ONLINE MUTATION REPORT

Novel germline mutations in the *adenomatous polyposis coli* gene in Polish families with familial adenomatous polyposis

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Familial adenomatous polyposis (FAP) is a genetically determined disorder that is inherited in an autosomal dominant manner. The occurrence of FAP is associated with mutations in the *APC* gene, which were described in 1991.[1] *De novo* mutations of the *APC* gene occur in one per 10 000 newborns. The *APC* gene is localised on chromosome 5q21 and consists of 21 exons. In most cases, mutations of the *APC* gene are small deletions or insertions: the AAAGA deletion at codon 1309, which occurs in 10% of families with FAP, and the ACAAA deletion at codon 1061, which occurs in 5% of families with FAP, are the most frequent mutations. Ninety-two percent of all mutations in the *APC* gene lead to truncations of the APC protein product. Dysfunction of the *APC* gene causes the accumulation of B-cathenin and the expression of genes that promote cell division.

The FAP syndrome contributes only a relatively low percentage of all colorectal carcinomas (1–2%) and is characterised by the presence of numerous (at least 100) polyps that line the mucosa of the large intestine and rectum. The occurrence of other gastrointestinal adenomas, cutaneous sebaceous cysts, osteomas (mostly in the jaw, scapula, and long bones), connective tissue neoplasms, desmoid tumours, and, in some cases, coexisting duodenal and thyroid carcinomas (which together are classified as Gardner syndrome) and less common structural deformations in the teeth may also be observed.[2 3] The DNA bank of Polish families with FAP was established in 1997 at the Institute of Human Genetics, Polish Academy of Science in Poznań.

This study reports a spectrum of mutations of the *APC* gene in Polish patients with FAP.

Key points

- The aim of this study was to investigate mutations in the *adenomatous polyposis coli* (*APC*) gene in the Polish DNA bank for families with FAP.
- 120 Polish families with familial adenomatous polyposis (FAP) were screened for mutations in the part of the *APC* gene that encompasses exons 5–8, exons 10–14, and the 3′ part of exon 15. DNA was screened with heteroduplex analysis, single strand conformational polymorphism methods, and DNA sequencing.
- Mutations in the *APC* gene were found in 42 (35%) Polish families with FAP, and 22 types of mutation in the *APC* gene were identified. Of these, 15 mutations were deletions of 1-11 base pairs, five were insertions of 1–8 base pairs, and two were substitutions. Overall, 14 of the identified mutations were not seen in other populations. *De novo* mutations occurred in three families; one of these mutations had not been described before.
- Most of the detected mutations in 88% are localised at the 5′ end of exon 15 of the *APC* gene.

MATERIAL AND METHODS

Patients

Clinical diagnoses of FAP in patients were established in genetic centres or gastroenterology clinics in Poznań, Szczecin, Kraków, Wrocław, Gdańsk, Warszawa, and Lódź, appropriate to the patients' place of residence. Families with FAP came from the following regions of Poland: 79 from the central west, 17 from the northwest, seven from the south east, six from the southwest, two from the north, and nine from the central east. To date, samples from 315 people from 120 families with FAP have been collected in the Polish FAP DNA bank. In this group, 140 patients had typical FAP, while eight patients had atypical FAP. Mutations in the *APC* gene were analysed in 120 probands. If mutations were identified in a proband, the members of the proband's families were also screened (if available).

Molecular methods

We extracted DNA from peripheral blood cells with the classical phenol purification method. We screened fragments of the *APC* gene that encompassed exons 5–8, exons 10–14, and the fragment from A to L of exon 15 of the *APC* gene for mutations with heteroduplex analysis and single strand conformational polymorphism methods. DNA fragments that showed heteroduplex in heteroduplex analysis or additional patterns in single strand conformational polymorphism analysis were sequenced by direct polymerase chain reaction product sequencing and analysed with ALFExpress (Amersham-Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's specifications. New mutations were confirmed to be absent in 50 unrelated people in the control group, which consisted of 25 unrelated women and 25 unrelated men randomly chosen from the Polish population.

RESULTS AND DISCUSSION

We initially analysed DNA from 120 probands in the DNA bank for mutations in the *APC* gene. Mutations were found in 42 (35%) probands (table 1). We identified 22 types of mutations in the group studied; 14 of these were not seen in other populations. Five mutations recurred in the families that we examined (table 2). In three families, probands had *de novo* mutations.

