

## SHORT REPORT

# Attenuated familial adenomatous polyposis manifests as autosomal dominant late-onset colorectal cancer

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Colorectal cancer (CRC) risk is well defined for families of patients with classical familial adenomatous polyposis (FAP). However, the risk for those with an attenuated form of FAP is less well characterised. In this study, we estimated CRC risks for carriers of a novel germline mutation in the *APC* gene that causes attenuated FAP (AFAP). We performed genetic testing on 53 individuals from seven AFAP families harbouring an identical *APC:c.288T>A* mutation. Using a modified segregation analysis, we estimated relative and absolute CRC risks for mutation carriers. Twenty-three individuals harboured the disease causing mutation. CRC occurred in 28 individuals (mean 61.7 years, range 32–80 years). The estimated CRC relative risks for mutation carriers aged 60–69 and  $\geq 70$  years were 19 (95% CI: 1.77–204.08) and 45 (95% CI: 11.32–180.10), respectively, while the absolute CRC lifetime risk for men was 94% (95% CI: 67.5–99.9%), and for women, 84% (95% CI: 50.9–99.0%). This study shows that AFAP can manifest as autosomal dominant late-onset CRC. These findings highlight a subgroup of inherited CRCs that require new criteria for identification and surveillance.

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## INTRODUCTION

Colorectal cancer (CRC) is one of the commonest forms of cancer in the developed world. Moreover, it is the third most common cancer in UK and is the second leading cause of cancer-related deaths.<sup>1</sup> Although accounting for ~5% of cases, carriers of inherited germline mutations leading to conditions such as hereditary non-polyposis CRC (HNPCC, MIM #609310) and familial adenomatous polyposis (FAP, MIM #175100), form an important clinical subgroup. In this group, genetic testing and active treatment can prevent CRC in at-risk individuals.<sup>2</sup>

FAP, arising from a germline mutation in the adenomatous polyposis coli (*APC*) gene at 5q21-q22, is characterised by the onset of hundreds to thousands of small adenomatous polyps throughout the entire length of the colon with almost complete penetrance by the fourth decade.<sup>3</sup> A subset of these exhibits a phenotype different to classical FAP. Termed attenuated FAP (AFAP), patients with this phenotype are described as having fewer than 100 polyps, right-sided polyps with rectal sparing, and a typically later onset of polyps and CRC.<sup>4</sup>

Mutations associated with AFAP have been found at the 5' end, exon 9, and the distal 3' end of the *APC* gene.<sup>4</sup> It has been suggested that alternatively spliced transcripts with deletions of exons 3 and 4 result in a reduced amount of mutant *APC* transcript or impaired transcript function.<sup>5</sup> Although extra-intestinal manifestations are limited in AFAP,<sup>4</sup> higher incidences of desmoid tumours have been associated with 3' *APC* mutations.<sup>6</sup>

CRC risk is well defined for families of individuals with adenomatous polyps.<sup>7</sup> However, the risk for those with AFAP is less well characterised and has, to our knowledge, only been evaluated for a

single AFAP-associated mutation.<sup>8</sup> In this study, we identified a novel germline *APC* mutation present in seven families from the Tayside region of Scotland with a presentation of autosomal dominant late-onset CRC. In addition, we have been able to link three of these families to a pair of common ancestors, suggesting the presence of a founder effect in this population. Furthermore, we characterised the risk of CRC in these mutation carriers. Ultimately, these data enhance understanding of AFAP and informs the development of effective guidelines in its clinical management.

## MATERIALS AND METHODS

### Patients

This study was conducted with Caldicott Guardian approval for analysis of anonymised data. Families were ascertained through affected probands. Probands were referred to the clinic on the basis of a history of rectal bleeding, right-sided polyps, and/or family history of CRC. Classical FAP phenotype was excluded, and DNA microsatellite instability analyses in seven probands excluded HNPCC or Lynch syndrome. No cases of CRC were detected on surveillance colonoscopy.

The seven families in this study comprise 255 individuals. The mean (SD) age was 52.5 years (20.7) whereas the mean (SD) age of CRC onset was 61.7 years (10.5), range 32–80 years. In addition to the 23 *APC:c.288T>A* mutation carriers, 13 individuals were identified as obligate mutation carriers. Data used for analysis are summarised in Table 1. Furthermore, three families were identified as sharing a pair of common ancestors (Figure 1) and for the purposes of the modified segregation analysis were treated as a single pedigree.

