Circadian clock gene *PER1* mutations in colorectal cancer (CRC)

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Introduction

- The clock machinery regulates the circadian expression of up to 20% of genes expressed in any particular cell or tissue, modulating key cellular pathways including cell proliferation and apoptosis, DNA damage repair, cellular senescence, metabolic homeostasis, inflammatory and immune response[1].
- Colorectal carcinogenesis and CRC progression have been linked to the disruption of the molecular clockwork by experimental data showing deregulation of the circadian machinery and clock gene expression, both in preclinical models and in colonic neoplastic tissue[2].
- *PER1* encodes for one of the main negative regulators of the clock machinery[3], with an apparent tumor-suppressor role suggested by in vitro studies and animal models[4,5].
- Downregulation of *PER1* has been observed in CRC samples compared to matched healthy tissues, and lower expression levels of PER1 have been associated with poor survival and increased incidence of liver metastases[6-8].
- Few data are available on *PER1* gene mutations (*PER1*mut) in CRC. Therefore, we aimed to explore the clinical and molecular differences between *PER1* mutated versus wild-type (WT) CRCs.

Methods

- A cohort of 4079 CRCs tested with comprehensive genomic profiling by Caris Life Sciences (Phoenix, AZ) was identified from a retrospective database and included in this analysis.
- NextGen sequencing (NGS) was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the NextSeq platform (Illumina, Inc., San Diego, CA) on 592 genes.
- Microsatellite instability high (MSI-H) status was tested by NGS in 3996 tumors. The threshold to determine MSI-H was determined to be 46 or more microsatellite loci with somatic insertions or deletions to generate a sensitivity of greater than 95% and specificity of greater than 99% (MSI-equivocal, 43–45 altered loci; microsatellite stable (MSS), $\leq$ 42 loci).
- Pathogenicity of *PER1*mut was estimated using Poly-Phen and SIFT predictive scores.
- *PER1*mut prevalence comparisons in each groups were performed using Fisher-Exact test; while Chi-square deviance test (i.e. likelihood ratio test) on nested logistic regression models was performed for multivariate analysis. P<0.05 was considered statistically significant for all tests performed.

References

1. Sahar S et al. Nat Rev Cancer 2009; 2. Karantanos T et al. World J Gastroenterol 2014; 3. Shearman LP et al. Science 2000; 4. Yang X et al. Integr Cancer Ther 2009; 5. Yang X et al. Chronobiol Int 2009; 6. Mazzoccoli G et al. Chronobiol Int 2011; 7. Karantanos T et al. Int J Biol Markers 2013; 8. Oshima T et al. Oncol Rep 2011.

Results

- Overall, 185 unique *PER1*mut/variants were identified in 304 samples (7.45%); 45 were classified as pathological/possibly pathological (PATHmut) according to predictive scores.
- *PER1*mut were significantly associated with right-sided tumor location (p<0.001) and MSI-H (p<0.001).
- Overall incidence of *PER1*mut and PATHmut in the MSI-H group were 24% and 3.4%, respectively.
- In the multivariate analyses PATHmut were independently associated with mutations in *ARAF, BAP1, CHEK2, NF1, PIK3CA* and *POLE.*

1. Basic characteristics of the 4079 tumors interrogated for *PER1* mutation status.

CRC cohort (N=4079)			
		N	%
Gender	Female	1942	48%
	Male	2137	52%
Age	Median	61	
	Range	16~90	
Location	Right	1117	27%
	Left	1777	44%
	NOS	1185	29%
Primay/Met	Primary/Local	2083	51%
	Mets	1988	49%
	Unclear	8	0%
MSI	MSI-High	262	6%
	MSS/Eq*	3734	92%
	Unknown	83	2%
RAS/RAF	All WT	1579	39%
	KRAS/NRAS MT	2178	53%
	BRAF MT	322	8%

*22 cases included in our entire cohort were classified as MSI-equivocal by NGS. 10 of these were MSS by fragment analysis, 3 were MSI-Low, 8 were MMR proficient according to IHC, 1 had no additional testing.

2. *PER1* mutation rates in different groups. *P* values of significant differences are shown.

