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POT1 mutation spectrum in tumor types commonly diagnosed among POT1-associated hereditary cancer syndrome families

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Abstract

Background—The shelterin complex is composed of six proteins that protect and regulate telomere length, including protection of telomeres 1 (POT1). Germline *POT1* mutations are associated with an autosomal dominant familial cancer syndrome presenting with diverse malignancies including glioma, angiosarcoma, colorectal cancer, and melanoma. Although somatic *POT1* mutations promote telomere elongation and genome instability in chronic lymphocytic leukemia, the contribution of *POT1* mutations to development of other sporadic cancers is largely unexplored.

Methods—We performed logistic regression, adjusted for tumor mutational burden, to identify associations between *POT1* mutation frequency and tumor type in 62,368 tumors undergoing next-generation sequencing.

Results—A total of 1,834 tumors harbored a non-benign mutation of POT1 (2.94%), of which 128 harbored a mutation previously reported to confer familial cancer risk in the setting of germline POT1-deficiency. Angiosarcoma was 11 times more likely than other tumors to harbor a *POT1* mutation ($P=1.4 \times 10^{-20}$), and 65% of *POT1*-mutated angiosarcoma had >1 mutations in

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POT1. Malignant gliomas were 1.7 times less likely to harbor a *POT1* mutation ($P=1.2\times 10^{-3}$) than other tumor types. Colorectal cancer was 1.2 times less likely to harbor a *POT1* mutation ($P=0.012$), while melanoma showed no differences in *POT1* mutation frequency versus other tumors ($P=0.67$).

Conclusions—These results confirm a role for shelterin dysfunction in angiosarcoma development, but suggest that gliomas arising in the context of germline *POT1* deficiency activate a telomere lengthening mechanism that is uncommon in gliomagenesis.

Keywords

POT1 mutation; telomere; hereditary cancer syndrome families; TERT; ATRX

INTRODUCTION

Eukaryotic telomeres, located at the ends of linear chromosomes, function to protect against the loss of genomic content during repeated rounds of cellular replication.¹ The shelterin complex is a protein complex that binds and protects human telomere ends and regulates telomere length.^{2,3} Protection of telomeres 1 (POT1) is one of six proteins in the shelterin complex and one of the core components that directly bind to telomeres. *POT1* loss causes telomere uncapping and leads to unprotected telomere ends, aberrant telomere lengthening, and chromosomal fusions.^{4,5} As such, telomere dysregulation caused by *POT1* deficiency is associated with tumorigenesis.⁶

POT1 is frequently mutated in aggressive forms of chronic lymphocytic leukemia (CLL).^{7–11} Because *POT1* mutations arise early in CLL pathogenesis, they are believed to function as drivers in disease progression.^{7–11} Furthermore, germline *POT1* mutations have been shown to underlie a number of hereditary familial cancer syndromes involving CLL,¹¹ glioma,¹² melanoma^{13–18} and colorectal cancer.¹⁹ Furthermore, a deleterious germline missense variant in *POT1* (p. R117C) was identified in three Li-Fraumeni-like (LFL) families with angiosarcomas,²⁰ and additional *POT1* variants were subsequently identified in sporadic angiosarcoma patients.²¹

Tumor-based next-generation sequencing (NGS) panels are increasingly common in clinical practice, and disease-associated variants identified from tumor sequencing can provide clinical insights.^{22–24} The frequency and spectrum of *POT1* mutations in solid tumors, especially those that are associated with *POT1* germline deficiency, have not been rigorously evaluated. In the current study, we obtained NGS data from 62,368 tumors and reviewed *POT1* mutation frequency in pan-cancer analyses.

METHODS

Next Generation Sequencing

This study involved collection of existing data and publicly available diagnostic specimens, and the information gathering process precludes direct and indirect identification of subjects, which therefore exempts it from requiring institutional review board approval under HHS regulations at 45 CFR 46.101(b). *POT1* sequencing data were obtained for 62,368 tumors

from Caris Life Sciences. Each tumor was reviewed by board certified pathologists and has been classified into one of 172 tumor categories based on World Health Organization (WHO) classification guidelines.