### Mutation analysis

Mutation analysis of the *APC* gene (NM\_000038.5) was performed using standard clinical protocols. These included PCR amplification and

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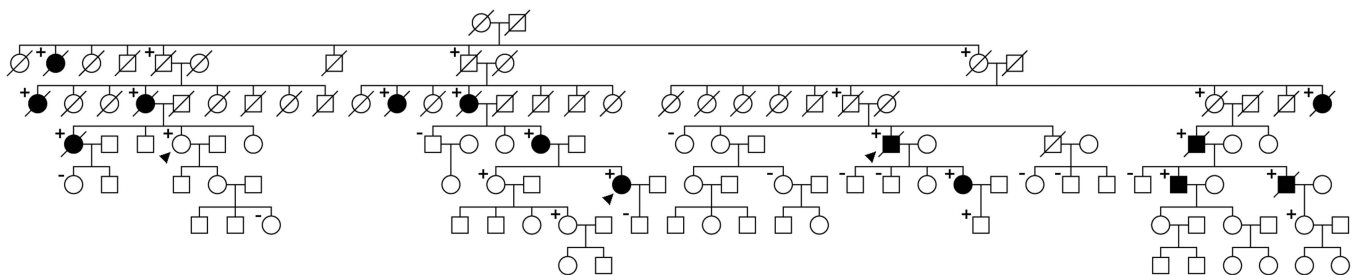
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**Table 1** Segregation analysis for *APC:c.288T>A* mutation

Model	Group	Not tested	Non-carriers	Carriers	RR (95% CI)	P	LRT		
							$\chi^2$	P	
Standard	Unaffected	181	30	16	30.78 (12.12–78.16)	5.65 × 10 <sup>-13</sup>			
	Affected	21	0	7					
Sex-specific	Male	Unaffected	84	13	9	20.35 (3.60–115.08)	6.53 × 10 <sup>-4</sup>	0.692	0.405
		Affected	10	0	4				
	Female	Unaffected	97	17	7	52.67 (14.33–193.60)	2.40 × 10 <sup>-9</sup>		
		Affected	11	0	3				
Age-specific (years)	20–49	Unaffected	134	16	10	33.02 (0.97–1122.12)	0.0519	0.271	0.965
		Affected	3	0	1				
	50–59	Unaffected	7	6	3	22.65 (0.75–680.28)	0.0722		
		Affected	2	0	4				
	60–69	Unaffected	11	4	2	19.01 (1.77–204.08)	0.0150		
		Affected	10	0	2				
	≥ 70	Unaffected	29	4	1	45.15 (11.32–180.10)	6.76 × 10 <sup>-8</sup>		
		Affected	6	0	0				

Abbreviations: CI, confidence interval; LRT, likelihood ratio test. Unaffected/affected indicates colorectal cancer disease status. Not tested/non-carriers/carriers reflects *APC:c.288T>A* mutation status.



**Figure 1** Pedigree of three *APC:c.288T>A* attenuated familial adenomatous polyposis families. Probands are indicated with an arrow. Shading refers to colorectal cancer. Positive symbols indicate *APC:c.288T>A* mutation carriers, while negative symbols indicate confirmed non-mutation carriers.

bi-directional sequencing of each exon (according to NM\_000038.5) of the *APC* gene.<sup>9</sup> Variant data were submitted to the Leiden Open Variation Database (<http://www.lovd.nl/APC>).

