Allele

JK

2019 9 27

BRCA result

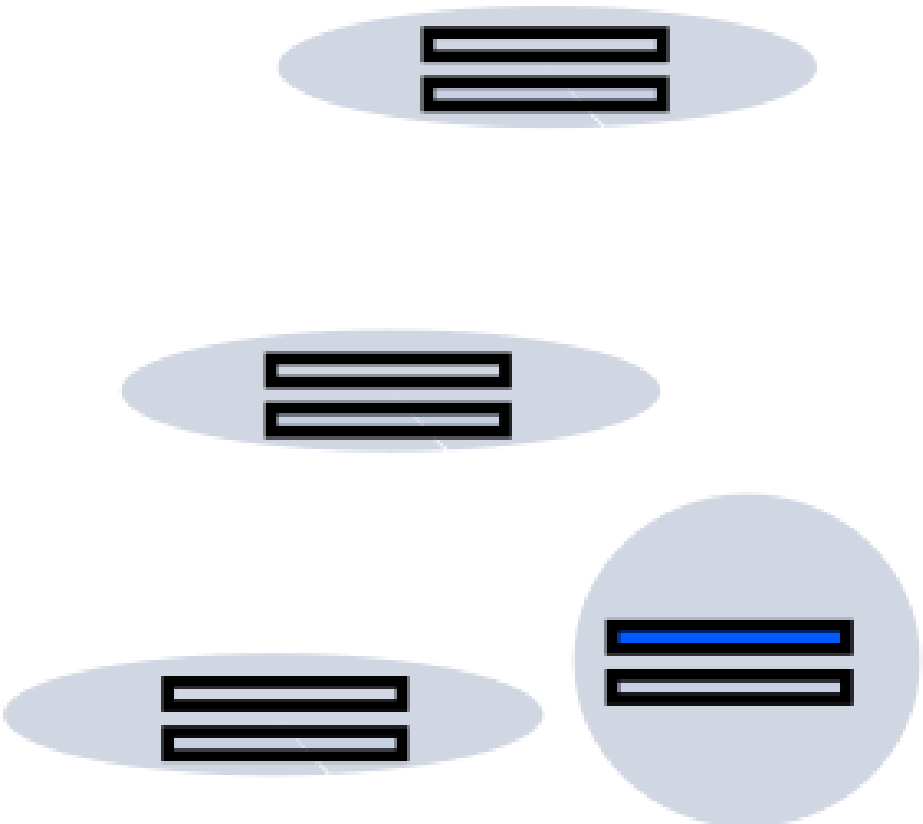
- BRCA1 mutation: Positive - p.Glu649Ter (c.1945G>T)
- Variant allele frequency 63.84%
- Tumor cell percentage: 70%
- BRCA negative in blood sample

