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SHORT REPORT

Marfan-like habitus and familial adenomatous polyposis in two unrelated males: a significant association?

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Familial adenomatous polyposis (FAP) can be considered as a condition of the whole body as extracolonic features derived from all the three embryonic lineages are recorded with varying frequency in addition to the presence of multiple adenomas in the large intestine. Here, we describe two unrelated cases of FAP with unusual extracolonic phenotypes, namely several abnormalities of mesodermal origin strongly resembling Marfan syndrome (MFS) or a Marfan-like habitus. Conventional cytogenetic and FISH analysis did not reveal any gross chromosomal rearrangement on the long arm of chromosome 5 where the *APC* and *FBN2* genes were located. However, in case 2 the FAP-causing mutation in the *APC* gene was found in the donor splice site of exon 4 and was shown to result in a frameshift and a premature termination codon. We propose that such connective tissue abnormalities may result from germline *APC* mutations in combination with specific genetic and/or environmental modifying factors.

Keywords: APC; FAP; MFS; Marfan syndrome; adenomatous polyposis

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterised by the presence of more than 100 adenomatous polyps in the colon and rectum that inevitably progress to adenocarcinoma before the age of 40 to 50. The disease is not limited to the large bowel, as FAP patients are known to be at high risk for a broad spectrum of extracolonic manifestations derived from all three embryonic lineages.[1] FAP is caused by mutations in the tumour suppressor gene *APC* located on chromosome 5q21–q22. Recently, it has been proposed that the *APC* gene acts as a gatekeeper of colonic epithelial cell proliferation and that its inactivation is a rate-limiting step for tumour formation in the colon and rectum.[2]

Hereditary disorders of the connective tissue include a broad spectrum of skeletal, ocular and cardiac abnormalities ranging from very mild conditions to the most severe Marfan syndrome (MFS).[3] Molecular analysis of these Marfan-like conditions has revealed

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that patients with congenital contractural arachnodactyly (CCA or Beals syndrome) carry germline mutations of the fibrillin 2 (*FBN2*) gene on chromosome 5q23–q31.[4]

Here, we report on two unrelated patients affected by FAP with marfanoid features and discuss the possibility that this represents a novel association.

Case Reports

Case 1

A 28-year-old Romanian man was admitted because of bloody diarrhea and a one-year history of recurrent left calf deep vein thrombophlebitis. There was no familial history of note. On examination, the patient appeared tall and thin (height 184 cm and weight 60 kg) compared to his father (aged 62, 165 cm/68 kg), mother (aged 56, 158 cm/62 kg), brother (aged 26, 168 cm/65 kg) and sister (aged 24, 161 cm/55 kg). Several facial features were recorded: malar hypoplasia, a narrow and high arched palate with crowding of teeth, dental abnormalities (deficient implantation of teeth and multiple caries, but no extra teeth), and inferior maxillary retrognathism. Musculoskeletal abnormalities were also noticed: moderate thoracic kyphoscoliosis, bilateral high pedal arches, moderate hypermobility of all joints and skin hyperextensibility (Figure 1a and b). Cardiovascular changes comprise a systolic murmur in the tricuspid area and signs of left calf deep vein thrombosis. Moderate mental retardation was also assessed.

The flexible rectosigmoidoscopy and the enema examination of the large bowel identified polyposis of the whole colon and a rectal stenosis. Pathologic analysis of the specimens obtained from the protrusions revealed the presence of tubular adenomas. On biplane echocardiography a tricuspid insufficiency was diagnosed without any aortic or mitral abnormalities. During abdominal ultrasound and upper digestive endoscopy no extracolonic manifestations were recorded. X-ray films including mandibular radiography did not reveal any changes in bone structure and confirmed the kyphoscoliosis. The ophthalmology examination was normal and no congenital hypertrophy of the retinal pigment epithelium (CHRPE) was detected. No slit-lamp examination was performed. During laparotomy, an invasive rectal adenocarcinoma was discovered and a palliative sigmoidostomy was made. The patient was lost to follow-up 8 months later.

The clinical and rectosigmoidoscopic examination of all first degree relatives of the patient showed no abnormalities at both colonic and extracolonic levels.