NGS was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina, Inc., San Diego, CA). Tumor specimens were collected via manual microdissection and harvesting targeted tumor tissue. Matched normal tissue was not sequenced. A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets, including *POT1* (Agilent Technologies, Santa Clara, CA). All variants were detected with >99% confidence based on allele frequency and amplicon coverage with an average sequencing depth across all amplicons >500x and an average depth across *POT1* amplicons >750x. DNA sequencing results are validated per Clinical Laboratory Improvement Amendments (CLIA) and Internal Organization for Standardization (ISO) requirements. All analyses were completed in the same testing environment with the same sequencing and analytic pipeline, and in compliance with CLIA and ISO regulations.

Genetic variants were categorized according to the American College of Medical Genetics and Genomics (ACMG) standards (Richards et al., 2015, Tables 3–5) as: “pathogenic”, “presumed pathogenic”, “variant of unknown significance”, “unclassified”, “presumed benign”, or “benign”, according to ACMG guidelines.²⁵ “Presumed benign” and “benign” mutations were excluded from *POT1* mutation frequency analyses. The remaining categories are collectively referred to in this study as “non-benign”. A flowchart of variant classification is included in Supplementary Figure 1. A sensitivity analysis was also performed limiting to only the variants classified as “pathogenic” and “presumed pathogenic”.

Tumor Mutation Burden

Tumor mutation burden (TMB) was measured by counting all non-synonymous single-nucleotide variants found per tumor (592 genes). TMB was calculated in accordance with the Friends of Cancer Research TMB harmonization project.²⁶ Nonsynonymous, nonsense, in-frame deletion and frameshift variants were added after filtering out presumed germline variants determined from a cosmopolitan pooled reference database including: the Genome Aggregation Database (release 2.1), the Single Nucleotide Polymorphism Database human build 137, and the Caris in-house benign database. Per tumor sample, a total of 1.4 Mb was sequenced.²⁷ Although comparison of tumors to a reference database rather than to matched germline specimen may inflate estimates of TMB, the similar treatment of all sequenced samples in these analyses and the comparison of these samples to a common reference database suggests that modest inflations of TMB are unlikely to create substantial biases in downstream analyses across samples.

Statistical Analysis

Data were analyzed using multivariate logistic regression in SPSS v25.0 (SPSS Inc., Chicago, IL, USA). *POT1* mutation status (mutated/non-mutated) was used as the outcome of interest and tumor classification was used as the predictor of interest, adjusting for TMB

to prevent passenger mutations in highly-mutated tumor types (*e.g.*, melanoma) from confounding associations. TMBs of tumors were compared among different *POT1* mutation categories using the non-parametric Mann-Whitney U-test.

RESULTS

Each of 62,368 tumors was classified into one of 172 tumor categories, with 83 tumor categories including more than 50 cases (Supplementary Table 1). A total of 1,834 tumors harbored a non-benign mutation of *POT1* (2.94%), with all mutations having variant allele frequencies $\geq 7\%$ (mean 34.8%, median 33%). Of these, 128 tumors harbored a mutation previously reported to confer familial cancer risk in the setting of germline *POT1*-deficiency (Figure 1, Supplementary Table 2). Logistic regression analyses were conducted in tumor types previously associated with germline *POT1* deficiency, including: 2,200 malignant gliomas, 1,735 melanomas, 8,904 colorectal cancers, and 86 angiosarcomas. The database is almost exclusively comprised of solid tumors, so hematologic malignancies (N=306), including CLL (N=10), were not assessed.

After adjustment for TMB, angiosarcoma was 11 times more likely than all other tumors combined to harbor a *POT1* mutation (N=20/86, 23%; $P=1.4 \times 10^{-20}$) (Table 1). Deleterious mutations were found on both the DNA oligonucleotide/oligosarccharide and the TPP-1-binding domains of *POT1* (Figure 2, Supplementary Table 3). Of these 20 *POT1*-mutated angiosarcoma, 12 tumors harbored two *POT1* mutations and 1 harbored three *POT1* mutations. No angiosarcoma harbored >1 frameshift or nonsense mutation (Supplementary Table 4). However, the average variant allele frequency difference between *POT1* mutations within the same tumor was 13.5%, suggesting a mutational process wherein a “second hit” in *POT1* arises later during clonal evolution of the tumor. We did not observe any evidence that second hits in *POT1* were more or less likely to affect the same *POT1* protein domain (*i.e.* DNA-binding versus TPP1-binding) as the more clonal mutation.