**Statistical methods**

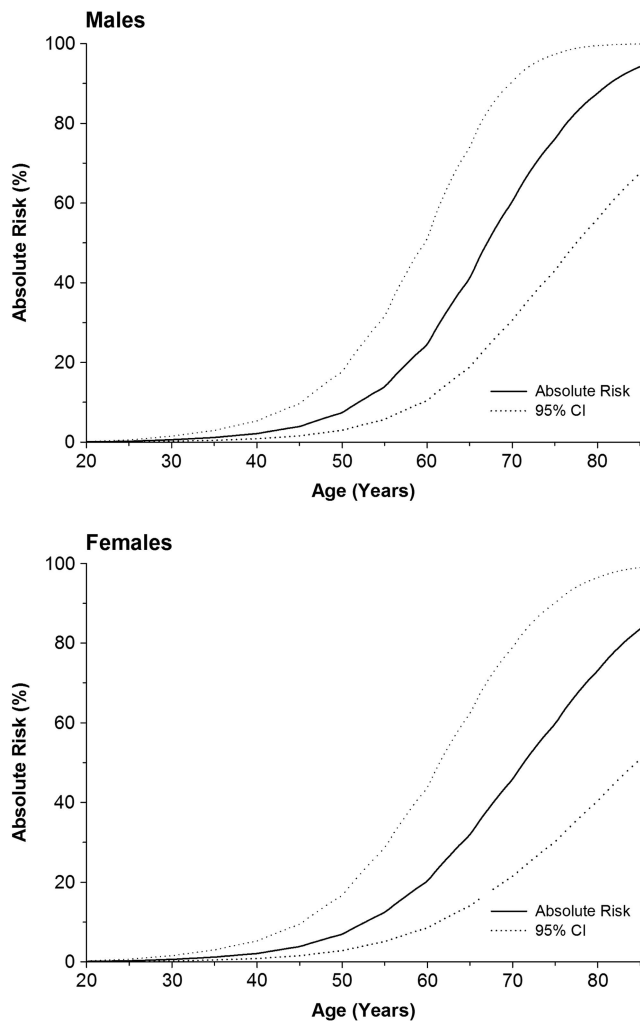
For all analyses, we considered time to CRC diagnosis for each family member. We assumed a censoring process such that an individual was followed from birth until the age at CRC diagnosis, age of death, age at last observation, or age 85 years – whichever occurred first. All individuals with censoring ages ≥ 85 years were censored as unaffected at age 85 years. In the instance of no available age information, we censored at age 0 years. All tests of statistical significance were two-sided, and we used a statistical significance threshold of  $P=0.05$ .

We used a modified segregation analysis to estimate CRC relative risks (RRs) and absolute risks for carriers of an *APC:c.288T>A* mutation. For this purpose, we modelled the retrospective likelihood of observing pedigree genotypes and phenotypes of all family members conditional on pedigree disease phenotypes of all family members and genotype of the first identified mutation carrier in each family. This is in line with the sequential ascertainment adjustment procedure of Cannings and Thomson<sup>10</sup> allowing us to obtain unbiased parameter estimates. We assumed that the prevalence of the mutation in the population was rare (prevalence = 0.001), but our results were not sensitive to this assumption. All models were parameterised in terms of the log-relative risk parameters and three models were fitted: (i) a standard model estimating a single RR parameter; (ii) a sex-specific model that estimated associations separately for men and women; and (iii) an age-specific model that estimated associations for age strata 0–49 years, 50–59 years, 60–69 years,

and ≥ 70 years. The RR was assumed to be 1 for individuals aged < 20 years. The CRC incidences were constrained over all genetic effects to agree with population incidences,<sup>11</sup> and CRC population- and gender-specific incidences were obtained from the Cancer Incidence in Five Continents publications (<http://ci5.iarc.fr/CI5i-ix/ci5i-ix.htm>). Parameters were estimated using maximum likelihood estimation. Likelihood ratio tests (LRT) were performed to compare the various models and identify the most parsimonious model. Segregation analyses were performed using pedigree analysis software MENDEL.<sup>12</sup>

**RESULTS**

Table 1 displays the segregation analysis results. The estimated RR of CRC in *APC:c.288T>A* mutation carriers was 30.78 (95% CI: 12.12–78.16,  $P=5.65 \times 10^{-13}$ ). The sex-specific model revealed a larger RR for women (RR = 52.67, 95% CI: 14.33–193.60) compared with men (RR = 20.35, 95% CI: 3.60–115.08) but the difference in male and female RRs was not significant. There were statistically significant increased risks for individuals aged 60–69 (RR = 19.01, 95% CI: 1.77–204.08,  $P=0.0150$ ) and ≥ 70 years (RR = 45.15, 95% CI: 11.32–180.10,  $P=6.76 \times 10^{-8}$ ), highlighting an increased incidence of late-onset CRC. LRTs showed that there was no evidence to suggest that the sex- or age-specific models fit better than the standard single RR model. Under the most parsimonious model, the estimated RRs translate to a lifetime CRC risk in men of 94.2% (95% CI: 67.5–99.9%) and 83.6% (95% CI: 50.9–99.0%) in women (Figure 2).



