Molecular tumor testing in patients with Lynch-like syndrome reveals a de novo mosaic variant of a mismatch repair gene transmitted to offspring

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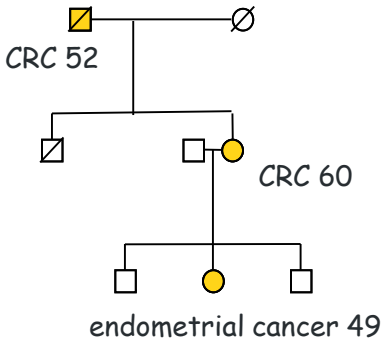
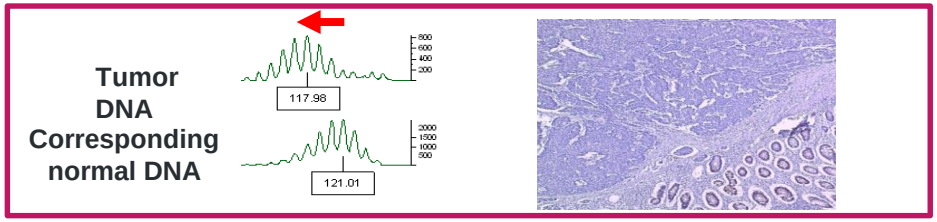
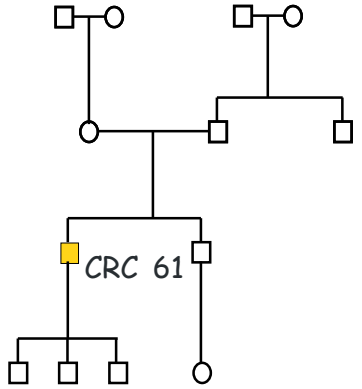
Lynch like syndrome

Somatic results in favor of LS (MSI /loss of expression by IHC)







With no germline pathogenic variant found
(Excluding somatic methylation of *MLH1* promotor)

- Technical limits (splicing mutation, mutation in regulatory elements…)
- Other genes mimicking LS at a somatic level (*POLE/POLD1, MUTYH*…)
- Other ?

LS is not excluded
No presymptomatic test in relatives
Screening as LS

Double somatic events

Especially if
- « Old » age at diagnosis
- No familial history of cancer

Sporadic case
No LS
Stop screening as LS

Tumoral analysis in Lynch-like patients

Fam Cancer. 2013 Mar;12(1):27-33

I. Sourrouille et al.

Table 2 Summary of tumoral hits found in *MLH1* in patients with *MLH1* loss of expression

	Somatic hit no 1	Somatic hit no 2	Diagnosis
Patient no 1	c.2155insT (p.Ile719TyrfsX4)	–	–
Patient no 3	c.872dupT (p.Leu292ProfsX15)	–	–
Patient no 5	c.5C>A (p.Ser2X)	–	–
Patient no 7	c.588delA (p.Lys196AsnfsX6)	c.676C>T (p.Arg226X)	Double somatic mutation

Table 3 Summary of tumoral hits found in *MSH2* in patients with *MSH2* loss of expression

	Somatic hit no 1	Somatic hit no 2	Diagnosis
Patient no 8	c.942 + 3A>T	–	–
Patient no 9	c.664delA (p.Ile222PheX2)	c.1147C>T (p.Arg383X)	Double somatic mutation
Patient no 10	c.2541delA (p.Lys847fsX44)	Deletion *MSH2* and *EpCAM*	Somatic mosaicism
Patient no 11	c.1165C>T (p.Arg389X)	–	–
Patient no 12	c.2038C>T (p.Arg680X)	c.942 + 3A>T	Lynch syndrome
Patient no 14	c.645 + 1G>A (alternate splicing)	c.1069G>T (p.Glu357X).	Double somatic mutation
Patient no 16	c.1823-1827delGCTTT	–	–
Patient no 17	Deletion of *MSH2* and *MSH6*	–	–