Glioblastoma and other malignant gliomas were 1.7 times less likely than all other tumors combined to harbor a *POT1* mutation (N=38/2200, 1.73%; $P=0.0012$). Of the 38 *POT1*-mutated gliomas, only 3 harbored a second *POT1* mutation. Colorectal cancer was 1.2 times less likely to harbor a *POT1* mutation than all other tumors combined (N=231/8904, 2.59%; $P=0.012$), with 14/231 harboring a second *POT1* mutation. Melanoma showed no difference from other tumor types in *POT1* mutation frequency (N=64/1735, 3.69%; $P=0.67$), and 5/64 *POT1*-mutated melanomas harbored a second *POT1* mutation. Sensitivity analyses limiting to only those variants classified as “pathogenic” or “presumed pathogenic” were largely consistent with results from our primary analyses, with *POT1* associations being strengthened in the angiosarcoma group, attenuated in the glioma group, and remaining approximately the same in the colorectal cancer and melanoma groups (Supplementary Table 5). *All POT1 variants classified as “pathogenic” or “presumed pathogenic” across all tumor histologies are listed in Supplementary Table 6.*

Among glioma histologies, glioblastoma was 1.56 times less likely than other tumors to harbor a *POT1* mutation (N=30/1602, 1.87%; $P=0.019$) after adjustment for TMB. Neither anaplastic astrocytoma nor diffuse astrocytoma displayed significant differences in the

frequency of *POT1* mutation (N=6/230, 2.61%; P=0.82 and N=1/125, 0.8%; P=0.23). There were no *POT1* mutations identified in oligodendrogliomas or anaplastic oligodendrogliomas (N=0/156) (Table 2).

Overall median TMB for 62,368 tumors was 7 mutations/Mb. The median TMB for tumors harboring a *POT1* mutation characterized as “non-benign” was 11 mutations/Mb, significantly higher than the median TMB for tumors lacking a *POT1* mutation (P<0.0001) and the median for tumors harboring a *POT1* mutation characterized as “benign or presumed benign” (median=8.5; P<0.0001) (Figure 3). Because TMB was higher in tumors with non-benign *POT1* mutations than those with benign *POT1* mutations, the observed association is unlikely to be attributable to more-highly mutated tumors simply harboring more mutations in any queried gene.

In addition to affecting genomic instability, *POT1* mutations have been shown to augment telomere length,²² helping cells escape replicative senescence. Although *TERT* promoter mutation data were unavailable, *ATRX* mutations that lead to the alternative lengthening of telomeres (ALT) phenotype were available for analysis. Across all 62,368 tumors, logistic regression revealed that *ATRX*-mutated tumors were 1.7 times less likely to have a *POT1* mutation after adjusting for TMB (P=0.001). However, this association was not observed when analyses were limited to angiosarcoma (P=0.999), glioma (P=0.398), colorectal cancer (P=0.398), or melanoma (P=0.369).

DISCUSSION

Our study is the first to report the prevalence of *POT1* mutations in a diverse set of solid tumors, revealing that angiosarcomas are 11 times more likely than other tumor types to harbor a *POT1* mutation, often harboring multiple *POT1* mutations. This indicates that shelterin complex dysfunction may be a key contributor to angiosarcoma development. Pathogenic mutations were detected in both the oligonucleotide/oligosarccharide and the TPP-1-binding domains of *POT1*, previously associated with abnormally long telomeres and telomere fragility,^{10,15,28–29} genomic instability, and tumorigenesis.^{4,30–32} Further studies are required to reveal which of the reported *POT1* mutations are particularly damaging and which *POT1*-dependent molecular mechanisms are critically involved. Notably, five deleterious *POT1* variants have been reported in sporadic angiosarcoma patients and angiosarcoma patients in the context of Li-Fraumeni-Like Syndrome (LFLS).^{20–21} These *POT1* mutations were distributed across all conserved domains. Our analyses of angiosarcoma tumor genomes revealed a high frequency of *POT1* mutations distributed in a similar pattern, complementing other emerging studies that have observed a high frequency of *POT1* mutations in angiosarcoma.³³ Because the database lacks family history data, the cases profiled in the current study may contain patients meeting criteria for LFLS.