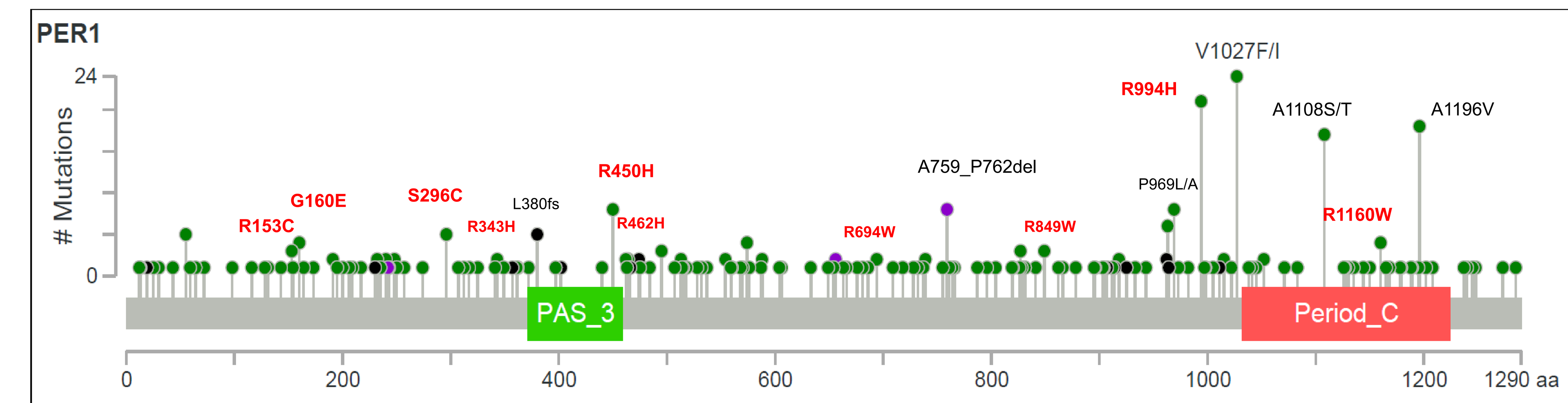
PER1 mutated-CRC (N=304)					
		PER1 Mut	Per1 %	PATHmut	PATHmut %
Gender	Female	151	**8%**	38	2%
	Male	153	**7%**	48	2%
Age	Median	60		61.5	
	Range	24~90		38~89	
Location	Right	111	**10%**	31	3%
	Left	114	**6%**	29	2%
	NOS	79	**7%**	26	2%
Primay/Met	Primary/Local	164	**8%**	51	2%
	Mets	139	**7%**	35	2%
	Unclear	1	**13%**	0	0%
MSI	MSI-High	63	**24%**	9	3%
	MSS/Eq	237	**6%**	75	2%
	Unknown	4	**5%**	2	2%
RAS/RAF	All WT	116	**7%**	31	2%
	KRAS/NRAS MT	153	**7%**	47	2%
	BRAF MT	35	**11%**	8	2%

p=0.0008 (Location: Right vs Left); p<0.0001 (MSI-High vs MSS/Eq); p=0.0175 (KRAS/NRAS MT vs BRAF MT); p=0.0412 (All WT vs BRAF MT)

0% 5% 10% 15% 20% 25% 30%

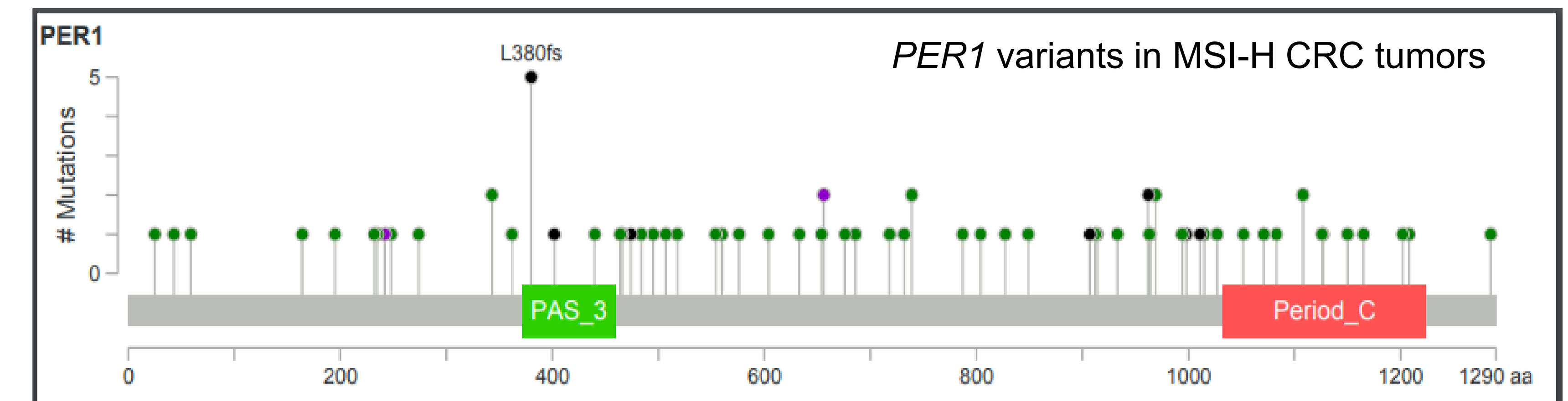
- *BRAF* mutation was significantly associated with *PER1*mut;
- *PER1*mut rates in MSI-H tumors with or without *BRAF* mutation were similar (25% vs 23%); the same in MSS/Eq tumors (5.1% vs 6.4%).