Question

Is it possible ***64% allele frequency*** *of somatic mutation?*

Variant allele frequency in clinical tumor sample

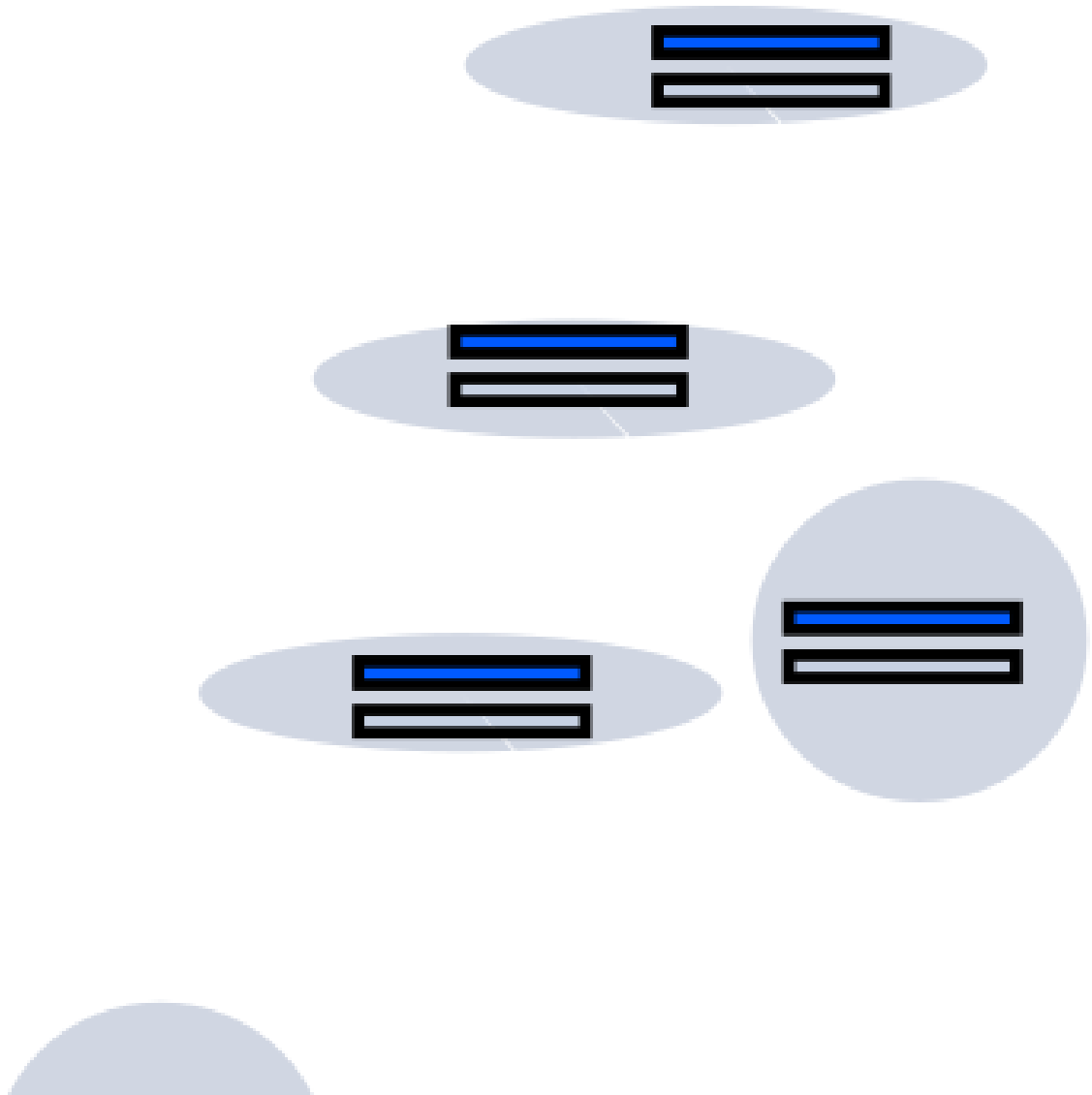
$$Allele frequency \approx Readcount$$