We previously identified three germline *POT1* variants in association with familial glioma.¹² While all affected individuals in these families were found to have oligodendroglioma or mixed oligoastrocytoma, a subsequent molecular analysis revealed that at least one proband had a molecular astrocytoma according to revised 2016 WHO criteria (*i.e.* IDH-mutated, 1p/19q intact).³¹ Here we have identified *POT1* mutations in the tumor genome of

astrocytic-lineage gliomas (e.g. glioblastoma, anaplastic astrocytoma) but not oligodendroglioma. The frequency of *POT1* mutations was lower in glioma, particularly high-grade malignant glioblastoma, relative to other tumor types, indicating that *POT1* mutations are likely not a major contributor to gliomagenesis in non-familial cases. In our previous observations of 20 familial glioma patients, those harboring a germline mutation in the shelterin complex genes *POT1* or *TERF2* were the only patients not to have a somatic *TERT* promoter or *ATRX* mutation.³¹ As the vast majority of gliomas maintain telomere length by reactivating *TERT* expression through hotspot *TERT* promoter mutations³⁴ or by activating ALT through loss of *ATRX*,³⁵ it is possible that, in rare instances, *POT1* mutations activate a non-canonical mechanism of ALT via shelterin dysfunction. As pathways of telomere maintenance are associated with clinical features, including age at diagnosis³⁶ and glioma patient survival,³⁵ gliomas caused by shelterin dysfunction may also harbor unique clinical presentations and therapeutic vulnerabilities.

The frequency of *POT1* mutations appear lineage specific and colorectal cancer was another cancer type that was less likely to harbor a *POT1* mutation. Three putatively pathogenic germline *POT1* variants have been identified in colorectal cancer patients.¹⁹ One of these variants, D617Efs was also identified in familial glioma.^{12,19} A shared risk allele could confer pleiotropic risk of both colorectal cancer and glioma through a telomere-dependent mechanism. This has precedent, as specific polymorphisms in the telomerase component *TERC* have been associated with longer telomere length and increased risk of both colorectal cancer and high-grade glioma.^{37–38}

Although *CDKN2A* is the primary high-penetrance melanoma predisposition gene,^{39–40} germline *POT1* variants have been reported in melanoma-prone families by several groups.^{13–18} More than a dozen pathogenic germline mutations of *POT1* have been identified in *CDKN2A*-wild-type melanoma kindreds, distributed across the DNA-binding and TPP1-binding domains. Melanoma has among the highest TMB of any cancer due to its association with UV-induced DNA damage.⁴¹ In our dataset, melanoma had a TMB of 21.36 mutations/Mb, substantially higher than the average TMB across all tumors (10.12 mutations/Mb). Associations between *POT1* mutation frequency and tumor histology can be confounded by TMB due to the presence of passenger mutations that do not have biological or phenotypic consequences.⁴² However, our analyses adjusted for TMB to reduce the possibility of this confounder and observed no differences in *POT1* mutation frequency in melanoma versus other tumor types.

A limitation to our study was that the NGS data were processed in the setting of tumor-only sequencing, which precludes the ability to definitively differentiate rare germline variants from somatically-acquired mutations.²³ As a result, a portion of the mutations identified in our data may indeed be rare or previously-unreported germline variants. It is worth mentioning that many identified *POT1* mutations – including in angiosarcoma – were detected in substantially less than 50% of sequencing reads, suggesting a somatic rather than a germline origin and potentially a role for *POT1* in disease progression as opposed to simply tumor initiation. Although the inability to definitively distinguish germline from somatic variants does not impact our interpretation that *POT1* dysfunction is a hallmark of angiosarcoma development and an uncommon driver of tumorigenesis in sporadic glioma,

melanoma and colorectal cancer, it does limit our ability to directly translate findings to familial cancer syndromes or risk counseling. NGS using paired tumor-normal tissue would help to separate germline from somatic mutations and should be a focus of future research efforts, especially in angiosarcoma. Additionally, our *POT1* sequencing data included only the coding region of the gene. This approach may miss functionally-relevant mutations in non-coding regions. However, with the exception of splice-site mutations, pathogenic non-coding variants are typically activating in nature (*e.g. TERT* promoter mutations), whereas relevant *POT1* mutations appear to be loss-of-function or hypomorphic variants.