Most mutations were found in the 5′ region of exon 15; in addition, two mutations were found in exon 5, one in exon 8, and one in exon 11. No mutations outside exon 15 had been described previously. In exon 11, we saw insertion T in position 1491 in two families. In both families, differences

Table 1 Mutations of *APC* gene detected in Polish families with FAP

Case	Family #	Patient #	Age of onset	Cancerous features outside the colon	Exon and fragment	Mutation	Stop codon
1	045	9045	NDA	–	5	601delG	204
2	017	9017	26	–	5	636delA	218
3	089	9089	NDA	–	8	892–893delCA	325
4	001	9001	35	Desmoid tumour and cerebral flax tumour	11	1491insT	608
		9002	37	–	11	1491insT	608
		9003	47	–	11	1491insT	608
		9004	36	–	11	1491insT	608
		9005	16	–	11	1491insT	608
		9033	36	–	11	1491insT	608
5	129	9129	10	Brain fibromatosis and ossis tempoparietalis	11	1491insT	608
		9129,1	12	–	11	1491insT	608
		9129,2	12	–	11	1491insT	608
6	050	9050	NDA	NDA	15 B	2413C>T Arg>Stop	805
7	023	9023	16	NDA	15 C	2509delC	915
8	031	9031	NDA	NDA	15 C	2626subC>T	876
9	043	9043	30	–	15 C	2626subC>T	876
		9043,1	–	–	15 C	2626subC>T	876
		9043,5	–	–	15 C	2626subC>T	876
10	130	9130	NDA	NDA	15 D	2922insG	975
11	051	9051	NDA	NDA	15 D	3119–3126insCTCTGGAA	1058
12	102	9102	–	–	15 E	3164–3168delTAATA	1057
13	028	9028	28	–	15 E	3183–3187delACAAA	1062
		9028,1	–	–	15 E	3183–3187delACAAA	1062
14	067	9067	19	NDA	15 E	3183–3187delACAAA	1062
15	093	9093	–	–	15 E	3183–3187delACAAA	1062
16	108	9108	–	–	15 E	3183–3187delACAAA	1062
17	111	9111	–	–	15 E	3183–3187delACAAA	1062
18	088	9088	NDA	–	15 E	3202–3205del TCAA	1124
19	106	9106	26	–	15 E	3202–3205del TCAA	1124
20	011	9011	19	Thyroid	15 F	3473–3474delGA	1162
21	041	9041	13	–	15 E	3371delA	1225
		9041,4	–	–	15 E	3371delA	1225
		9041,5	–	–	15 E	3371delA	1225
22	058	9058	36	–	15 F	3515delA	1181
23	083	9083	NDA	–	15 F	3578–3581delCAGT	1264
24	080	9080	NDA	–	15 F	3613delA	1264
25	146	9146	8	–	15 G	3921–3924Del AAAA	1319
		9146,1	4	Duodenal polyps	15 G	3921–3924Del AAAA	1319
26	059	9059	18	–	15 G	3927–3931del AAAGA	1312
27	065	9065	36	–	15 G	3927–3931del AAAGA	1312
		9065,1	12	–	15 G	3927–3931del AAAGA	1312
28	069	9069	NDA	NDA	15 G	3927–3931del AAAGA	1312
29	071	9071	36	NDA	15 G	3927–3931del AAAGA	1312
30	075	9075	NDA	NDA	15 G	3927–3931del AAAGA	1312
31	032	9032	NDA	NDA	15 G	3927–3931del AAAGA	1312
32	036	9036	13	–	15 G	3927–3931del AAAGA	1312
33	016	9016	20	–	15 G	3927–3931del AAAGA	1312
34	103	9103	26	Liver and lung	15 G	3927–3931del AAAGA	1312
35	104	9104	–	–	15 G	3927–3931del AAAGA	1312
36	105	9105	20	–	15 G	3927–3931del AAAGA	1312
37	118	9118	NDA	–	15 G	3927–3931del AAAGA	1312
38	147	9147	NDA	–	15 G	3927–3931del AAAGA	1312
39	149	9149	NDA	–	15 G	3927–3931del AAAGA	1312
40	030	9030	31	Desmoid tumour and stomach polyps	15 H	4266–4276delTCTTCCAGATA	1433
41	123	9123	NDA	NDA	15 H	4386–4387Ins GA	1462
42	027	9027	45	Desmoid tumour and stomach polyps	15 I	4667INS A	1558

NDA, no data available; –, not observed.

were seen in the age of onset of polyps. In family 9129, symptoms were noted when patients were 10 and 12 years old, while in family 9001, symptoms were noted later (table 1). Different genetic backgrounds in these families must have influenced the time of onset of the disease. In both families, probands had brain cancer, and proband 9001 also had a desmoid tumour, which is linked with mutations located between codons 1403 and 1578p; in both cases in our study, the affected proband had a mutation in exon 11.[2–4]