Case 2

A 38-year-old Romanian man was referred to the Gastroenterology Department for alternate constipation and diarrhoea. The medical history of the patient was fairly uneventful. Physical examination revealed an unusually tall and thin man (height 192 cm and weight 66 kg), with a great arm span (arm span to height ratio of 1.05). Dental abnormalities (two supernumerary teeth, one in each premolar area of maxilla) and clubbing of fingers and toes were recorded. A diastolic murmur in the aortic area was heard.

On echocardiography, dilatation of the aortic root (45 mm diameter) without aortic regurgitation was detected (Figure 1c). Other findings were a mitral valve prolapse with insignificant regurgitation, a tricuspid valve balonisation and the presence of an atrial septal aneurysm. On colonoscopy, a diffuse carpeting of the large bowel with polyps ranging from 1–2 mm to 3–4 cm in diameter was recorded (Figure 1d). The

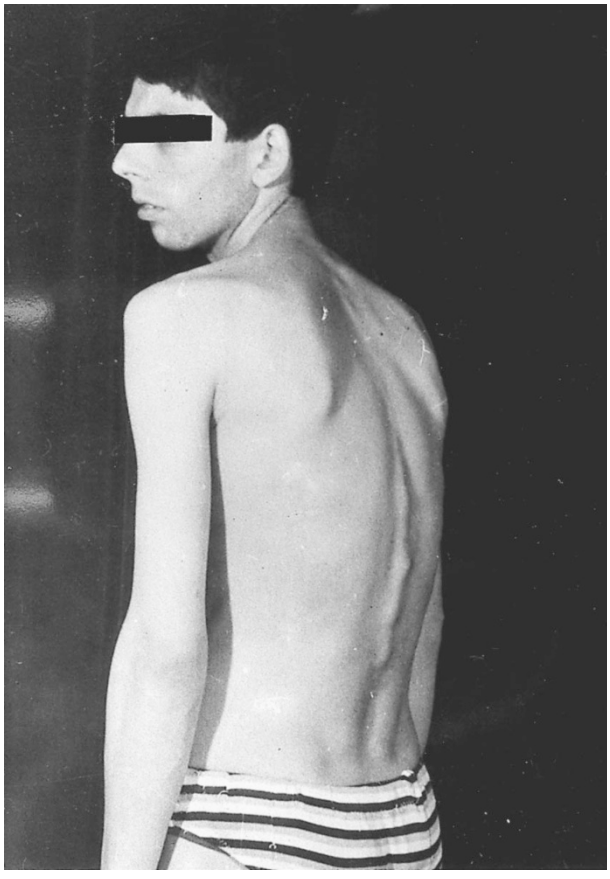

Figure 1a *Thoracic kyphoscoliosis and inferior maxillary retrognathism (partially corrected in childhood)*

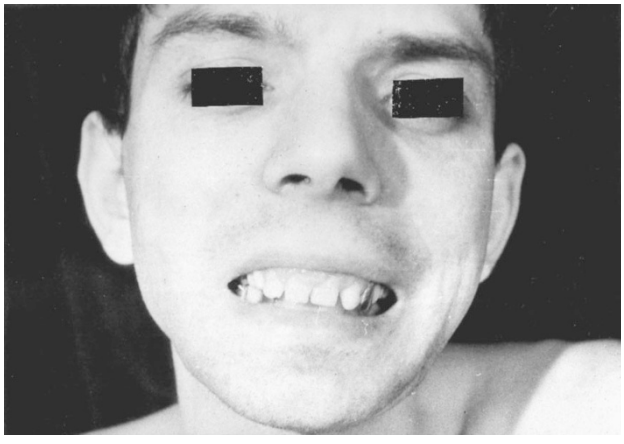

Figure 1b *Facial aspect and dental abnormalities*

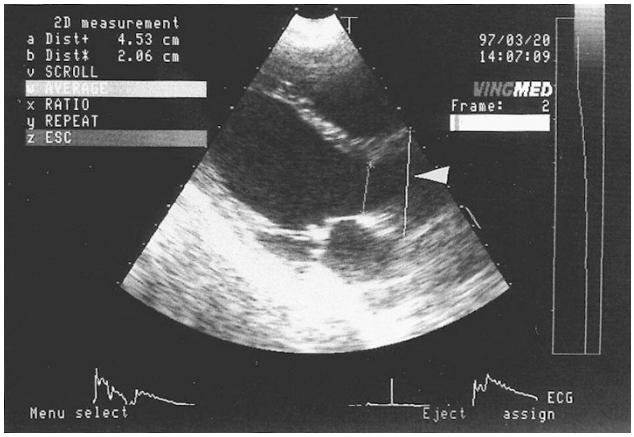

Figure 1c *Biplan echocardiography (parasternal longitudinal section) showing aortic root dilatation (4.53 cm in diameter – see arrow)*