In summary, we evaluate *POT1* mutation status in a pan-cancer analysis and report the prevalence of *POT1* mutations across tumor types, including those previously associated with germline *POT1* mutations. The high prevalence of *POT1* mutations in angiosarcomas suggests that loss of *POT1* function is a major driver of angiosarcoma development and that angiosarcoma sequencing studies should prioritize *POT1* analyses. The observation that glioblastoma and anaplastic astrocytoma, and to a lesser extent colorectal cancers, were less likely to harbor *POT1* mutations than other tumor types suggests that when these tumors arise in the context of germline *POT1* deficiency they are utilizing non-canonical pathways of telomere maintenance. This is underscored by our observation that *POT1*-mutated tumors had higher TMB, fewer *ATRX* mutations, and our previous report that glioma patients with germline *POT1* mutations lacked a hallmark telomere-maintenance mutation in either *ATRX* or the *TERT* promoter.³¹ Our results provide insight into the various roles that *POT1* may play across a diverse group of tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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^aRobles-Espinoza et al., 2014, Shi et al., 2014, Wilson et al., 2017, Pellegrini et al., 2017, Wong et al., 2019, Potrony et al., 2019
^bBainbridge et al., 2015
^cCalvete et al., 2015, 2017
^dChubb et al., 2016

Figure 1:
Summary of reported *POT1* mutations in *POT1*-associated hereditary familial cancer syndromes.

Angiosarcoma

Figure 2:
Summary of *POT1* mutations in angiosarcoma. Deleterious mutations were found on both the DNA oligonucleotide/oligosaccharide and the TPP-1-binding domains of *POT1*. Variants are listed with complete HGVS nomenclature in Supplementary Table 3.

Figure 3:
Box plots of average TMB for different categories of *POT1* mutation status.

Table 1: Association of *POT1* mutation frequency with tumor type among cancers known to be associated with germline *POT1*/mutations.

Tumor Type	N	Average Tumor Mutation Burden (mutations/Megabase)	Percent with <i>POT1</i> mutation ^a	Odds Ratio (95% Confidence Interval) ^b	P-value
Angiosarcoma	86	8.91	23.26%	10.99 (6.62–18.18)	1.40×10^{-20}
Malignant glioma ^c	2200	8.03	1.73%	0.58 (0.41–0.80)	1.2×10^{-3}
Colorectal cancer	8904	10.48	2.59%	0.83 (0.72–0.96)	0.012
Melanoma	1735	21.36	3.69%	0.95 (0.73–1.23)	0.67

^aThe following are included in the calculation of percent with *POT1* mutation: “mutated, pathogenic”, “mutated, presumed pathogenic”, “mutated, variant of unknown significance”, and “unclassified mutation”. 20 angiosarcomas harbored a *POT1*/mutation, of which 12 harbored a second *POT1* mutation and 1 harbored three *POT1* mutations. 38 gliomas harbored a *POT1* mutation, of which 3 also harbored a second *POT1* mutation. 231 colorectal cancers harbored a *POT1* mutation, of which 14 also harbored a second *POT1* mutation. 64 melanomas harbored a *POT1* mutation, of which 5 harbored a second *POT1* mutation.

^bAssociation is adjusted for tumor mutation burden.

^cIncludes glioblastoma, anaplastic astrocytoma, diffuse astrocytoma, anaplastic oligodendroglioma, and oligodendroglioma.

Table 2:

Association of *POT1* mutation frequency with tumor type among glioblastoma and other malignant gliomas.

Tumor Type	N	Percent with <i>POT1</i> mutation ^a	Odds Ratio (95% Confidence Interval) ^b	P-value
Glioblastoma, WHO grade IV	1602	1.87%	0.64 (0.44–0.93)	0.019
Anaplastic Astrocytoma	230	2.61%	0.91 (0.40–2.07)	0.822
Diffuse Astrocytoma	125	0.8%	0.30 (0.04–2.14)	0.229
Oligodendroglioma and anaplastic oligodendroglioma	156	0%	N/A	N/A

^aThe following groups are included in the calculation of Percent with *POT1* mutation: “mutated, pathogenic”, “mutated, presumed pathogenic”, “mutated, variant of unknown significance”, and “unclassified mutation”.

^b Association is adjusted for tumor mutation burden.