. This group consisted of consecutively collected surgical cases of CRC ($n = 377$). All of the cancers were examined for evidence of defective DNA MMR as described elsewhere (24). Of the 377 cases, 314 (83%) showed intact MMR, whereas 63 (17%) demonstrated defective MMR.

Cases: Group 2. Group 2 consisted of patients with 4–100 lifetime adenomas ($n = 145$). These patients included patients who were referred to Mayo Clinic for *APC* mutation testing ($n = 620$), and where germ-line mutations in *APC* were excluded by a protein truncation test for exon 15 and conformation sensitive gel electrophoreses for exons 1–14.

Cases: Group 3. This group consisted of Mayo Clinic patients with 1–3 lifetime adenomas and no personal history of CRC ($n = 86$).

Controls: Group 4. Group 4 consisted of spouses of Mayo Clinic CRC patients who participated in an international cooperative registry (Cooperative Family Registry for Colon Cancer Studies; $n = 362$). The registry CRC patients were not included in group 1 above.

Controls: Group 5. Group 5 consisted of patients with normal colon mucosa at screening colonoscopy and no personal history of CRC or adenomas ($n = 317$).

Clinical and family history data were coded and entered into a database. Subjects were coded as family history positive if they reported at least one first-degree relative with a history of CRC. We had previously excluded spouse controls from this study if the only first-degree relative they reported was an offspring whose other parent was a CRC proband in the registry. Blood samples were processed and stored at -70^{o} until retrieved for genotyping.

Genotyping Method. Genomic DNA was isolated using the Puregene nucleic acid isolation kit. Amplification was performed using 3 μl of 50 ng/μl DNA template in a 25 μl PCR mixture containing 0.2 μl Taq Gold polymerase, 1.25 μl of each forward and reverse primers (10 μM), 2 μl deoxynucleotide triphosphates (1.25 μM), and 2 μl $MgCl_2$ (25 μM), in a 10× buffer. PCR was performed in Perkin-Elmer Gene-Amp PCR System 9600 thermal cycler (Perkin-Elmer, Branchburg, NJ) under the following condition: 94° for 12 min followed by 35 cycles of 94° for 20 s, 51° for 30 s, and 72° for 1 min, followed by one 10 min extension at 72°. A 147-bp product spanning a portion of exon 15 was obtained. The primers needed for the PCR included the forward primer 5′-AGCTGAAGATGAAATAG- 3′ and the reverse primer 5′- AACTAGAACCCTGCA -3′.

E1317Q genotyping was performed on the PCR product by Pyrosequencing (Pyrosequencing AB, Uppsala, Sweden;[4] Ref. 25) using 1.25 μl (20 μM) sequencing primer 5′- AGC TGA AGA TGA AAT AG -3′. Pyrogram results from Pyrosequencing for the two genotypes (G/G or G/C) are represented in Fig. 1. Investigators reading these results were blinded to the patient classification. Patients were classified as carriers if they were found to have G/C genotype.

Statistical Analysis. χ^2 tests and t tests were initially performed to assess differences between patient groups for sex and age, respectively. Because differences were found to exist, age- and sex-adjusted odds ratios and 95% Wald confidence intervals were computed for comparisons of the E1317Q variant frequency between patient groups using logistic regression. Assuming $\alpha = 0.05$ and $\beta = 0.8$ and previously reported frequencies of the E1317Q variant, the study was determined to have sufficient power to detect statistically significant differences between cases and controls. Statistical analyses were conducted using Statistical Analysis Software (SAS), version 8 (SAS Institute, Cary, NC).

Results

A total of 1287 participants (608 cases and 679 controls) were screened for the *APC* E1317Q variant. Subject characteristics

[4] Internet address: http://www.pyrosequencing.com.

Table 1 Description of study samples

	n	APC E1317Q Carriers (%)	% Males	Mean Age ± SD (range)	Positive family history of CRC[a] (%)
Cases					
Surgical CRC[b] cases	377	9 (2.4%)	58.9	66.1 ± 11.5 (28–92)	14.3
4–100 lifetime adenomas	145	2 (1.4%)	57.9	46.2 ± 12.9 (20–74)	NA
1–3 lifetime adenomas	86	3 (3.5%)	73.3	64.5 ± 11.5 (24–87)	20.3
Controls					
Spouses	362	10 (2.8%)	42.3	56.5 ± 11.0 (22–82)	10.4
Normal colonoscopy	317	1 (0.3%)	54.6	59.4 ± 11.3 (20–82)	33.5

[a] Positive family history, having one or more first degree relatives with CRC.
[b] CRC, colorectal cancer; NA, not available.

