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TITLE: Long-Read DNA-Sequencing and Targeted RNA-Seq to Identify Previously Undetectable Classes of Mutations in Families with Lethal Prostate Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer has a significant heritable component, with 10-15% of patients with advanced disease harboring pathogenic germline mutations in DNA repair genes. It is critical for these patients to know their genotypes, because mutations in *BRCA2* and *PALB2*, and to a more modest degree *BRCA1* and *ATM*, predict favorable response to poly-ADP ribose polymerase inhibitors (PARPi) and platinum chemotherapy. In May 2020, the FDA approved two PARPi drugs for men with metastatic-castration resistant prostate cancer and mutations in these genes. However, many patients with metastatic prostate cancer and severe family histories remain without a detected germline pathogenic mutation. These patients cannot benefit from molecularly-directed therapies, because these drugs are useful only to patients with damaging mutations in DNA repair genes, and hence are targeted only to them. Our proposal aims to identify previously undetectable classes of pathogenic mutations in 450 patients for whom no pathogenic mutation has been found by gene-panel sequencing. We will use two newly developed genomic techniques: 1) CRISPR excision and long-read sequencing to identify complex structural mutations and 2) targeted RNA-Seq to evaluate patient RNA for cryptic regulatory and splice variants.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

Our research aims to identify, in 450 patients with metastatic prostate cancer and their families, classes of mutations in BRCA1, BRCA2, and other DNA-repair genes that have been previously undetectable, even by the best current sequencing approaches. We directly address the Overarching Challenge of reducing lethal prostate cancer for men who are genetically predisposed to the disease and would potentially benefit from targeted therapy, but precluded from these therapies because no causal mutation has been identified for them. We will use two newly developed genomic techniques: 1) CRISPR excision and long-read sequencing to identify complex structural mutations and 2) targeted RNASeq to evaluate patient RNA for cryptic regulatory and splice variants.

2. KEYWORDS:

Metastatic prostate cancer, genetic testing, sequencing, DNA repair gene, mutation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1. Major Task 1: Obtain regulatory approval. Milestone Achieved (Month 3).

Major Task 2: Enroll study participants and their informative relatives.
Subtask 1 (Months 4-30): To date, 130 patients have been recruited and returned their consent documents and blood kits. Subtask 2 (Months 4-30): Lymphoblast cell lines have successfully been established on all 128 participants recruited so far.

Specific Aim 2.
Major Task 1: Prepare, sequence SMRT-CATCH libraries
Subtask 1, 2, 3 (Months 5-32): We have performed SMRT-CATCH on 48 participants so far.
Major Task 2: Identify structural variants
Subtask 1, 2, 3 (Months 8-36): We have analyzed the SMRT-CATCH on 40 participants so far

Specific Aim 3.
Major Task 1: Perform targeted RNA-Seq
Subtask 1, 2, 3 (Months 5-36): We have performed RNA-Seq on 36 participants so far.

What was accomplished under these goals?

We are on pace with patient recruitment, established lymphoblasts, extracted DNA and RNA and initiated DNA and RNA sequencing analysis.

Our preliminary results include: 1) the identification of large deletion of BRCA2 encompassing the promoter region. We are currently analyzing the SMRT-CATCH sequencing reads to pinpoint the exact breakpoints, 2) we have found a deep intronic variant in BRCA1 intron 16 that introduces a cryptic donor site. The RNA-Seq data demonstrate that the cryptic donor site leads to the inclusion of part of intron 16 making a 'pseudoexon' that is included in the BRCA1 mRNA and predicted to lead to a premature truncation.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

For the next reporting period we will continue with the experiments outlined in Specific Aims 2 and 3 of the SOW.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We have identified two potentially pathogenic mutations, one in BRCA1 and one in BRCA2 in two different participants.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

No changes from the personnel Tasks outlined in the approved SOW.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: