



HAL
open science

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

Arnaud Lagarde, Etienne Rouleau, Anthony Ferrari, Tetsuro Noguchi, Jinghua Qiu, Adrien Briaux, Violaine Bourdon, Virginie Rémy, Pascaline Gaildrat, José Adelaïde, et al.

► To cite this version:

Arnaud Lagarde, Etienne Rouleau, Anthony Ferrari, Tetsuro Noguchi, Jinghua Qiu, et al.. Germline APC mutation spectrum derived from 863 genomic variations identified through a 15-years medical genetics service to French FAP patients. *Journal of Medical Genetics*, 2010, 47 (10), pp.721-10.1136/jmg.2010.078964 . hal-00557398

HAL Id: hal-00557398

<https://hal.science/hal-00557398v1>

Submitted on 19 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Lagarde Arnaud^{1*}, Rouleau Etienne^{2*}, Ferrari Anthony^{1,3**}, Noguchi Tetsuro^{3**}, Qiu Jinghua³, Briaux Adrien², Bourdon Violaine³, Rémy Virginie³, Gaildrat Pascaline⁴, Adélaïde José^{1,3}, Birnbaum Daniel^{1,3}, Lidereau Rosette², Sobol Hagay^{3,5}, Olschwang Sylviane^{1,3}

*/** Equally contributed to the work

Corresponding author : Dr. Sylviane Olschwang

Institut Paoli-Calmettes, Centre de Recherche en Cancérologie de Marseille, 232 boulevard Sainte-Marguerite 13009 Marseille

Sylviane.olschwang@inserm.fr ; tel 33(0)491223406, fax 33(0)491223544

1 Centre de Recherche en Cancérologie de Marseille INSERM UMR891, Marseille France

2 Centre René-Huguenin, Saint-Cloud France

3 Institut Paoli-Calmettes, Marseille France

4 INSERM U614

5 Université Aix-Marseille II, France

Key words : *APC* gene, germline mutation, familial adenomatous polyposis, mutation spectrum, genomic rearrangements

Word count : 1041

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 F1STR 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 adenomatous 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.

ACKNOWLEDGMENTS

This work was supported by the French national cancer institute INCa through a grant dedicated to the PHRATries network (Prédispositions Héritaires Rares Aux Tumeurs, Réseau d'Identification Et de Soins). We are grateful to the French clinicians who faithfully entrust our institution with molecular genetics tests of the *APC* gene.

REFERENCES

- [1] Galiatsatos P, Foulkes WD. Familial adenomatous Polyposis. *Am J Gastroenterol.* 2006;101:385-398.

- [2] Spirio L, Olschwang S, Groden J, Robertson M, Samowitz M, Joslyn G, Gelbert L, Thliveris A, Carlson M, Otterud B, Lynch H, Watson P, Lynch P, Laurent-Puig P, Thomas G, Leppert M, White M. Alleles of the APC gene : an attenuated form of familial polyposis. *Cell*. 1993;75:951-957.
- [3] Knudsen AL, Bisgaard ML, Bülow S. Attenuated familial adenomatous polyposis (AFAP). A review of the literature. *Fam Cancer*. 2003;2:43-55.
- [4] Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, Seargent L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*. 1991;66:589-600.
- [5] Tournier I, Vezain M, Martins A, Charbonnier F, Baert-Desurmont S, Olschwang S, Wang Q, Buisine MP, Soret J, Tazi J, Frébourg T, Tosi M. A large fraction of unclassified variants of the mismatch repair genes MLH1 and MSH2 is associated with splicing defects. *Hum Mutat*. 2008;29:1412-1424.
- [6] Liu J, Xing Y, Rinds TR, Zheng J, Wenqing X. The third 20 amino acid repeat is the tightest binding site of APC for b-catenin. *J Mol Biol*. 2006;360:133-144.
- [7] Aretz S, Stienen D, Uhlhaas S, Pagenstecher C, Mangold E, Caspari R, Propping P, Friedl W. Large submicroscopic genomic APC deletions are a common cause of typical familial adenomatous polyposis. *J Med Genet*. 2005;42:185-192.
- [8] Aretz S, Uhlhaas S, Caspari R, Mangold E, Pagenstecher C, Propping P, Friedl W. Frequency and parental origin of de novo APC mutations in familial adenomatous Polyposis. *Eur J Hum Genet*. 2004;12:52-58.
- [9] Romero-Gimenez J, Dopeso H, Blanco I, Guerra-Moreno A, Gonzalez S, Vogt S, Aretz S, Schwartz S Jr, Capella G, Arango D. Germline hypermethylation of the APC

promoter is not a frequent cause of familial adenomatous Polyposis in APC/MUTYH mutation negative families. *Int J Cancer*. 2008;122:1422-1425.

[10] Hes FJ, Nielsen M, Bik EC, Konvalinka D, Wijnen JT, Bakker E, Vasen HF, Breuning MH, Tops CM. Somatic APC mosaicism: an underestimated cause of polyposis coli. *Gut* 2008;57:71-76.

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.

1. Licence statement required

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in JMG and any other BMJPGJL products and sublicences such use and exploit all subsidiary rights, as set out in the licence proposed at <http://group.bmj.com/products/journals/instructions-for-authors/licence-forms> .

2. Competing Interests: None declared