Table 2 Comparison of APC E1317Q carrier proportions among case and control groups

	n	APC E1317Q		Comparison to spouse control group		Comparison to normal colonoscopy group	
		Allele frequency	Carriers (%)	OR[a] (95% CI)	*P*	OR (95% CI)	*P*
Cases							
Surgical CRC cases, total	377	0.012	2.4	0.838 (0.311, 2.260)	0.727	9.015 (1.104, 73.588)	0.040
Surgical CRC cases with intact DNA MMR	314	0.014	2.9	0.974 (0.363, 2.614)	0.958	10.268 (1.263, 83.461)	0.029
Surgical CRC cases with defective DNA MMR	63	0.000	0.0	[b]	—	[b]	—
4–100 lifetime adenomas	145	0.007	1.4	0.523 (0.102, 2.676)	0.436	3.693 (0.244, 56.018)	0.346
1–3 lifetime adenomas	86	0.017	3.5	1.164 (0.284, 4.777)	0.833	16.590 (1.539, 178.814)	0.021
Controls							
Spouses of CRC patients	362	0.014	2.8	—	—	9.226 (1.163, 73.215)	0.036
Normal colonoscopy patients	317	0.002	0.3	—	—	—	—

[a] OR, age- and sex-adjusted odds ratios; CI, Wald confidence interval; MMR, mismatch repair; CRC, colorectal cancer.
[b] OR and CI unestimable because of lack of sample variability.

and the frequency of E1317Q carriers detected in each of the five groups are summarized in Table 1. Whereas there were no differences in gender distribution among the surgical CRC cases, the group with 4–100 adenomas, and the normal colonoscopy controls, with slightly over half the samples being males, the group of patients with 1–3 adenomas were mostly male (73.3%). Not unexpectedly, there were more females in the spouse control group (57.7%). The mean ages of the control groups were found to differ from the case groups, so all of the additional analyses were age- and sex-adjusted. Family history data were unavailable for the group with 4–100 adenomas. We observed that 14.3% and 20.3% of the CRC cases and the group with 1–3 adenomas, respectively, reported a first-degree relative with CRC. As expected, a high proportion, 33.5%, of the normal colonoscopy controls reported a family history of CRC, because this is an indication for this procedure. Among the spouse controls, 10.4% reported a positive family history.

Results of the APC E1317Q genotyping analyses are shown in Table 2. There were no statistically significant differences in E1317Q carrier frequencies between any of the case groups and the spouse controls. We additionally subdivided CRC cases according to their MMR status. None of the CRC cases with defective MMR were carriers of the variant, and there were no differences in either MMR subgroup compared with the spouse controls. However, when the intact MMR cases and the group with 1–3 adenomas were compared with the normal colonoscopy controls, significant differences in E1317Q frequencies were detected (Table 2). Individuals with CRC and those with ≤3 adenomas demonstrated a statistically significant higher E1317Q frequency compared with the normal colonoscopy control group ($P = 0.04$ and 0.021, respectively). Among the CRC cases, the E1317Q allele was detected more frequently in individuals with tumors with intact MMR compared with normal controls ($P = 0.029$). The E1317Q frequencies between the group with 4–100 adenomas and the normal colonoscopy controls were not statistically different. There were no differences in E1317Q frequencies between either adenoma group or the surgical CRC group (data not shown). The normal colonoscopy controls had a statistically significant lower frequency of E1317Q carriers than spouse controls ($P = 0.036$), after adjusting for age and sex. Only 1 person in the normal colonoscopy control group carried the variant allele, and this person did not have a family history of CRC.

Within the surgical CRC group and the subset of this group with intact MMR, we examined whether there were any differences in E1317Q frequency when stratified by age and family history status (Table 3). Specifically, we compared: (*a*) all of the surgical CRC cases ≤50 years at age of diagnosis *versus* age >50 years; (*b*) all of the surgical CRC cases with no family history *versus* those with positive family history; (*c*) surgical CRC cases with intact MMR ≤50 years of age at diagnosis *versus* age >50 years; and (*d*) surgical CRC cases with intact MMR with no family history *versus* those with positive family history. Not unexpectedly (26), a preliminary analysis revealed significant or nearly significant differences in MMR status by gender and age (data not shown). However, no gender difference in E1317Q frequencies was detected by age category (data not shown). Including age and sex adjustments for comparisons *b* and *d*, there were no differences in any of the four comparisons.