Variant allele frequency in clinical tumor sample

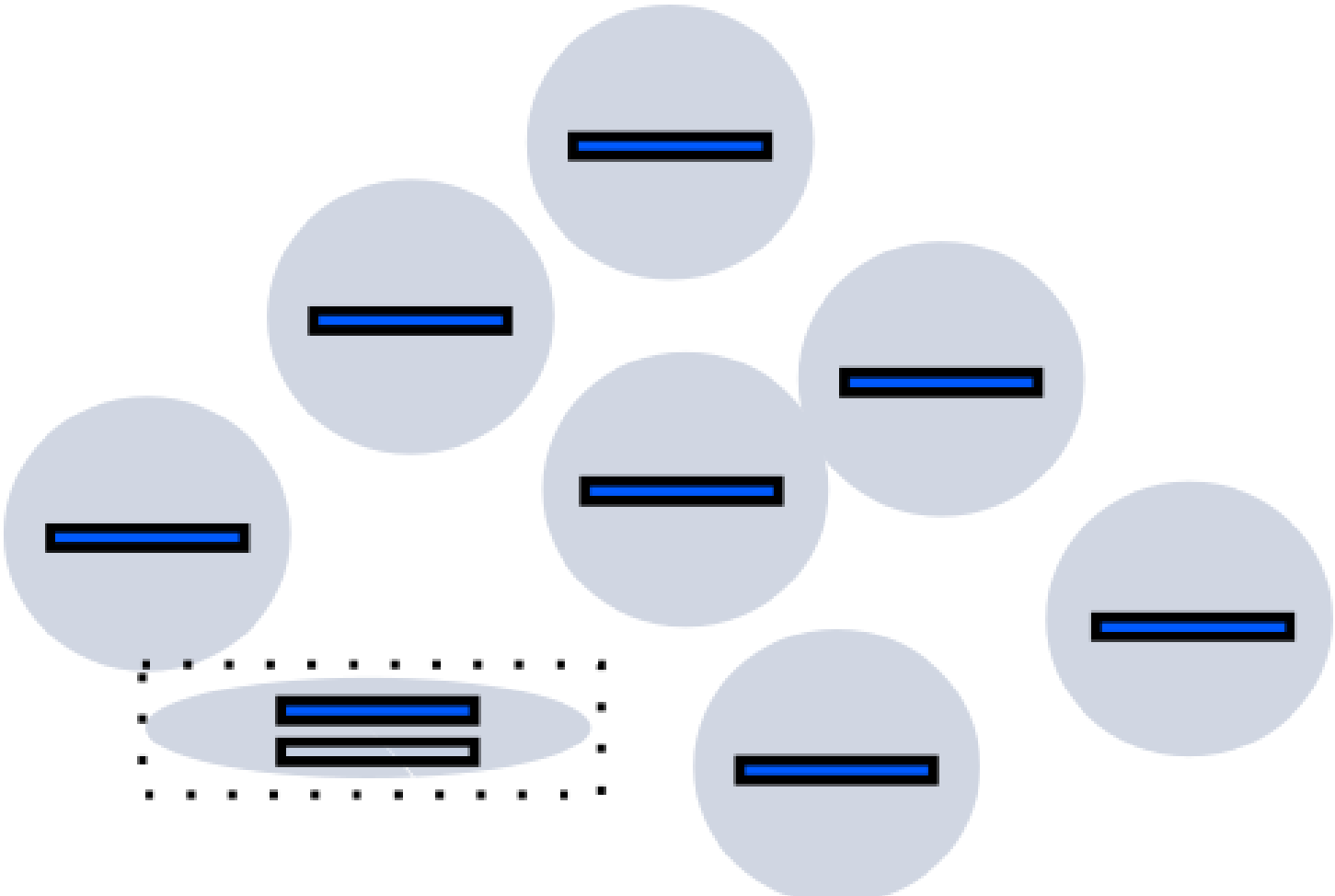
- Germline vs somatic
- Tumor cell proportion
- Copy number
- Loss of heterozygosity