The mutation 636delA in exon 5 is a new mutation that occurred *de novo* in family 9017. We detected two other *de novo* mutations in the Polish population: 2413C>T (Arg>Stop) and 4393–4394Ins GA both were described in 1996 by Dobbie and colleagues.[5] The youngest patient with FAP who we studied was a girl who at the age of four years already had numerous polyps in the colon and sparse duodenal polyps and who was a carrier for the known mutations 3921–3924delAAAA.[1]

The most frequent mutations in Polish families with FAP were 3927–3931del AAAGA (del 5 bp at 1309), which occurred in 15 (12.5%) families, and 3183–3187delACAAA (del 5 bp at 1061), which was seen in six (5%) families. The frequency of these mutations varies depending on populations. The frequency of the most common deletion, 5 bp at 1309, in other populations varies from 0% in northwest Spain through 2.4% in Australian populations, 5% in Dutch populations, and 7% in Israeli populations up to 16% in the group reported by Varsco and colleagues.[6–9] The deletion 5 bp at 1061 also occurs with a range of frequencies: for example, 0% in northwest Spain, 1.5% in Israeli populations, and 8.4%

Table 2 Types of mutations of *APC* gene detected in Polish population

Number	Exon	Mutations	Stop	Recurrence	References
1	5	601delG	204	1	New*
2	5	636delA	218	1	New
3	8	892–893delCA	325	1	New
4	11	1491insT	608	2	New
5	15	2413C>T Arg>Stop	805	1	5
6	15	2509delC	915	1	New
7	15	2626subC>T	876	2	13, 27–30
8	15	2922insG	975	1	New
9	15	3119–3126INS CTCTGGAA	1058	1	New
10	15	3164–3168delTAATA	1057	1	31, 32
11	15	3183–3187delACAAA	1062	6	1, 31, 33
12	15	3202–3204del TCAA	1124	2	14
13	15	3371delA	1225	1	New
14	15	3473–3474delGA	1162	1	New
15	15	3515delA	1181	1	New
16	15	3578–3581delCAGT	1264	1	New
17	15	3613delA	1264	1	New
18	15	3921–3924Del AAAA	1319	1	34
19	15	3927–3931del AAAGA	1312	15	1
20	15	4266–4276delTCTTCCAGATA	1433	1	New
21	15	4393–4394Ins GA	1462	1	New
22	15	4667INS A	1558	1	35, 36

*Mutation not in Thierry Soussi's database of mutations of the *APC* gene (http://p53.curie.fr/p53%20site%20version%202.0/APCdatabase.html) and Gene Bank.

in Australia.[6–8] A study of more than 100 Dutch families showed equal frequency of these most frequent mutations. In another study of 680 families from Germany, the two most frequent mutations had frequencies of 4.9% for deletion 5 bp at 1061 and 7% for deletion 5 bp at 1309.[10 11] In our group, the deletion 5 bp at 1309 occurred more than twice as frequently as the deletion 5 bp at 1061. In worldwide populations, differences in the frequency of these two mutations are seen. Polish populations of patients with FAP belong to the group in which both mutations occur with high frequency.[12]

In exon 15, we saw another two recurrent mutations, each in two families. One was a 2626C>T substitution and the other a 3202–3205delTCAA.[13 14] Mutations in the region between codons 1445 and 1578 are associated with the occurrence of numerous features outside the colon (desmoid tumours, osteomas, epidermoid cysts, and upper gastrointestinal polyps), which are classified as Gardner syndrome.[2 3] In this region of the gene, we identified two mutations: 4386–4387insGA in the 9123 family (*de novo*) and 4667insA in the 9027 family. One of those families (9027) had features of Gardner syndrome, while the patients from family 9123 did not have any cancerous features outside the colon. The second case of Gardner syndrome had a mutation outside the expected region: a TCTTCCAGATA deletion that started at codon 1422 and lead to the stop codon at codon 1433(fig 1). This may indicate that the region responsible for Gardner syndrome cannot be determined exactly. In Polish patients

