

Table 1. Characteristics of 35 patients with germline APC mutations in the three AFAP-associated regions (5', exon 9 and 3'; codons 1-177, 311-408 and >1580).

FS = frameshift; n/a = not available.

Patient ID	Germline APC mutation	Gender	Age at presentation	Polyp count	Polyps analysed
AFX MK	G126X	M	36	834	6
DFAP48	141FS	F	56	2	1
AVC.III.2	Q163X	M	51	2100	4
554.iv.2/1112	170FS	F	32	1357	11
554.iii.2	170FS	M	50	1077	5
1464/1	173FS	M	39	"multiple"	3
673.iii.3/1132	R332X	F	49	200-300	6
1571.ii.2	R332X	F	68	5	5
578.AA	R332X	F	n/a	n/a	4
578.FPL	R332X	F	27	20	6
578.iii.9	R332X	M	41	n/a	17
578.iv.1	R332X	F	43	130	14
578.iv.4	R332X	F	27	n/a	9
578.iv.7	R332X	M	n/a	n/a	15
DFAP16	R332X	M	52	"multiple"	1
DFAP81	R332X	M	32	<100	2
344-40	1597FS	M	47	99	4
344-44	1597FS	M	43	50	3
01/266	1738FS	F	53	29	19
2233/3	1919FS	n/a	n/a	n/a	15
MD2976	1919FS	F	43	>100	21
77-11	1943FS	M	66	500	24
77-12	1943FS	M	56	500	2
77-40	1943FS	M	39	500	2
1460/6	1982 FS	F	65	>100	5
1460/28	1982 FS	F	33	8	1
1460/42	1982 FS	F	33	>100	4
1460/88	1982 FS	M	48	<100	1
1489/10	1982 FS	F	29	n/a	1
1624/04	1982 FS	F	40	<100	3
S73119	2078FS	F	52	<100	1
DW20284	2078FS	F	n/a	n/a	1
J42424	2078FS	F	n/a	n/a	3
L12562	2078FS	F	60	"numerous"	3
110.2.vi	2078FS	F	n/a	33	14

Table 2. Numbers of tumours with evidence of ‘third hits’ (somatic mutation of germline mutant allele) at APC in exon 9- and 3’mutant patients’ polyps

20AAR1 = truncating mutation before first 20AAR, *etcetera*. Note that these are minimum estimates of the true frequency, not only because we could not screen the entire gene for mutations in small archival polyps, but also because it was not possible to assign all mutations to the germline mutant or wildtype allele.

Somatic mutation on germline wildtype allele ('second hit')	Somatic mutation on germline mutant allele ('third hit')	No. 5'-mutant patients	No. exon 9-mutant patients	No. 3'-mutant patients
LOH	20AAR3	0	6	0
LOH	20AAR2	0	0	2
20AAR0	20AAR3	0	2	1
20AAR1	20AAR3	0	2	0
20AAR0	LOH	0	0	1
20AAR1	LOH	2	4	3
20AAR2	LOH	0	0	2
Not found	20AAR3	0	20	0
Not found	20AAR2	0	2	2
Not found	LOH	0	0	9

Table 3. Observed and expected frequencies of somatic mutant APC alleles in AFAP polyyps with ‘three hits’ for ‘kick-start’ and ‘stepwise’ models of tumorigenesis. α_{gl} = proportion of germline wild-type allele in polyp; β_{gl} = proportion of germline mutant allele in polyp; β_{som} = proportion of somatic mutant allele in polyp. Observed α_{gl} β_{gl} frequencies were determined from LOH ratios. Observed β_{som} frequencies were similarly determined from LOH ratios generated by PCR amplification of a region encompassing the somatic insertion/deletion and subsequent Genescan analysis (using constitutional DNA from patients with germline mutations identical to the somatic change for normalisation). The observed ‘third’ to ‘second hit’ ratio for polyp 3 was determined by sequencing 58 clones of a PCR product encompassing both somatic changes.

A. Polyp with one somatic insertion/deletion and loss of the germline wild-type allele.

Model	Allele frequencies	Polyp 1	Polyp 2	Polyp 3	Polyp 4	Polyp 5	Polyp 6	Polyp 7	χ^2_6
	Observed α_{gl}	0.21	0.27	0.21	0.29	0.30	0.26	0.28	
	Observed β_{som}	0.64	0.52	0.49	0.52	0.33	0.40	0.27	
‘Kick-start’, LOH first	Expected $\beta_{som} = [(1-2\alpha_{gl})/2]$	0.29	0.23	0.29	0.21	0.20	0.24	0.22	1.59
‘Kick-start’, LOH second	Expected $\beta_{som} = (1-2\alpha_{gl})$	0.58	0.46	0.58	0.42	0.40	0.48	0.44	0.14
‘Stepwise’	Expected minimum β_{som}^*	0.20	0.20	0.20	0.20	0.20	0.20	0.20	2.72
	Expected maximum $\beta_{som} = [(1-2\alpha_{gl})/2]$	0.29	0.23	0.29	0.21	0.20	0.24	0.22	1.59

* Assuming that the somatic insertion/deletion can be detected if it comprises >20% of all alleles in the polyp.

B Polyp with one somatic insertion/deletion and loss of the germline mutant allele.

Model	Allele frequencies	Polyp 8	Polyp 9	Polyp 10	χ^2_3
	Observed β_{gl}	0.33	0.10	0.24	
	Observed β_{som}	0.41	0.83	0.61	
‘Kick-start’ (LOH must be second)	Expected $\beta_{som} = (1-2\beta_{gl})$	0.34	0.80	0.52	0.03
‘Stepwise’	Expected minimum $\beta_{som} = (1-2\beta_{gl})$	0.34	0.80	0.52	0.03
	Expected maximum $\beta_{som} = (1-\beta_{gl})$	0.67	0.90	0.76	0.14

C. Two truncating somatic mutations.

Model	‘Third’ to ‘second hit’ ratios	Polyp 11	χ^2_1
	Observed	27:31	
‘Kick-start’	Expected	1:1	0.03
‘Stepwise’	Expected minimum	1:4	14.4
	Expected maximum	1:1	0.03

* Assuming a detection sensitivity of 20% for the somatic ‘third hit’.