3. Mutation map of all *PER1* variants identified in all CRC tumors. PATHmut that occurred in 2 or more tumors are shown in red.

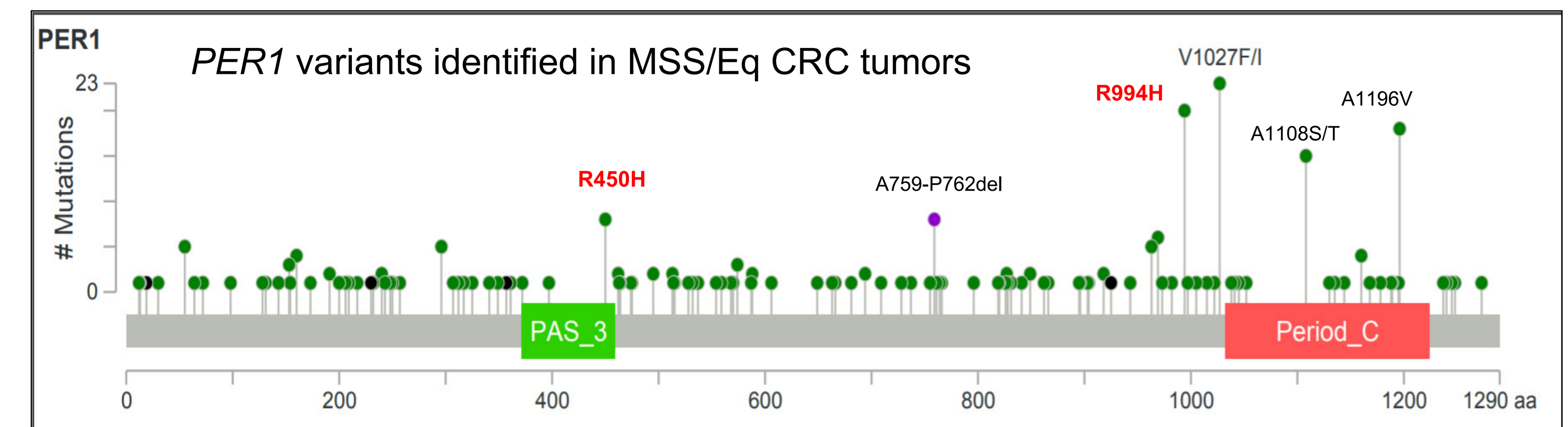


*(lollipop figure made using cBioPortal mutation mapper v 1.0.1)

4. *PER1* variant distribution in MSI-H and MSS/Eq CRC tumors.

