Somatic APC mosaicism

Luigi Ricciardiello, MD
Department of Medical and Surgical Sciences
University of Bologna, Italy
Germline mutations in the *APC* or *MutYH* genes cause familial adenomatous polyposis (FAP) or *MutYH*-associated polyposis (MAP), respectively. Kinzler et al, 1991; Croitoru 2004; Aretz 2006

Recently, inherited mutations in *POLE, POLD1, NTHL1* and *MSH3* genes have been associated to different forms of polyposis. Palles et al, 2013; Weren et, 2015; Adam et al, 2016

However, ~30-50% of polyposis remains genetically unsolved.
Frequent clinical scenario - Challenge

• The majority of the cases referred for germline mutation are patients with 5–20 adenomas or without a family history of FAP.

• This group is genetically heterogeneous and only a fraction of these cases are germline APC, MUTYH or other predisposing mutations responsible for the phenotype.

• In 10–25% of the index patients with FAP, a de novo APC mutation is identified.

Kanth et al. Am J Gastroenterol. 2017;112(10):1509-1525
Frequent clinical scenario - Challenge

• How should we tackle patients with unexplained predisposition to continuously grow multiple adenomas?

• Endoscopically managed as FAP but what is the risk for family members?
Mosaic and Mosaicism

- In art, a **mosaic** is a piece of art or image made from the **assembling of small pieces** of colored glass, stone, or other materials.
- In genetics, a **mosaic**, or **mosaicism**, is due to the presence of **two or more populations of cells with different genotypes** in one person who has developed from a single fertilized egg.
Somatic Mosaicism as a cause of diseases

Somatic mosaicism being recognized due to a high de novo mutation:

- Hemophilia
- Duchenne muscular dystrophy
- Neurofibromatosis 1 and 2
- Tuberous sclerosis
- von Hippel-Lindau disease
- Retinoblastoma
- Sporadic early onset Alzheimer’s disease
- Autism

Adenomatous Polyposis

O’Roak BJ, Nat Genet 2011, 43:585-589
Sanders SJ, Neuron 2011, 70:863-885
Embrioblast separation into layers

https://opentextbc.ca Embriobyc Development
Somatic Mosaicism and Cancer

Vijg J. Current Opinion in Genetics & Development 2014, 26:141–149
Somatic mutations are part of the logic of life. But while germ line mutagenesis is a sine qua non for the perpetuation of life, mutations in the soma are not needed and could very well lead to an error catastrophe.

Early errors can lead to disaster later on
Somatic Mutations During Embryogenesis

Finding Somatic APC mosaicism

75 cases with an assumed or confirmed de novo mutation. Prescreening methods (protein truncation test [PTT], DHPLC) indicated the presence of somatic mosaicism in eight cases (11%).