Germline variant, Two copy, Heterozigosity, Tumor cellularity 25%

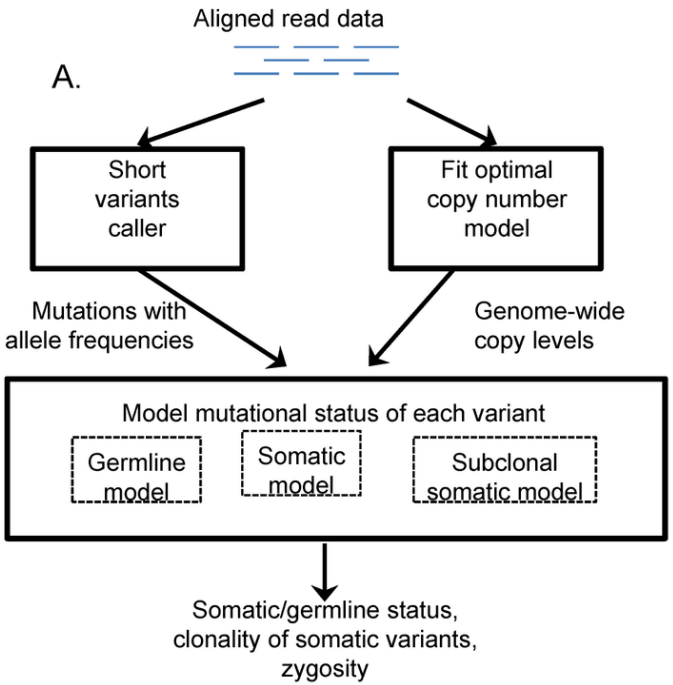


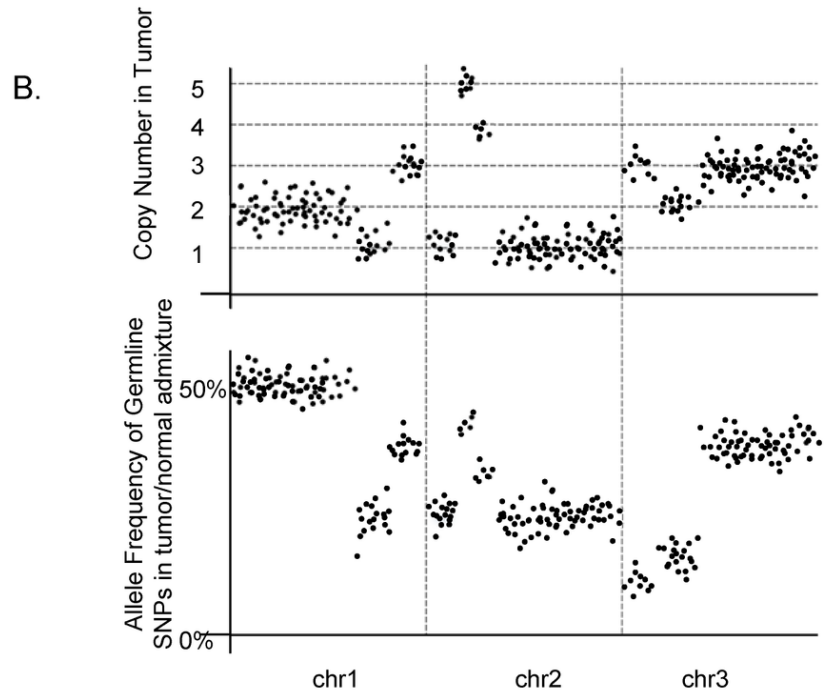
Germline variant, One copy, LOH, Tumor cellularity 100%

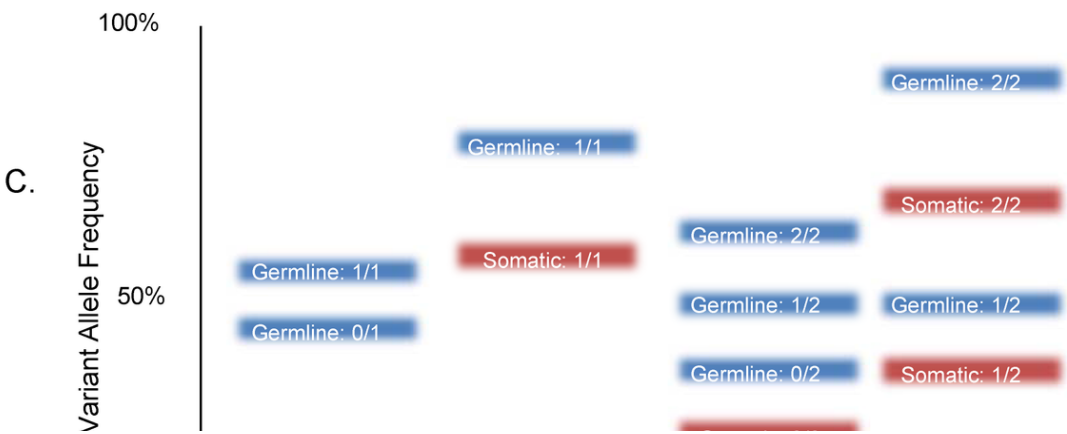
Allele frequency 100% ***hetero germline*** *variant*



Allele frequency in Somatic vs Germline in tumor only sample[1]







Allele frequency in Somatic vs Germline in tumor only sample

- BRCA1 mutation: Positive - p.Glu649Ter (c.1945G>T)
- Variant allele frequency 63.84%
- Tumor cell percentage: 70%

Allele frequency[1]

$$AF_{germline} = \frac{pV+1-p}{pC+2(1-p)} \; AF_{somatic} = \frac{pV}{pC+2(1-p)}$$

- Given copy number (C)
- Variant allele count (V)
- Sample purity (p)
- Variant status (somatic or germline)

Table of expected mutational allele frequencies[1]

copy number	LOH status	status of variant	p: sample purity 0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
C=1	LOH	V=0 germline	50	49	47	46	44	43	41	39	38	35	33	31	29	26	23	20	17	13	9	5	0
		V=1 somatic	0	3	5	8	11	14	18	21	25	29	33	38	43	48	54	60	67	74	82	90	100
		V=1 germline	50	51	53	54	56	57	59	61	63	65	67	69	71	74	77	80	83	87	91	95	100
C=2	LOH	V=0 germline	50	48	45	43	40	38	35	33	30	28	25	23	20	18	15	13	10	8	5	3	0
		V=2 somatic	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
		V=2 germline	50	53	55	58	60	63	65	68	70	73	75	78	80	83	85	88	90	93	95	98	100
	het	V=1 somatic	0	3	5	8	10	13	15	18	20	23	25	28	30	33	35	38	40	43	45	48	50
		V=1 germline	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
C=3	LOH	V=0 germline	50	46	43	40	36	33	30	28	25	22	20	18	15	13	11	9	7	5	3	2	0
		V=3 somatic	0	7	14	21	27	33	39	45	50	55	60	65	69	74	78	82	86	89	93	97	100
		V=3 germline	50	54	57	60	64	67	70	72	75	78	80	82	85	87	89	91	93	95	97	98	100
	het	V=1 somatic	0	2	5	7	9	11	13	15	17	18	20	22	23	25	26	27	29	30	31	32	33
		V=1 germline	50	49	48	47	45	44	43	43	42	41	40	39	38	38	37	36	36	35	34	34	33
		V=2 somatic	0	5	10	14	18	22	26	30	33	37	40	43	46	49	52	55	57	60	62	64	67
		V=2 germline	50	51	52	53	55	56	57	57	58	59	60	61	62	62	63	64	64	65	66	66	67
C=4	LOH	V=0 germline	50	45	41	37	33	30	27	24	21	19	17	15	13	11	9	7	6	4	3	1	0
		V=4 somatic	0	10	18	26	33	40	46	52	57	62	67	71	75	79	82	86	89	92	95	97	100
		V=4 germline	50	55	59	63	67	70	73	76	79	81	83	85	88	89	91	93	94	96	97	99	100
	het	V=1 somatic	0	2	5	7	8	10	12	13	14	16	17	18	19	20	21	21	22	23	24	24	25
		V=1 germline	50	48	45	43	42	40	38	37	36	34	33	32	31	30	29	29	28	27	26	26	25
		V=3 somatic	0	7	14	20	25	30	35	39	43	47	50	53	56	59	62	64	67	69	71	73	75
		V=3 germline	50	52	55	57	58	60	62	63	64	66	67	68	69	70	71	71	72	73	74	74	75

Limited information

- What we know
  - Tumor cell percentage
  - Variant allele frequency
- What we don‘t know
  - Copy number
  - LOH

Error

- Tumor cell percentage
- Allele frequency

$$Allele frequency \not\approx Readcount$$

Allele frequency in Somatic vs Germline in tumor only sample

- BRCA1 mutation: Positive - p.Glu649Ter (c.1945G>T)
- Variant allele frequency 63.84%
- Tumor cell percentage: 70% -> 80%

$$AF_{somatic} = \frac{pV}{pC+2(1-p)} = 0.67$$

BRCA somatic mutation with LOH[2]