18 CRC (parafin) MSI/loss of expression with no germline mutation

Guillerm et al. EJHG accepted

Patient n°	cancer type (age)	Somatic results of MMR analysis	LOH
1	CRC (59)	*MLH1* c.794 G>C, p.(Arg265Pro) [77%]	*MLH1*
2	CRC (39)	WT	WT
3	CRC (56)	*MLH1* c.134_135dup, p.(Ser46Glnfs*5) [41%]	*MLH1*
4	CRC (35)	*MLH1* c.677+1G>A, p.? [71%]	*MLH1*
5	CRC (61)	*MLH1* c.790+1G>A, p.? [14%]	WT
6	CRC (60)	*MLH1* c.1219C>T, p.(Gln407*) [60%]	WT
7	CRC (33)/UC (38)	WT	WT
8	CRC (63)	WT	NA
9	CRC (50)	WT	WT
10	CRC (78)	*MSH6* c.361del, p.(Arg121Alafs*28) [27%]	WT
11	CRC (57)	*MSH2* c.1165C>T, p.(Arg389*) [28%]	*MSH2*
12	CRC (28)	*MSH2* c.1770dup, p.(Pro591Thrfs*7) [10%]	NA
13	CRC (24)	*MSH6* c.3261del, p.(Phe1088Serfs*2) [18%]	WT
14	CRC (64)	*MSH2* c.1738G>T, p.(Glu580*) [21%]	*MLH1*
15	EC (45)/CRC (60)	*MSH2* c.1269del, p.(Lys423Asnfs*15) [37%]	*MSH2*
16	CRC (46)	WT	*MLH1*