Table 3 Comparison of APC E1317Q carrier proportions among subgroups of CRC cases

Comparison		*n*	APC E1317Q carriers (%)	OR[a] (95% CI)	*P*
	Surgical CRC cases ($n = 377$)				
A	Age ≤50 years at diagnosis	38	2.6		
	vs.			0.894 (0.109, 7.350)	0.917
	age >50 years at diagnosis	339	2.4		
B	No family history	323	2.2		
	vs.			2.043 (0.389, 10.724)	0.465
	Positive family history of CRC[b]	54	3.7		
	Surgical CRC cases with intact DNA MMR $n = 314$)				
C	Age ≤50 years at diagnosis	36	2.8		
	vs.			1.037 (0.126, 8.533)	0.973
	Age >50 years at diagnosis	278	2.9		
D	No family history	275	2.6		
	vs.			2.176 (0.417, 11.348)	0.356
	Positive family history of CRC	39	5.1		

[a] OR, odds ratio; for comparisons B and D, OR are age- and sex-adjusted; CI, confidence interval; CRC, colorectal cancer; MMR, mismatch repair.
[b] Positive family history, self report of a first degree relative with CRC.

Discussion

The *APC* variant E1317Q has been reported to occur more frequently in individuals with sporadic colorectal adenomas (17, 21), multiple polyps (15, 18), and CRC (21) when compared with controls (19), suggesting that it has a pathogenic effect. The E1317Q variant has been detected in both the general population (15) and in a spouse control group (19) that was clinically characterized, but not endoscopically verified as adenoma- and CRC-free. The results of published studies are contradictory, and most of the studies were small or lacked comparable controls. In some studies, the controls were pooled with those from other studies to demonstrate statistically significant differences in E1317Q frequency, thus risking variation in comparability. In our evaluation of a large population of unrelated Caucasian patients undergoing medical evaluation at Mayo Clinic, we did not detect a statistical difference in E1317Q frequency in individuals with adenomas or CRC compared with healthy spouse controls who had not undergone endoscopic evaluation. We conclude that E1317Q is not associated with increased risk for colorectal neoplasia in the general population.

Our decision to base our conclusion using the spouse control group comparison deserves elaboration, particularly in the context of an ongoing debate as to what constitutes "correct" controls in colorectal neoplasia studies (27–29). The presence of asymptomatic colorectal polyps or even early stage CRC cannot be ruled out in our (unexamined) spouse controls. This potential misclassification might be sufficient to attenuate an association between E1317Q and colorectal neoplasia, and is a well-recognized weakness of case-control studies. For example, studies generally have shown that cigarette smoking has a strong relationship with colorectal adenomas, but generally is not associated with CRC (30, 31). In light of the well-known adenoma-carcinoma sequence for colorectal neoplasia (23), the explanation for this paradox is unknown, but misclassification has been argued to play a role (28). This being said, we still maintain that the spouses represent the appropriate comparison group in this study, following the epidemiological principle that controls in this type of analysis should have the same distribution or risk of "exposure" as cases and have the same probability of having been in the case sample (27, 32).

In contrast to the results based on spouse controls, our comparisons of allele frequencies that used colonoscopy patients did show statistically significant differences. It can be construed that this is because of the rarity of the E1317Q variant in the latter control group, which differed even from the spouse control frequency. The colonoscopy controls represent a group that is proven by clinical examination to be free of colonic tumors, although one-third of this group had a first-degree relative with CRC. The low frequency of the E1317Q variant in this group appears at first to counter the hypothesis that E1317Q is a risk factor. However, this control group was selected for being free of colonic neoplasia, and interestingly the phenotype could be argued to obviate the impact of family history as well. Thus, there is some consistency between our observation that E1317Q is rarer in this "super-healthy" group and our observation based on spouse controls that there is no association of the variant with colorectal neoplasia.