Table 3 *BRCA1/2* Defects in the COBRA Cohort

ID	Histology	Gene	cDNA change*†	Amino acid change‡	T%	VAF tumor	VAF normal	LOH wild-type allele
Germline variants								
p18	HGSC	*BRCA1*	c.1881C>G§	p.Val627=	70	0.80	NA	Yes
p32	HGSC	*BRCA1*	c.2685_2686delAA	p.Pro897fs	85	0.98	NA	Yes
p56	HGSC	*BRCA1*	c.5277+1G>A	p.?	80	0.74	NA	Yes
p30	HGSC	*BRCA2*	c.4576dupA	p.Thr1526fs	80	0.97	0.48	Yes
p62	HGSC	*BRCA2*	c.5117A>C§	p.Asn1706Thr	80	0.54	NA	No
CNV-MLPA, germline								
p41	HGSC	*BRCA1*	Deletion of exon 22	p.?	30	NAP	NAP¶	Yes
Somatic variants								
p24	HGSC	*BRCA1*	c.3718C>T	p.Gln1240*	80	0.76	Not present	Yes
p11	HGSC	*BRCA1*	c.3858_3861delTGAG	p.Ser1286fs	70	0.56	Not present	Yes
p52‖**	HGSC	*BRCA1*	c.4868C>G§	p.Ala1623Gly	40	0.37	Not present	Yes††
p39	HGSC	*BRCA1*	c.5366C>T§	p.Ala1789Val	95	0.65	Not present	Uncertain
p12	HGSC	*BRCA2*	c.209_210delCT	p.Ser70fs	70	0.82	QCF	Yes
MS-MLPA								
p7	HGSC	*BRCA1*	Promoter hypermethylation	p.?	80	NAP	NA	Uncertain
p15	HGSC	*BRCA1*	Promoter hypermethylation	p.?	35	NAP	NA	Yes
p17	HGSC	*BRCA1*	Promoter hypermethylation	p.?	80	NAP	NA	Yes
p23	HGSC	*BRCA1*	Promoter hypermethylation	p.?	85	NAP	NAP	Yes
p25	HGSC	*BRCA1*	Promoter hypermethylation	p.?	70	NAP	NAP	Yes
p36	HGSC	*BRCA1*	Promoter hypermethylation	p.?	95	NAP	NAP	Yes
p58	HGSC	*BRCA1*	Promoter hypermethylation	p.?	70	NAP	NA	Uncertain
p59	HGSC	*BRCA1*	Promoter hypermethylation	p.?	70	NAP	NA	Uncertain

All variants had a coverage well above 100 reads, reaching >1000 reads in 10 of 11 cases (91%).
*Only class 3 (variant of unknown significance), class 4 (likely pathogenic), and class 5 (pathogenic) variants are reported.
†Reference sequences: NM_007294.3 for *BRCA1* and NM_000059.3 for *BRCA2*.
‡NP_009225.1 for BRCA1 and NP_000059.3 for BRCA2.
§Variant of unknown significance.
¶CNV-MLPA not performed on normal DNA sample.
‖DNA concentration too low to perform MS-MLPA.
**Not enough tumor to perform CNV-MLPA.

References

1. Sun, J.X., He, Y., Sanford, E., Montesion, M., Frampton, G.M., Vignot, S., Soria, J.-C., Ross, J.S., Miller, V.A., Stephens, P.J., et al. (2018). A computational approach to distinguish somatic vs. Germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. PLOS Computational Biology *14*, e1005965.

2. Jonge, M.M. de, Ruano, D., Eijk, R. van, Stoep, N. van der, Nielsen, M., Wijnen, J.T., Haar, N.T. ter, Baalbergen, A., Bos, M.E.M.M., Kagie, M.J., et al. (2018). Validation and Implementation of BRCA1/2 Variant Screening in Ovarian Tumor Tissue. The Journal of Molecular Diagnostics *20*, 600–611.