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Germline APC mutation spectrum derived from 863 genomic variations identified through a 15-years medical genetics service to French FAP patients

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ABSTRACT
Heterozygous *APC* germline alteration is responsible for familial adenomatous Polyposis, a colon cancer predisposition with almost complete penetrance. Point mutations generally lead to truncated proteins or no protein at all. They mainly involve exon 3 to codon 1700 (exon 15). The work presented here precisely delineates the *APC* mutation spectrum through a 15-years systematic molecular screening that identified 863 independent alterations in the French population.

COMMUNICATION
Familial adenomatous polyposis (FAP) is a well-described, autosomal dominant inherited syndrome with near complete penetrance associated with *APC* mutation.\[1\] The phenotypic hallmark of FAP is the presence of more than 100 colorectal adenomas in combination with extracolonic manifestations; a subset of *APC* mutation carriers presents with fewer colorectal adenomas, and, with the exception of desmoid tumors, lacks extradigestive manifestation. This variant disease has been initially referred to as “attenuated adenomatous polyposis coli” (AAPC) and more recently included in a clinical entity called “attenuated familial adenomatous polyposis” (AFAP).\[2-3\] We present here the genotypic description of 863 FAP patients who present with a germline *APC* genomic alteration.

Patients referred from the French outpatient genetic clinics for molecular screening to document colonic adenomatous polyposis from 1993 and 2008 were systematically screened for point mutations of the *APC* gene from exon 3 to codon 1700 of exon 15. Negative cases exhibiting at least one extra-intestinal manifestation of FAP, as they were also negative for *MYH* mutations (data not shown), were selected for additional screening, including search for point mutations upstream of exon 3 and downstream of codon 1700 and large genomic rearrangements. All patients or legal representatives signed an informed consent for genetic
analyses related to their disease. DNA was extracted from peripheral blood cells using standard procedure after cell lyses and proteinase K digestion. Several techniques of genomic analysis were successively applied and compared. Exons of the APC gene (NM_000038, NT033772.6) and splicing junctions were analyzed by sequencing after PCR amplification using primers reported by Groden et al.[4] Search for large genomic rearrangements was performed first applying the QMPSF (Quantitative Multiplex PCR of Short Fragments) technique. Results were validated and more precisely characterized by CGH on 244K oligoarrays (Agilent) and a 11,000-oligoarray specially designed, encompassing nucleotides 111,999,686 to 112,306,766 from the chromosome 5 hg17 build. Exons were fully covered by overlapping and duplicated probes, with an average of one oligonucleotide every 12 bases. Introns and adjacent regions were covered with one oligonucleotide every 350 to 420 bases. Sequences analysis was driven using the PhredPhrapConsed package v11 and CGH data were analyzed with CGH-analytics® from Agilent Technologies.

A total of 784 patients had variants predicted to shorten the length of the APC protein. The functional effect of variants observed in 48 additional patients was more ambiguous. In addition to in silico analysis, nucleotide substitutions were tested after PCR-amplification from patient genomic DNA together with approximately 150 bp of flanking sequences then inserted into a splicing reporter minigene according to Tournier et al.[5] Fifteen variants found in 22 patients had an impact on the splicing process and were subsequently classified as deleterious. The g.20,377,206A>T substitution located in exon 0.1, identified in 8 patients, cosegregated in two families with a cumulate LODscore of 5.6. A 40-bp deletion was also found in exon 0.1, that removed one splicing junction. Sixteen unique variants from 17 patients remained unclassified, among which none occurred in highly conserved domains involved in beta-catenin binding or degradation.[6]
Finally a total of 815 point mutations were retained to delineate the \(APC\) mutation spectrum, corresponding to 390 different mutations (Supplementary table 1, Figure 1). All will be referred to the \(APC\) mutation database developed with LOVD v2.0 Build 17, available at http://fap.taenzer.me/home.php?select_db=APC, where 91 are already described. All but 9 mutations were located upstream to codon 1700 thus detected through the standard screening. No mutation was found in exons 1 and 2. Focussing on the 286 non-sense mutations, half (140) involved one of the 15 CGA codons leading within this part of gene (with the exception of codon 348 located in the alternatively transcribed part of exon 9), up to 25 times for codon 213. The majority of insertions were duplications of one nucleotide. Most of the deletions involved short tandem repeats of 1 to 5 nucleotides. The two hot-spots described at codons 1061 and 1309 were involved 56 and 92 times respectively., i.e. one third of the ins/del mutations. One larger deletion was found twice, also flanked by a 5-bp repeat.

Large genomic deletions were detected by QMPSF in 48 patients, i.e. 5.5% of all germline alterations (Figure 1). No duplication was observed. All are also detected with the P043 MLPA kit (MRC-Holland). Thirty-five deletions were studied on CGH arrays. All were confirmed on the dedicated array. Five deletions spanning exon 9 (2 cases) and 11 to 13 (3 cases) were missed on the 244K array, no corresponding oligonucleotide being spotted on this array. The dedicated array allowed the characterization of the deletions boundaries, ranging from 2.7 Kb to over 17 Mb without any recurrent breakpoint as previously reported.[7] In two patients, the deletion of exons 11 to 13 was replaced with a 351-bp Alu Ya5 sequence.

In a sub-group of 341 sporadic patients, parents’ DNAs were available and correct assignation of parentage was confirmed using Amp FlSTR SGM Plus kit (Applied Biosystems), so that it was clear that the mutation identified in the index case arose de novo. This means that at least 40% of the detected pathogenic mutations arose as de novo mutations in the index case, a
proportion slightly lower than that observed in other rare cancer predispositions, but much higher than that usually announced in familial adenomatosis polyposis (FAP).[8] A total of 863 genomic variants were identified through this systematic approach applied to patients exhibiting at least one extra-colonic common manifestation of FAP. No APC genomic alteration was found in 71 cases, leading to a 93% mutation detection rate in FAP. One allelic imbalance was noticed after RT-PCR but the causative genomic event could not be documented. As RNA was not available for the majority of patients, we could stipulate that this phenomenon is probably more frequent and could be investigated in relation to promoter hypermethylation or somatic mosaicism, as possible causes of FAP.[9,10] The presence of desmoid tumour was registered for 110 patients, i.e. 13% as commonly reported. In summary, this experience highlights the strong yield of APC germline mutations in familial adenomatous polyposis. The spectrum of mutations is wide from point mutations to large rearrangements, which can be complex. Finally, the high frequency of de novo mutations should be considered to propose APC screening in MutYH negative cases of sporadic adenomatous polyposis patients even in absence of extradigestive manifestations.

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**LEGEND TO FIGURE 1**

Figure 1: APC germline mutation spectrum

The APC gene is represented from the 5’ genomic part on the left to the Stop codon on the right. Coding exons are shown in boxes. Introns are not dimensioned to scale. Vertical bars indicate mutations according to the number of events found independently; frequent events (more than 20) are countered next to the corresponding position. Nucleotide substitutions within the coding sequence (in black) or involving consensus splicing regions (in grey), and short deletions/insertions are positioned above and under the exons respectively. Large genomic deletions are shown horizontally under the encompassed genomic region.
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