16 CRC (frozen) MSI/loss of expression with no germline mutation

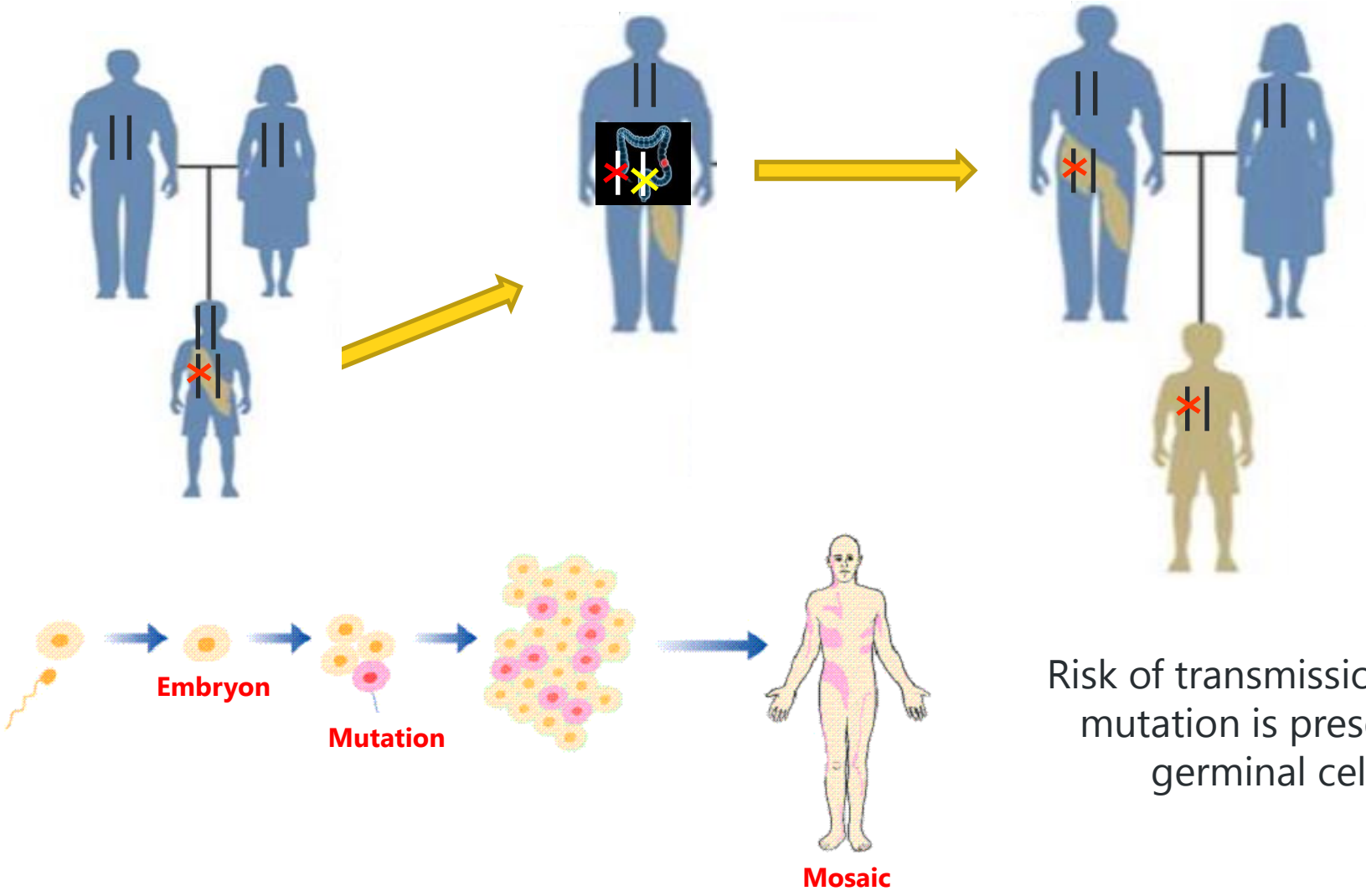
If one or two event(s) in tumor

- Re –analyze of germline results by NGS if done in Sanger seq
- Targeted search of identified mutations in adjacent normal tissue