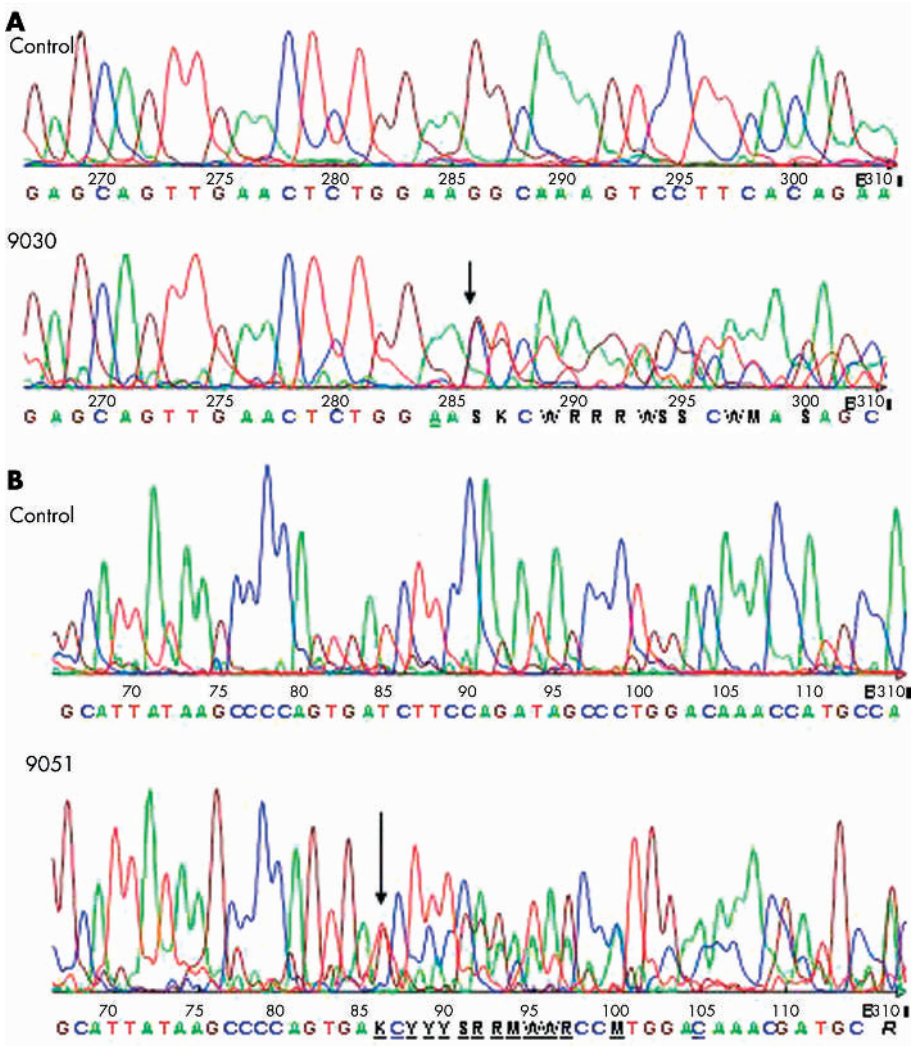

Figure 1 Mutations detected in Polish population: largest insertions detected in Polish population 3119–3126insCTCTGGAA (A) and largest deletions detected in Polish population 4266–4277delTCTTCCAGATA (B).

with FAP, most of the detected mutations were localised at the 5′ end of exon 15 of the *APC* gene. Of the detected mutations, 50% were in the fragment that encompassed nucleotides 1040 to 1309. In our group of patients, we did not see mutations in exons 6, 7, 10, 12, 13, and 14, in which mutations were expected with the frequency of 1–2% on the basis of their occurrence in other populations.[15]

In our study, we examined 120 Polish families with FAP for the occurrence of mutations in the *APC* gene. For cost-efficiency reasons, we screened only the part of the *APC* gene in which mutations were most expected. Exon 9 of the *APC* gene was omitted, because of the absence of late onset of the disease and of variations in phenotypic manifestations in the identified families, which are characteristic for mutations in exon 9.[16–18] The study region was chosen on the basis of previous studies in other populations, especially the report published by Wallis and colleagues, which considered >200 families with FAP from the United Kingdom.[1 15 19 20]

We found mutations in 35% of the families we studied. A study in the biggest referred group from Germany reported mutations in 48% of 680 studied families.[11] The percentage of detected mutations in our study was lower, which could have been caused by the occurrence of mutations outside the studied region or the lower efficiency of the methods we used to detect mutations. In all of the abovementioned studies in other populations, *in vitro* translation was used for mutation screening, and differences in rates of mutation detection ranged from 30% to 85%.[11 15 21 22] In our study, known polymorphic single base substitutions were visible during the analysis (data not shown), but the detected rate of substitutions in the studied group was low compared with the large German study (3/120 *v* 87/680).[11] This can be attributed to the efficiency of the applied methods or the occurrence of mutations specific for Polish families with FAP.

To rule out the occurrence of large deletions, structural rearrangements of *APC* gene, or reduced expression of one allele of the *APC* gene in Polish patients with FAP as a frequent cause of FAP in Poland is impossible.[23 24] In addition, the occurrence of recessive mutations in other genes (for example, the *MYH* gene), especially in *de novo* cases (>25% in our group), may contribute to the occurrence of the disease.[25 26] Further studies to look at the remaining regions of the *APC* gene and to search for large deletions in Polish families with FAP will be necessary. A study of recessive mutations of the *MYH* gene will have to be performed in families who lack mutations in the *APC* gene as the next step towards explaining causes of FAP in Poland.

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