**Figure 2** Absolute risks of colorectal cancer for men and women by age 85 years for *APC:c.288T>A* mutation carriers.

## DISCUSSION

The *APC:c.288T>A* mutation results in a premature stop codon (p.Tyr96\*). The resulting truncated transcript is expected to account for the observed AFAP phenotype in our families. There are a number of possible explanations as to why a premature stop codon would produce an attenuated phenotype. The premature stop codon in the mutant transcript may lead to nonsense mediated decay, so that there can be no dominant negative effect that has been shown for other classical mutations. Alternative splicing or translation initiation downstream of the mutation can remove the mutant exon and allow the coding of peptides with partial functionality from the mutant allele.<sup>5,13,14</sup> It has also been proposed that other loci may have an influence on *APC* gene expression.<sup>14</sup> Thus, one or a combination of these molecular mechanisms is expected to explain this observed attenuated phenotype.

Found within exon 3, this *APC:c.288T>A* mutation is consistent with studies that link 5' *APC* mutations to an AFAP phenotype with fewer extra-intestinal features.<sup>4</sup> While some studies have shown extra-intestinal manifestations associated with the 5' region, these are not as numerous as those associated with 3' mutations of the *APC* gene.<sup>6,14</sup> In our families, there were no reports of extra-intestinal features, although patients were not exhaustively examined for these.

In agreement with other reports,<sup>8,15</sup> we show the mean age of CRC onset to be 61.7 years as opposed to that of 40 years in classical FAP.<sup>4</sup> However, our lifetime CRC risk estimates are not in keeping with that of reported by Burt *et al*<sup>8</sup> (69%, 95% CI: 41–84%) for a different AFAP *APC* mutation. Although these differences may be mutation specific, we argue that unlike the Kaplan–Meier approach used by Burt *et al*,<sup>8</sup> which assumes random ascertainment and only utilises information on genotyped family members—ignoring the phenotypes of un-genotyped family members and their relationships, we address these issues by using a modified segregation analysis. Indeed, our own Kaplan–Meier estimate (data not shown) yields a similar biased lifetime CRC risk of 72.6% (95% CI: 35.1–88.5%) in mutation carriers.

While we observe a late-onset CRC phenotype in mutation carriers, the large confidence intervals for those aged <60 years is attributed to our limited sample size. In addition, although we witness a reduction in the age of onset of CRC across generations (Figure 1; generations II–V, mean range 70–58 years, respectively), these differences were not statistically significant. Finally, identifying common ancestors for three of the seven families strongly supports a founder effect for this mutation.

The late-onset CRC phenotype observed in our pedigrees has no clear distinguishing features. We observe highly variable colonic polyp frequencies ranging from 0 to ~100 polyps. Indeed, this reduces the likelihood of identifying these families as AFAP, with the risk of missing an important genetic diagnosis that guides treatment.

In FAP, surveillance strategies are broadly based on the age distribution of CRC cases, with lifelong endoscopic screening often commenced in the early-to-mid teens in individuals with a known mutation, or continued until age 50 years in families with no known mutation.<sup>16</sup> Surveillance strategies are more difficult to design for AFAP given the phenotypic variability and limited amount of data available to guide management. In AFAP, colonoscopy may delay the requirement for colectomy, and patients may receive greater benefit from aspirin chemoprophylaxis.<sup>17</sup>

While individuals with an increased risk of CRC as a result of highly penetrant genetic disorders are often identified as a result of familial clustering and pathology,<sup>18</sup> the observation of late-onset CRC in this study does not fulfill usual diagnostic criteria for FAP or HNPCC. As per current British Society of Gastroenterology CRC screening and surveillance guidelines,<sup>18</sup> the familial clustering of CRC in this study would fall into a high-moderate risk category warranting 5-yearly colonoscopy from age 50 years. Adopting such a surveillance strategy would not suffice for our AFAP families given the extremely high relative risk of CRC after age 60 years. As such, and in light of a CRC diagnosis in an *APC:c.288T>A* carrier at age 32 years, at-risk individuals were offered biennial colonoscopy from age 25 years.

In conclusion, this study has identified a novel *APC* germline mutation associated with an attenuated form of FAP that manifests as autosomal dominant late-onset CRC, which is not easily identified with current risk stratification strategies. Furthermore, we have presented age-dependent penetrance data for CRC in *APC:c.288T>A* mutation carriers. Pedigrees such as those in our study may account for a proportion of late-onset high penetrance CRC families not accounted for by HNPCC mutations. These findings suggest that there is a subgroup of individuals with dominantly inherited CRC risk that requires new criteria for identification and surveillance.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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