10 « double somatic events» cases
No risk of transmission

2 germline *de novo* mosaic cases

de novo mutation at a mosaic state

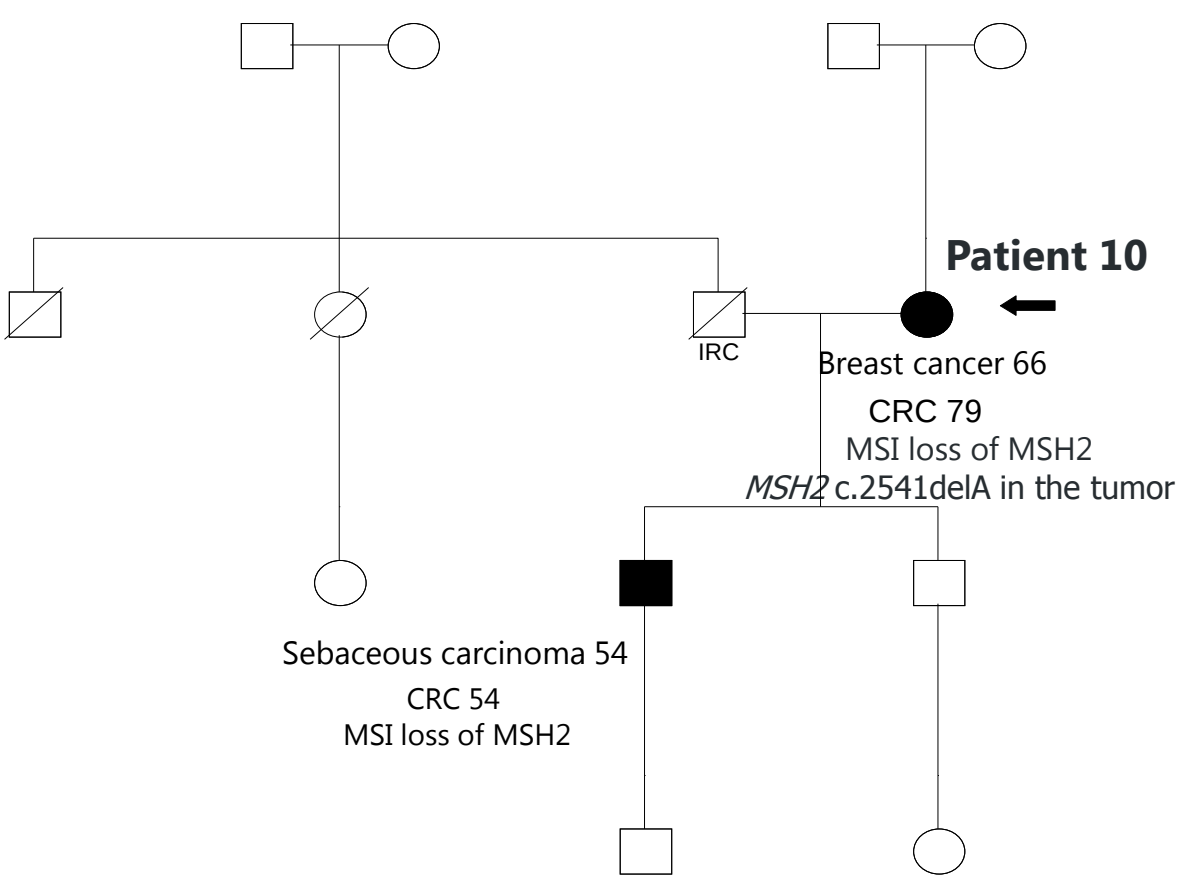


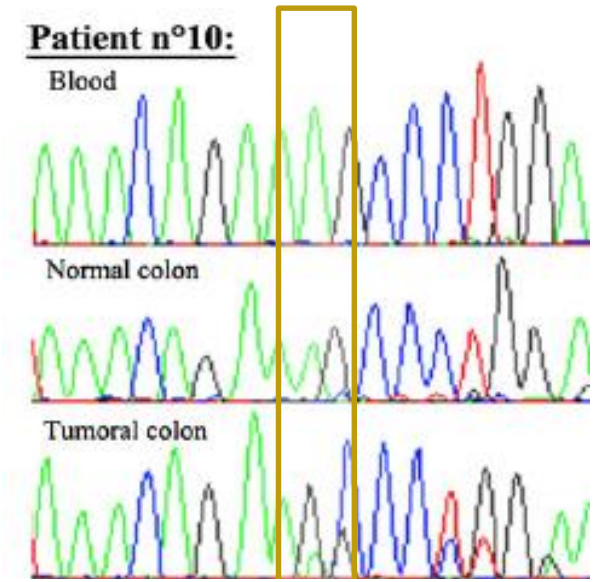
Risk of transmission if the mutation is present in germinal cells

The frequency of mosaic patients
is related to the frequency of *de novo* mutations (3 % in Lynch syndrome)