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Exon</th>
<th>Codon</th>
<th>Mutation</th>
<th>Blood</th>
<th>Normal mucosa</th>
<th>Adenomas</th>
<th>Age at diagnosis (years)</th>
<th>Mode of diagnosis</th>
<th>Number of colorectal polyps</th>
<th>Colorectal adenoma distribution</th>
<th>CRC at diagnosis</th>
<th>Duodenal adenomas</th>
<th>CHRPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>563</td>
<td>6</td>
<td>236</td>
<td>c.646G&gt;T; p.Arg216X</td>
<td>53%; 39%</td>
<td>n.a.</td>
<td>n.a.</td>
<td>50</td>
<td>Screening</td>
<td>&gt; 50</td>
<td>Predominantly proximal</td>
<td>No</td>
<td>No</td>
<td>No (53 years)</td>
</tr>
<tr>
<td>1374</td>
<td>4</td>
<td>293</td>
<td>c.847C&gt;T; p.Arg282X</td>
<td>40%; 42%</td>
<td>n.a.</td>
<td>66%; 80%</td>
<td>49</td>
<td>By chance</td>
<td>&lt; 100</td>
<td>Mostly proximal and distal</td>
<td>No</td>
<td>No</td>
<td>Multiple (17 years)</td>
</tr>
<tr>
<td>1312</td>
<td>5A</td>
<td>793</td>
<td>c.2307delG;p.Ala766 deletes</td>
<td>73%; 77%</td>
<td>n.a.</td>
<td>&gt; 100%;</td>
<td>37</td>
<td>Symptoms</td>
<td>50</td>
<td>Proximal</td>
<td>No</td>
<td>No</td>
<td>4 adenomas (28 years)</td>
</tr>
<tr>
<td>1312</td>
<td>5E</td>
<td>1127</td>
<td>c.3379C&gt;T; p.Gln1130X</td>
<td>40%; 44%</td>
<td>n.a.</td>
<td>71%; 72%</td>
<td>26</td>
<td>Symptoms</td>
<td>&gt; 50</td>
<td>Predominantly proximal</td>
<td>No</td>
<td>No</td>
<td>Multiple (60 years)</td>
</tr>
<tr>
<td>840</td>
<td>5E</td>
<td>1127</td>
<td>c.3379C&gt;T; p.Gln1130X</td>
<td>50%; 44%</td>
<td>n.a.</td>
<td>71%; 72%</td>
<td>37</td>
<td>Symptoms</td>
<td>&gt; 100</td>
<td>Mostly proximal and distal</td>
<td>No</td>
<td>No</td>
<td>Multiple (60 years)</td>
</tr>
<tr>
<td>477</td>
<td>15F</td>
<td>1152</td>
<td>c.3454C&gt;T; p.Glu1152X</td>
<td>27%; 29%</td>
<td>n.a.</td>
<td>71%; 72%</td>
<td>14</td>
<td>Symptoms</td>
<td>&gt; 100</td>
<td>Whole colorectum</td>
<td>No</td>
<td>No</td>
<td>No (41 years)</td>
</tr>
<tr>
<td>755</td>
<td>15G</td>
<td>1309</td>
<td>c.3925G&gt;T; p.Glu1309X</td>
<td>15%; 17%</td>
<td>n.a.</td>
<td>55%; 63%</td>
<td>7</td>
<td>Symptoms</td>
<td>&gt; 100</td>
<td>Whole colorectum</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>722</td>
<td>15G</td>
<td>1309</td>
<td>c.3925G&gt;T; p.Glu1309X</td>
<td>15%; 17%</td>
<td>n.a.</td>
<td>55%; 63%</td>
<td>41</td>
<td>Symptoms</td>
<td>&gt; 100</td>
<td>Whole colorectum</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
</tr>
<tr>
<td>1058</td>
<td>15H</td>
<td>1644</td>
<td>c.4301_4394delAG; p.Glu1484 Val608X</td>
<td>22%; 24%</td>
<td>35%; 45%</td>
<td>n.a.</td>
<td>21</td>
<td>Symptoms</td>
<td>&gt; 200</td>
<td>Whole colorectum</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

---

4/8 patients presented with an attenuated course (age at diagnosis 48 years and/or < 100 colorectal adenomas)

Aretz S et al. HUMAN MUTATION 2007; 28(10): 985-992
APC mosaicism in 242 families with germline APC mutations

Classic FAP is defined when a patient has >100 colorectal adenomas with early manifestations (i.e., 30–40 years of age). Attenuated FAP exerts a more variable phenotype including patients with a smaller number of adenomas (<100), and/or at a more advanced age of diagnosis.

10 mosaic cases (4%)
Finding a needle in a haystack

• Mutations present in less than 10% of all cells in a tissue difficult to pick up based on adopted technologies

• In previous studies somatic mosaicism could only be detected because the mutation occurred early during development thereby comprising a sizable fraction of cells

Solutions:

• Use stringent criteria to screen for somatic mosaicism

• Look for mosaicism in adenomas (significant number)

• New technologies
Low-level APC mutational mosaicism as a cause of adenomatous polyposis

Table 1: Detailed histological findings and results of APC mutation screening in blood and polyph samples of the seven cases with mosaic APC mutations.

Somatic APC mosaicism → Dutch experience

- Six patients with 5–20 adenomas, 18 patients with 21-100 adenomas, one patient with >1000 adenomas, two patients with multiple primary colorectal carcinomas and two positive controls.
- At least two adenomas or carcinomas with sanger, HRMA and NGS. Extension with NGS.
- 9 of the 18 patients with 21-100 (50%) and two positive controls with somatic mosaicism

Jansen AM et al. Gastroenterology 2017;152:546–549
Somatic APC mosaicism – Our experience

Inclusion criteria:
>20 adenomatous polyps by age 35 years old or
>50 adenomatous polyps by age 55 years
and
APC and/or MutYH genes negative tests