From a pathophysiologic perspective, our observation that there are significant differences between patients with 1–3 adenomas or CRC cases compared with the colonoscopy controls might suggest insights into mechanisms. Defective DNA MMR is detected in ~20% of sporadic CRC (24). The pathogenesis of tumors with defective MMR tumors has been studied extensively, and almost all such cases are because of functional loss of either *hMLH1* or *hMSH2*, the majority caused by hypermethylation of the *hMLH1* promoter (33). Somatic mutations in *APC* do occur in CRC with intact or defective MMR (34), although the frequency of somatic *APC* mutations may be lower in defective MMR tumors (35). However, germ-line *APC* mutations have not been reported to result in DNA MMR-defective CRC, and our findings are consistent with this observation. CRC with intact MMR showed a higher E1317Q frequency when compared with the normal colonoscopy control group, whereas tumors with defective MMR did not. Therefore, the *APC* variant E1317Q may play a role in tumorigenesis of CRC with intact MMR.

The details of *APC*-driven tumorigenesis are not fully understood, but the available data on genotype-phenotype correlation (7), and on possible APC structure and function correlations (36, 37) suggest that the severity of colorectal manifestation may, at least in part, be determined by the level of functional APC protein in the cell. The *APC* variants I1307K and E1317Q are in a region of the *APC* gene where sporadic CRCs frequently harbor mutations (38). The mode of action of the I1307K mutation was suggested by Laken *et al.* (14) to result in somatic hypermutability. The missense mutation at codon 1307 appears to create an unstable sequence susceptible

at the somatic level to chain-terminating mutations within adjacent sequences. Alternatively, a direct effect of the variant may be another mechanism of action. The I-to-K substitution at codon 1307 is in a region that bisects the β-catenin-binding sites and that is involved in binding at least two other proteins. Thus, the substitution could give rise to a mild dominant-negative effect, therefore reducing the amount of available functional *APC* protein enough to substantially increase the risk of polyp formation (15). The variant E1317Q, on the other hand, is a GAA to CAA point mutation within codon 1317 of exon 15 leading to the substitution of glutamine for glutamic acid and, therefore, substituting an uncharged hydrophilic amino acid for an acidic hydrophilic amino acid. This substitution may affect colorectal adenoma predisposition by the same mechanism suggested above for the I1307K. However, the possibility cannot be excluded that E1317Q is simply a polymorphism detected in patients and controls, and does not influence *APC* protein function.

Lamlum *et al.* (18) have estimated that germ-line *APC* variants account for ~10% of patients with multiple adenomas and that it is worthwhile to screen multiple adenoma patients for a restricted number of *APC* variants, namely E1317Q and I1307K (if of Ashkenazi descent) and truncating mutations 5′ to exon 5, in exon 9 and 3′ to codon 1580, if there is a positive family history for CRC. A family history of CRC is a well-recognized risk factor for CRC and seems to be associated with the presence of detectable *APC* germ-line variants in 4 of 18 Italian patients with multiple adenomas (39), yet no E1317Q polymorphisms were found in this small population. In another series, germ-line *APC* mutations were not detected in 79 individuals with MMR intact CRC, <4 lifetime polyps, a strong family history of CRC, and/or early age(<50 years of age) onset of cancer (40). In both the multiple adenoma population and the early onset and/or positive family CRC history cohorts studied, neither a positive family history for CRC (first- and/or second-degree relative) nor early age of CRC onset (<50 years) was associated with an increased frequency of E1317Q.

Genetic screening for *APC* variants in the general population or presymptomatic testing in persons at increased risk for CRC is very controversial, because the results are not predictive nor cannot be clearly interpreted. Whereas the I1307K allele is common in the Ashkenazim, CRC does not develop in most carriers. Individuals of Ashkenazi descent who do not have a family history of CRC seem to benefit the most from I1307K testing (41). Even so, the value of positive or negative screening tests for I1307K has yet to be confirmed (42). Because E1317Q in this study is not a risk factor for polyps or CRC in the general population, screening for E1317Q in patients with polyps, CRC at any age, or positive family history of CRC is not justified.

In summary, the *APC* variant E1317Q has a low frequency (1.4–3.5% in cases and 0.3–2.8% in controls) and does not appear to confer an increased risk for colorectal neoplasia in the general population. Genetic screening for E1317Q is not indicated. Still, in individuals with ≤3 adenomas or with intact DNA MMR tumors, the E1317Q frequency was significantly increased compared with individuals with normal colonoscopies, indicating that a possible role for E1317Q in colorectal tumorigenesis may exist.

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D. Hahnloser, G. M. Petersen, K. Rabe, et al.

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