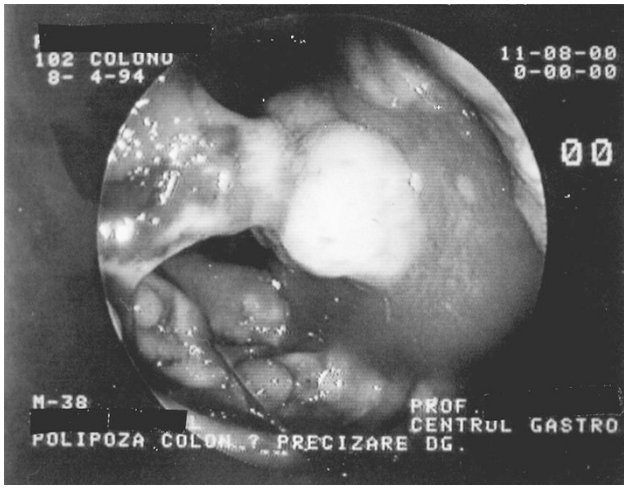

Figure 1d *Colonoscopic image showing diffuse polyposis*

rectum was free of polyps. The biopsy specimens were classified as tubular adenomas. X-ray analysis of the skull and skeleton did not reveal any changes in bone structure. Ophthalmological examination anormal retinal aspect with no abnormalities in the macular region, optic disc and vascular structures. Slit-lamp examination failed to detect any lens abnormality. A prophylactic total colectomy with ileorectoanastomosis was performed. A surgical biopsy from a small mass in the liver revealed a mesenchymal hamartoma. The patient is still alive 4 years after the surgical procedure with no rectal malignant development.

Considering that the patient's mother died at age 34 from an unknown 'digestive cancer' (she refused the diagnostic laparotomy) and was also likely to be affected by FAP, we performed a colonoscopic screening of the only first degree relative available. The clinical and rectosigmoidoscopic examination of the 36-year-old sister also showed the presence of polyposis. She is also tall and thin (height 180 cm and weight 53 kg), but with no other clinical abnormalities on examination and imaging.

Materials and Methods

Cytogenetic and FISH Analysis

Prometaphase and metaphase chromosomes were prepared from phytohemaglutinin-stimulated peripheral blood lymphocytes according to standard protocols.[5] Karyotyping was performed by GTG banding,[6] while FISH was performed as previously described.[5] For the FISH analysis of the *APC* gene, the digoxigenin-labelled cosmid probe cAPC3 was used.[7]

Molecular Analyses

Denaturing gradient gel electrophoresis (DGGE) of APC exons 1–14 and protein truncation test (PTT) analysis of exon 15 were performed as previously described.[8,9] RT-PCR amplifications were performed on total RNA isolated from lymphoblastoid cell lines by RNA InstaPure (Eurogentech, Seraing, Belgium) according to manufacturer's instructions. First strand cDNA synthesis, PCR conditions and the sequence of the RT-PCR primers V58 (exon 3) and V12 (exon 8) are as described elsewhere.[10,11] APC protein analysis was performed by western blot with the monoclonal antibody FE-9 (Oncogene Research Products, Cambridge, MA, USA) as described by the manufacturer.

Results

Cytogenetic Studies

Since the two genes responsible for FAP and Marfan-like conditions, *APC* and *FBN2* respectively, are located on the long arm of chromosome 5, cytogenetic GTG banding analyses were carried out to exclude the presence of gross structural chromosomal rearrangements encompassing both genes. However, no chromosomal abnormalities were observed in either patient or in the first degree relatives analysed (parents, brother and sister of patient 1, and sister of patient 2). Moreover, fluorescence *in situ* hybridisation (FISH) with cosmid clones encompassing the *APC* genomic locus[7] was carried out on EBV-transformed lymphocytes from case 2. Also in this case no abnormalities were observed and both *APC* alleles were present (data not shown).