1st case of mosaic *de novo* mutation with transmission to offspring

Sourrouille I et al; Fam cancer 2013



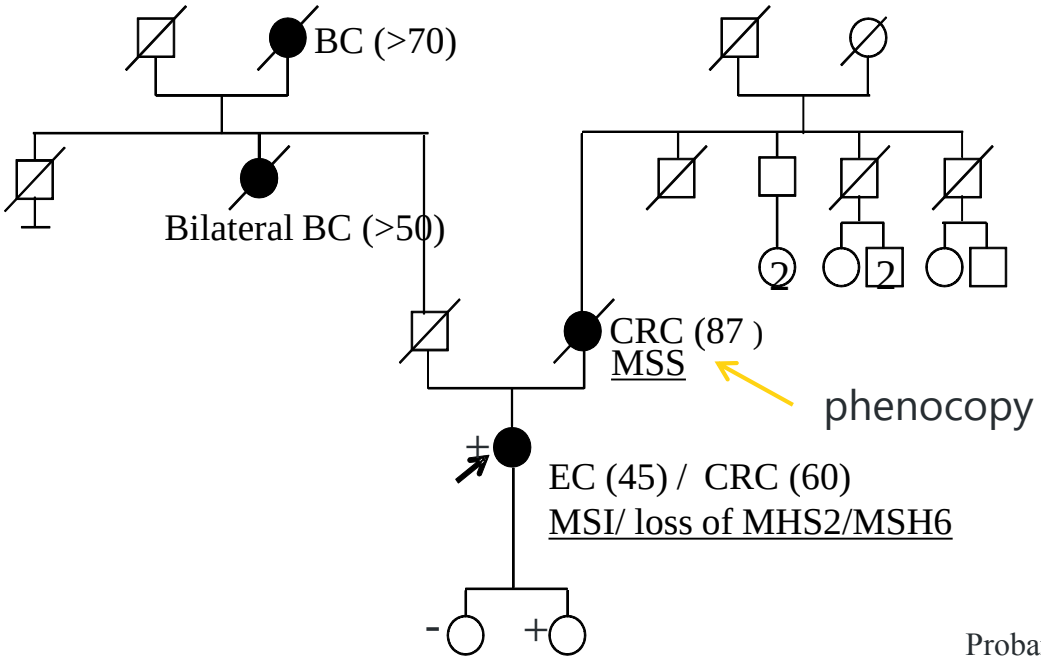


Deep NGS sequencing targeted on the mutation in the lymphocytes :

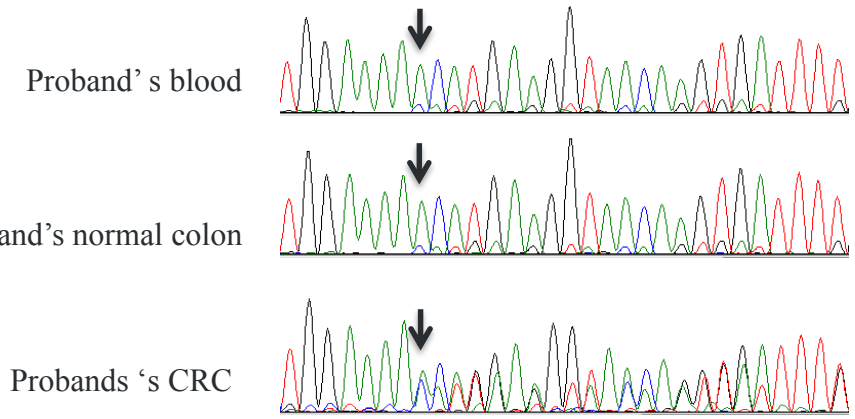
1.7% of reads (7000X)

2nd case of mosaic *de novo* mutation with transmission to offspring

Guillerm et al. EJHG accepted



Sanger sequencing
weak signal corresponding to the frameshift variant slightly higher than the background noise



Regular Sanger sequencing failed to identifie a deleterious variant

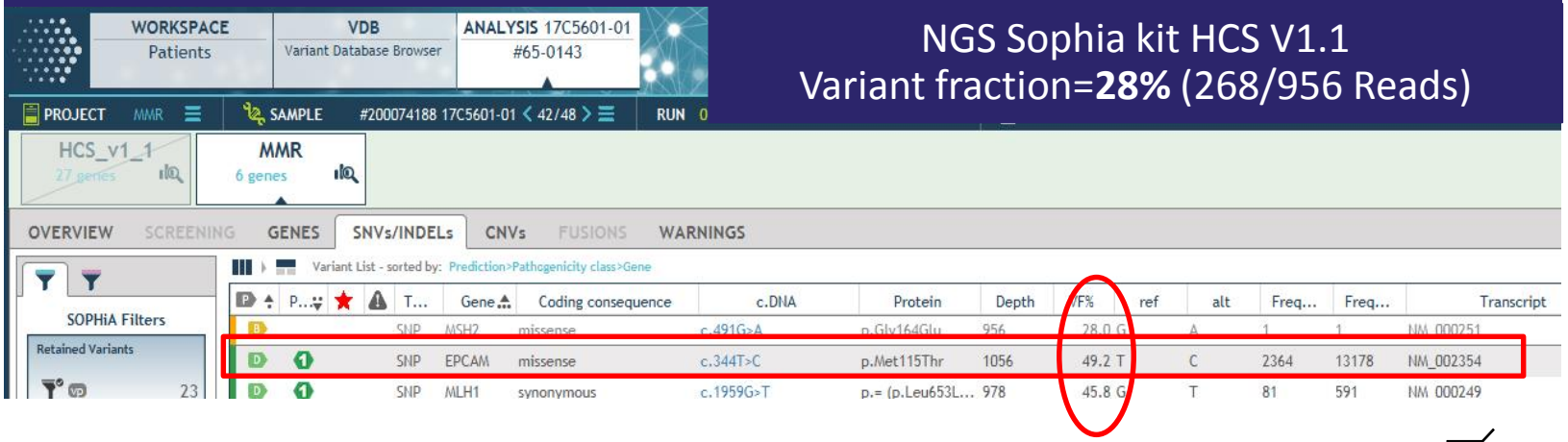
CRC by NGS :
MSH2 : c.1269delA,p.Lys423Asnfs*15 (37%)