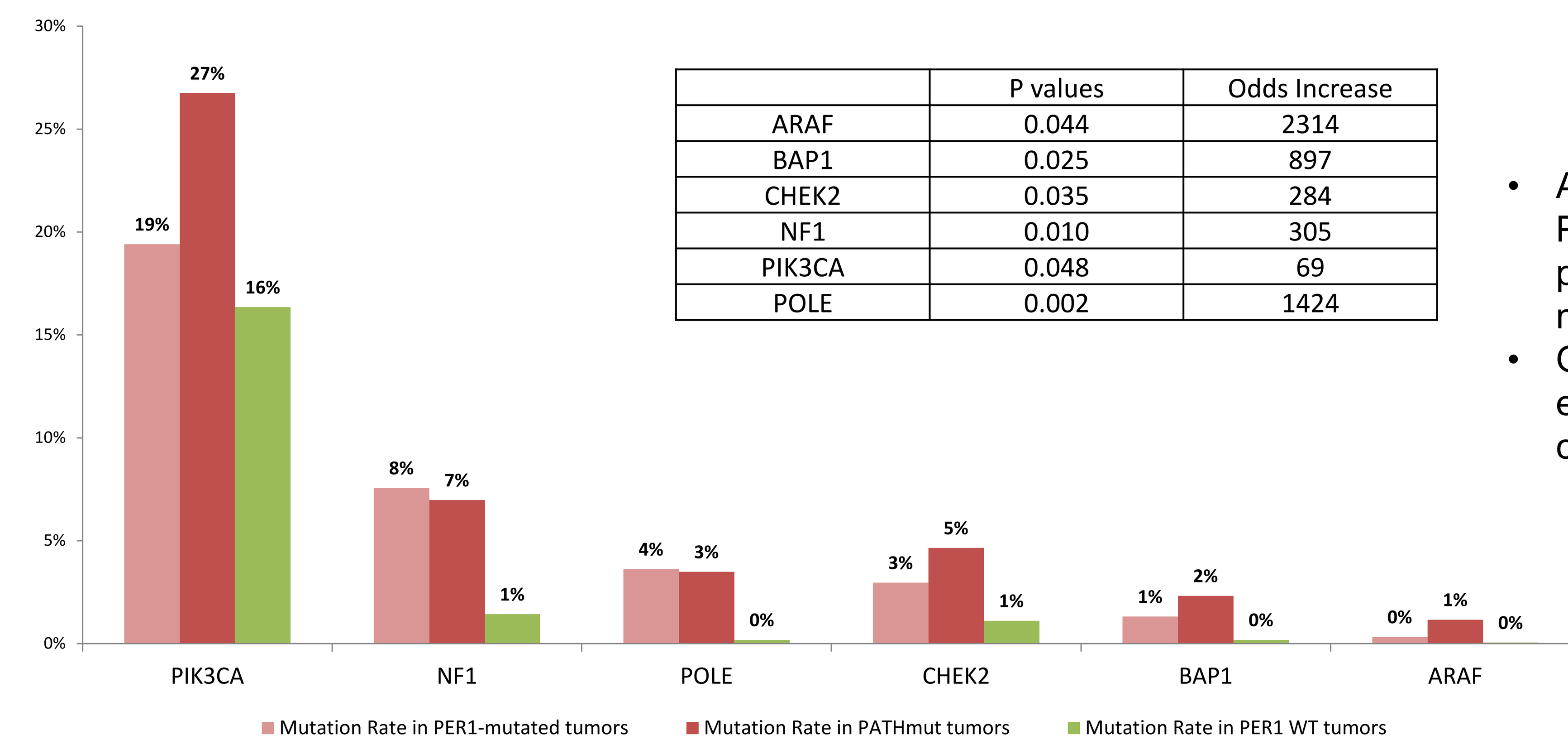


- Five L380fs were seen exclusively in the 262 MSI-H tumors while none was seen in MSS/Eq (p<0.0001).



- Point mutations that occurred in more than 5 tumors and were exclusive to MSS/Eq tumors included A1108S, A1196V, N55S, P963L, R450H and R296C, as well as small deletion A759_P762del.

5. *PER1* variants and association with additional markers. Shown are significant results after multivariate analysis.



	P values	Odds Increase
ARAF	0.044	2314
BAP1	0.025	897
CHEK2	0.035	284
NF1	0.010	305
PIK3CA	0.048	69
POLE	0.002	1424

- After adjusting for MSI status, PATHmut were significantly positively associated with mutations in 6 genes.
- Odds increases indicate the effect size of the positive correlation.

Conclusions

- Our results provide the first exploratory data on *PER1*mut association with clinical and molecular features in CRC, in a large population of patients with extensive genetic testing.
- A deeper understanding of *PER1*mut pathogenicity and functional role is necessary to guide future analyses. Nevertheless, our results suggest a significant association with MSI-H, possibly reflecting an interplay between mismatch repair status and the clock genes pathway in CRC, consistent with previous data and warranting further investigation.

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