Molecular Studies

Genomic DNA for mutation analysis was available from case 2 and his first degree relatives only. DGGE analysis of exon 4 revealed an aberrant electrophoretic pattern, indicative of the presence of a nucleotide variant (Figure 2). Sequence analysis of the corresponding PCR fragment revealed the presence of an A to C substitution at position +3 of the exon 4 donor splice site. DGGE analysis of the other available family members confirmed the presence of the mutation in his sister already diagnosed with FAP (I.2) and in two of

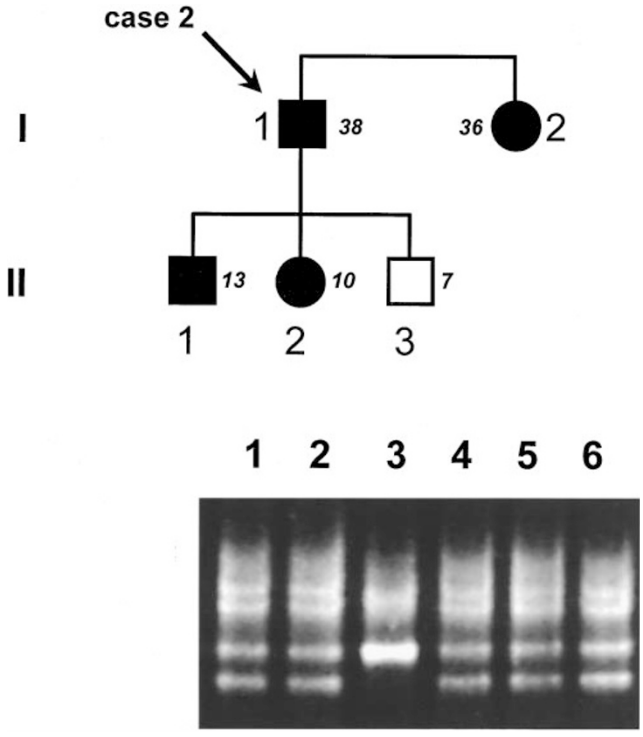

Figure 2 *Partial pedigree of case 2 (age at examination is shown in italics) and corresponding DGGE analysis of* APC *exon 4. The appearance of 4 bands (two homo- and two heteroduplexes) in individuals I.1 (lane 6), I.2 (lane 5), II.1 (lane 1), and II.2 (lane 2), is indicative of the presence of the single nucleotide substitution.*

her three as yet asymptomatic children (II.1 and II.2) (Figure 2).

Next we analysed the *APC* mRNA species from two lymphoblastoid cell lines derived from case 2 and his affected sister by RT-PCR with primers located in exons .3 and 8 (Figure 3a).[8,9] As shown in Figure 3a, in both cases two bands can be observed, namely the expected 935 bp wild type fragment and a 826 bp mutant cDNA. Nucleotide sequence determination of the latter showed that exon 3 is directly connected to exon 5 thus deleting exon 4 and resulting in a frameshift (Figure 3b). The truncated polypeptide predicted from this splice mutant is about 14 kDa. In order to test whether the predicted truncated APC protein is indeed stable *in vivo*, we performed western blot and immunoprecipitation analyses on protein extracts from the lymphoblastoid cell lines derived from case 2 and his affected sister with a monoclonal antibody directed against an NH_2-terminal epitope. No evidence for the presence of the predicted 14 kDa truncated peptide was found (data not shown).

Discussion

FAP can be regarded as a condition of the whole body as affected individuals are at high risk for a broad spectrum of extracolonic manifestations.[1] In spite of their apparently unrelated origin, both of the FAP patients here described presented with the same peculiarity, ie an extracolonic phenotype with several connective tissue abnormalities consistent with a Marfan-like phenotype. To our knowledge, this is the first report of such an association.

Marfan syndrome (MFS) is a highly variable dominant connective tissue disorder, the cardinal features of which affect three systems: skeletal, ocular and cardiovascular.[3] In spite of the fact that the phenotype of the present two cases is indicative of MFS, none of them fulfil the new clinical criteria for the diagnosis of MFS.[12] In the first case, the skeletal abnormalities were more conspicuous than the cardiac ones. In the second patient, the involvement of the cardiovascular system satisfied both a major (dilatation of the ascending aorta) and a minor (mitral valve prolapse with regurgitation) diagnostic criterion.