Blood by NGS :
MSH2 : c.1269delA,p.Lys423Asnfs*15 (9%)

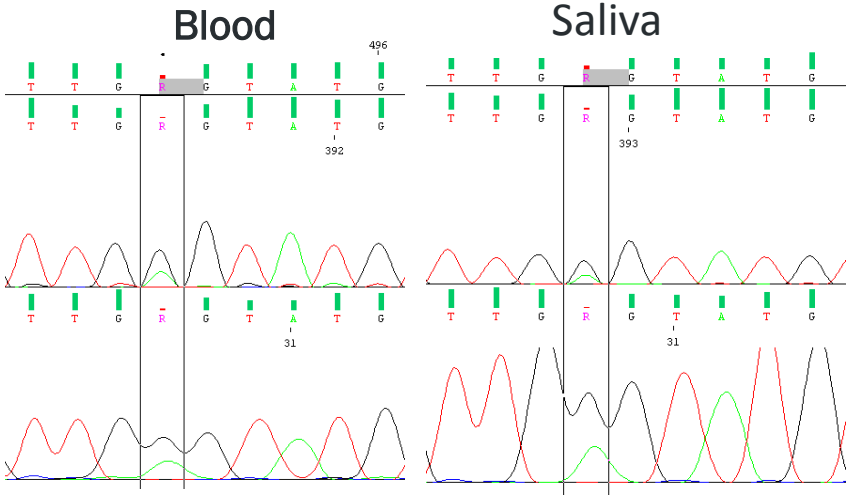
3rd case of mosaic *de novo* mutation

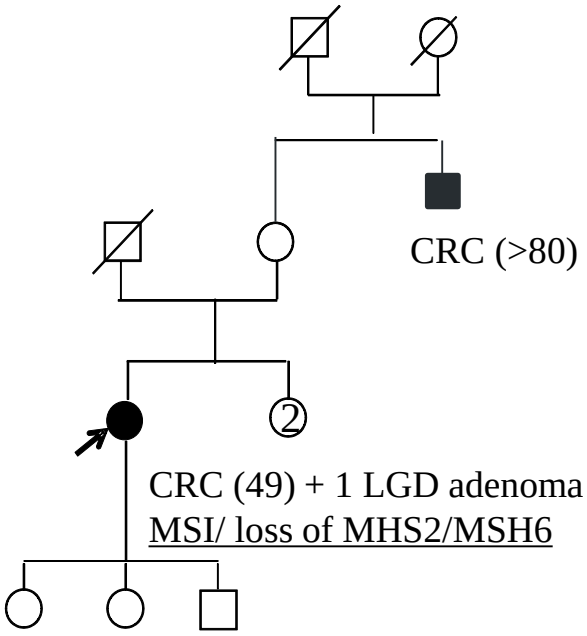
MSH2 : c.491G>Ap.(Gly164Glu)

NGS Sophia kit HCS V1.1
Variant fraction=**28%** (268/956 Reads)



SANGER sequencing





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What to do with « Lynch like » patients ?

The use of NGS techniques will facilitate the detection of mosaics in germline DNA (sufficient depth of reading in order to lower the validation threshold of variant allele frequencies)

Focus on the analysis of the youngest generations if several generations present with cancer/ advanced adenomas

Discuss to perform a **mutation search at the tumor level**

- systematically ?
- according to certain criteria (age, absence of family history, ...)?

Limits : availability of the tumor, quality of the tumor,
but techniques improve with development of theranostic analysis...

If a tumoral event is found

- Targeted search in normal tissue / lymphocytes with deep sequencing
- **Targeted search in offspring**

Identification of a mosaic case of Lynch Syndrome will have **significant impact on screening for both patient and relatives**

Thank you

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