**Somatic APC mosaicism – Our experience**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age*</th>
<th>Polyps</th>
<th>Additional clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F</td>
<td>32</td>
<td>&gt;50</td>
<td>No</td>
</tr>
<tr>
<td>P2</td>
<td>M</td>
<td>24</td>
<td>&gt;100</td>
<td>No</td>
</tr>
<tr>
<td>P3</td>
<td>F</td>
<td>47</td>
<td>&gt;150</td>
<td>Duodenal adenomas; Bilateral sensorineural hearing loss; Diabetes mellitus type II</td>
</tr>
<tr>
<td>P4</td>
<td>M</td>
<td>23</td>
<td>&gt;20</td>
<td>No</td>
</tr>
<tr>
<td>P5</td>
<td>M</td>
<td>29</td>
<td>&gt;20</td>
<td>Proctocolectomy; 2 synchronous adenocarcinomas</td>
</tr>
<tr>
<td>P6</td>
<td>M</td>
<td>54</td>
<td>&gt;50</td>
<td>Proctocolectomy; 2 synchronous adenocarcinomas</td>
</tr>
<tr>
<td>P7</td>
<td>M</td>
<td>55</td>
<td>&gt;50</td>
<td>Total colectomy; Urothelial Cancer</td>
</tr>
<tr>
<td>P8</td>
<td>F</td>
<td>40</td>
<td>&gt;50</td>
<td>Sub-total colectomy</td>
</tr>
</tbody>
</table>

* Age at diagnosis.

Screening for APC gene somatic mutations on colonic adenomatous tissues (n=8)

Yes Mutation (n=7)

Screening for APC mosaicism on at least 4 independent FFPE adenomatous polyps and 2 normal mucosa samples

No Mosaicism (n=3)

Yes Mosaicism (n=4)

APC gene mosaicism extension on other tissues
APC gene second hit search
WES on DNA from peripheral blood to exclude known polyposis genes

No Mutation (n=1)

In one patient (P8) no causative mutation in the APC gene was found in adenomatous tissue.

In 3/8 patients (P5-P7) a causative mutation in the APC gene was found in one sample but was absent in other FFPE adenomatous polyps of the same patient.

In 4/8 patients (P1-P4) a causative mutation in the APC gene was found in at least 4 independent adenomatous polyps => MOSAICISM.
Mosaicism was confined to the colon in 3 out of 4 patients (P1, P2 and P4); in patient P3 it extended to the duodenum and saliva. No extension in lymphocytes

Factors influencing detection

- Size and type of the clone of mutant mosaic cells
- Detection method → highly sensitive
- Adopted strategy: patient selection → number of polyps → test extension

Erickson RP. Current Opinion in Genetics & Development 2014, 26:73–78
Campbell IM et al. Trends Genet. 2015 July ; 31(7): 382–392
Open questions

Is the site of mutation determining disease severity?

• Mosaicism is frequently associated with a milder clinical manifestation.

• Clinical expression from mild to severely affected, based on the degree and distribution of mosaicism (mainly in the colon)

• Possible effect due to environmental factors?

Farrington SM, Dunlop MG. Am J Hum Genet 64:653–658
Somatic Mutations During Embryogenesis

Adenomatous polyposis based on APC somatic mutations

Tuohy and Burt Gut  2008; 57 (1): 10-12
Open questions

How should we manage patients and families?

• If only endoderm involved but no lymphocytes or ectodermal tissue involved → risk of transmission is low → Testing children anyway?

• When somatic mosaicism is found in a case of attenuated FAP → any affected children may be more severely affected → testing younger generation important

Other hereditary GI cancer syndromes due to somatic mosaicism?
CONCLUSIONS

• Somatic APC mosaicism is more frequent than previously thought and the cause of a significant (up to 50%) proportion of adenomatous polyposis with no family history.

• Restrictive selection criteria (patient age and number of polyps) could improve the identification of mosaic APC patients.

• Collection of multiple samples and use of highly sensitive technologies should be performed for the detection of mosaic APC patients.
ACKNOWLEDGEMENTS

• S.ORSOLA-MALPIGHI HOSPITAL
  Gastroenterology Unit
  Giulia Piauzzi
  Franco Bazzoli
  Pathology Unit
  Tiziana Balbi

Medical Genetic Unit
  Daniela Turchetti
  Michele Ciavarella
  Sara Miccoli
  Tommaso Pippucci
  Elena Bonora
  Francesco Buscherini
  Flavia Palombo
  Roberta Zuntini

• UNIVERSITY OF BOLOGNA
  Anna Prossomariti
  Claudio Ceccarelli

• IST-GENOA
  Viviana Gismondi
  Liliana Varesco

•GRANT SUPPORT
  Programma di Ricerca Regione-Università 2010-2012 Regione Emilia Romagna
  -Bando Giovani Ricercatori "Alessandro Liberati"- PRUa1GR-2012-007"