Other disorders share clinical similarities with FAP or MFS. Gardner syndrome is now known to be an allelic variant of FAP, caused by mutation in the *APC* gene.[1] However, the patients here described were not affected by the osteomas, soft tissue tumours or CHRPE characteristic of Gardner's patients. The absence of the cardiovascular and ocular complications diagnostic of MFS in our Case 1, together with the presence of various skeletal manifestations (especially kyphoscoliosis), raise the question of a missed CCA diagnosis.[12] This is unlikely to be the case as the patient lacked joint contractures and presented moderate hypermobility of all joints. Also, the association of moderate mental retardation and phlebitis in the first patient is indicative of homocystinuria, a diagnosis which cannot be excluded in the absence of biochemical tests. On the other hand, the recurrent phlebitis developed only a few months before hospital admission, can be explained as paraneoplasia in a patient with advanced colorectal cancer.[13]

The data here presented raise one essential question: does the Marfan-like habitus observed in the two polyposis patients result in part from the FAP extracolonic manifestations? Or is it the result of a casual association of FAP and MFS-causing mutations in two apparently unrelated individuals? Considering the incidence of these two conditions among Caucasian populations, the co-existence by chance of FAP with

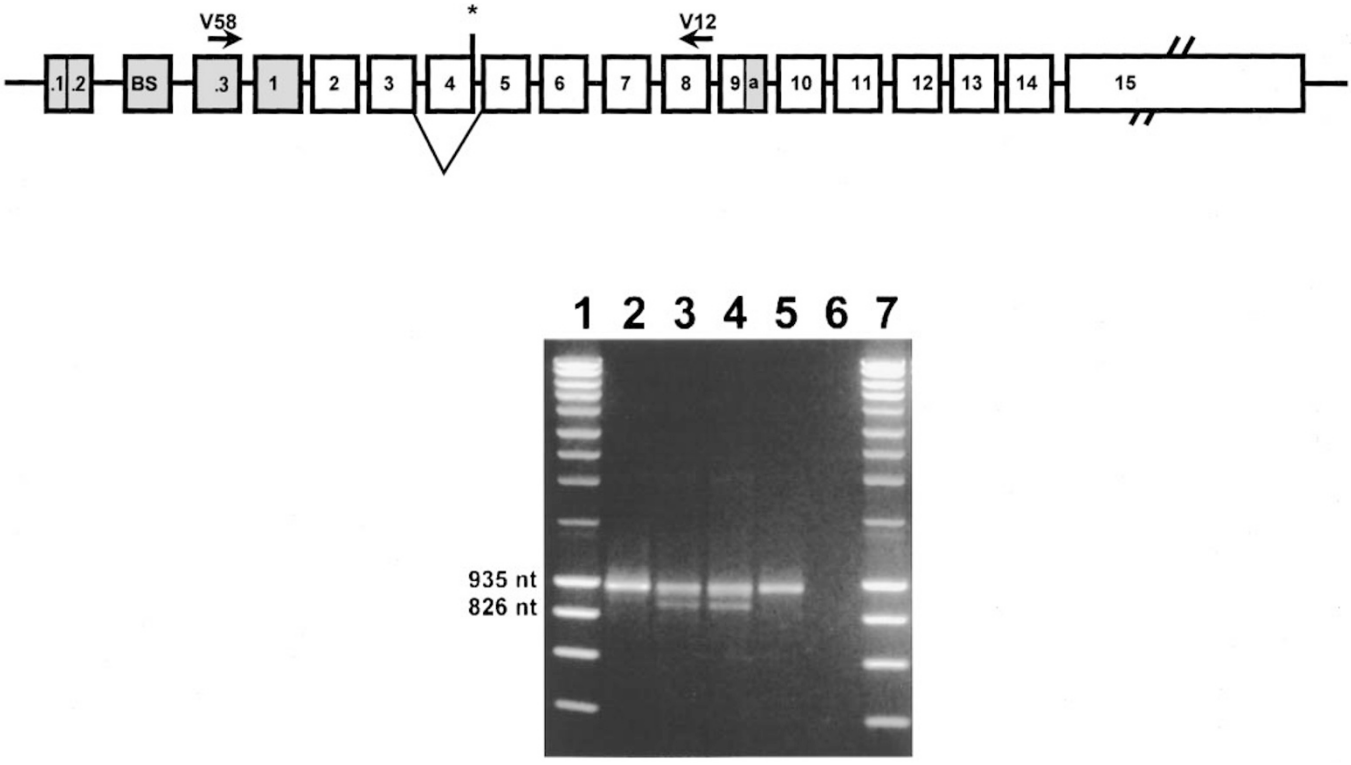

Figure 3a *RT-PCR analysis of total RNA derived from lymphoblastoid cell lines derived from non-affected individuals (lanes 2 and 5), individual I.1 (lane 3) and individual I.2 (lane 4). The schematic exon–intron organisation of the* APC *gene is not to scale. Alternatively spliced exons are depicted as grey boxes. RT-PCR primers V58 (exon .3) and V12 (exon 8) are depicted as arrows.*

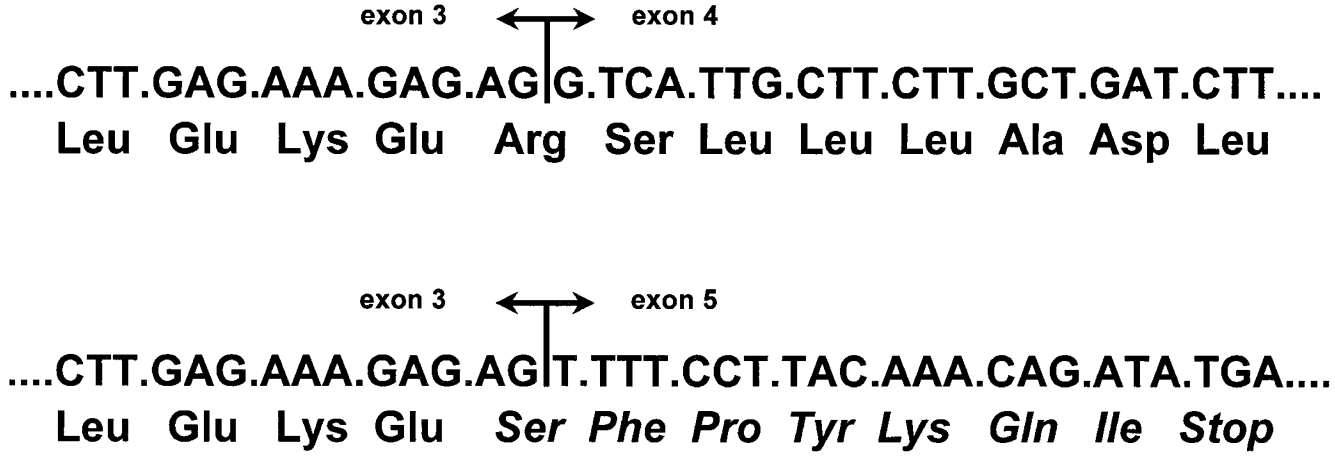

Figure 3b *Nucleotide sequence of the RT-PCR products at the exons 3–4–5 boundaries. From top to bottom: I) wild type sequence; II) mutant PCR product from case 2. The predicted aminoacid sequences are reported below the corresponding nucleotide sequences.*

Marfan syndrome seems highly unlikely (about 10^{-8}). Nevertheless, in the absence of extensive genealogical studies, we cannot exclude that the two cases are related and that the patients here presented are double heterozygous for *APC* and fibrillin-like gene mutations. Alternatively, the combination of *APC* germline mutations with specific genetic and/or environmental modifiers may result in a broad spectrum of connective tissues abnormalities reminiscent of a Marfan-habitus. The latter hypothesis is plausible in view of the well-known correlation between *APC* mutations and connective tissue abnormalities, as shown by the relatively

high risk of mesoderm-derived lesions such as desmoids, osteomas, liver tumours and dental abnormalities among FAP patients.

Although we cannot exclude the presence of a second *APC* mutation *in cis*, the *APC* exon 4 donor splice site mutation found in case 2 is interesting in view of its association with a florid polyposis phenotype. Germline *APC* mutations in exons 1–4 are usually associated with atypical FAP clinical features, namely low polyp multiplicity (< 100) and a delayed age of onset, often referred to as attenuated APC or AAPC.[14] AAPC-causing mutations have also been found in the 3' half of the *APC* gene, often in association with enhanced extra-colonic manifestations.[15] The exon 4 mutation here reported results in a full-blown colonic polyposis (Figure 1d) and, possibly, in atypical MFS-like abnormalities. Analysis of the resulting protein in lymphoblastoid cell lines failed to reveal the expected 14 kDa truncated polypeptide. Again, the complex interaction between the *APC* mutation and different genetic and environmental modifiers might result in unexpected phenotypes.

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