# Mutational Landscape of Candidate Genes in Familial Prostate Cancer

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**BACKGROUND.** Family history is a major risk factor for prostate cancer (PCa), suggesting a genetic component to the disease. However, traditional linkage and association studies have failed to fully elucidate the underlying genetic basis of familial PCa.
**METHODS.** Here, we use a candidate gene approach to identify potential PCa susceptibility variants in whole exome sequencing data from familial PCa cases. Six hundred ninety-seven candidate genes were identified based on function, location near a known chromosome 17 linkage signal, and/or previous association with prostate or other cancers. Single nucleotide variants (SNVs) in these candidate genes were identified in whole exome sequence data from 33 PCa cases from 11 multiplex PCa families (3 cases/family).
**RESULTS.** Overall, 4,856 candidate gene SNVs were identified, including 1,052 missense and 10 nonsense variants. Twenty missense variants were shared by all three family members in each family in which they were observed. Additionally, 15 missense variants were shared by two of three family members and predicted to be deleterious by five different algorithms. Four missense variants, *BLM* Gln123Arg, *PARP2* Arg283Gln, *LRCC46* Ala295Thr and *KIF2B* Pro91Leu, and one nonsense variant, *CYP3A43* Arg441Ter, showed complete co-segregation with PCa status. Twelve additional variants displayed partial co-segregation with PCa.
**CONCLUSIONS.** Forty-three nonsense and shared, missense variants were identified in our candidate genes. Further research is needed to determine the contribution of these variants to PCa susceptibility. *Prostate 74:1371–1378, 2014.* © 2014 Wiley Periodicals, Inc.

*KEY WORDS:* **exome sequencing; susceptibility; familial cancer**

## INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer deaths among American men [1]. Positive family history is one of the most recognized risk factors for PCa diagnosis, suggesting a genetic component to the disease [2]. However, the genetic basis of PCa is still poorly understood. Linkage studies focused on hereditary PCa pedigrees have provided inconsistent results. Genome-wide association studies of large case-control cohorts have identified dozens of single nucleotide variants (SNVs) which are consistently associated with

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PCa [3]. However, the risk elevation attributed to each of these variants is low (<1.3 per SNV), and together, they account for less than one quarter of familial PCa risk [4]. Targeted sequencing of a candidate linkage region on chromosome 17q21-22 led to the recent identification of the first rare, penetrant PCa susceptibility gene, *HOXB13* with the G84E mutation resulting in a 10- to 20-fold increased risk of PCa [5]. Taken together, PCa is likely a highly genetically heterogeneous disease, with rare PCa susceptibility variants yet to be discovered.

Massively parallel sequencing technologies now provide the ability to interrogate large amounts of genomic DNA, including entire genes and/or exomes, and to comprehensively catalog rare DNA variation on an individual basis. Here, we use a candidate gene approach to analyze whole exome sequencing data from 33 men with PCa in 11 unrelated hereditary PCa pedigrees to identify new PCa risk variants.

## MATERIALS AND METHODS

### Patient Selection

All individuals/families are participants in the University of Michigan Prostate Cancer Genetics Project (UM PCGP). Eleven pedigrees, each with three or more confirmed affected individuals, including at least one distant affected relative pair (e.g., cousins or an avuncular pair), were selected for whole exome sequencing. Germline DNA from three men with PCa per family was sequenced. Selection of the cases for sequencing in each pedigree was based on earliest age of PCa diagnosis with some preference given to aggressive cases. Genomic DNA was prepared using standard protocols. PCa diagnoses were confirmed by medical record review and reports were received from all cases selected for sequencing. Some additional cases were confirmed by two independent family members when records were not retrievable. Written informed consent was obtained from all subjects, and the research project was approved by the University of Michigan Institutional Review Board.

### Exome Sequencing

Exome capture was performed with a SureSelectXT Human All Exon 50mB kit (Agilent Technologies, Santa Clara, CA). Enriched libraries were then sequenced on an Illumina HiSeq2000 (Illumina, Inc., San Diego, CA). Sequence data was aligned to the reference sequence using the Burrows–Wheeler Aligner (BWA) [6], and variant calls were made using The Genome Analysis Tool Kit (GATK) [7]. Called variants were then annotated using ANNOVAR [8] which included classification of variants as benign/non-conserved or deleterious/conserved by five separate in silico bioinformatics tools (SIFT, PolyPhen, PhyloP, MutationTaster, and LRT).

### Candidate Gene/Variant Selection

Candidate genes included genes previously associated with risk, development or progression of prostate and/or other cancers as identified through literature review. Genes involved in DNA damage repair pathways were identified through the Gene Ontology (GO) database and also included as candidates. Additionally, genes in the 2-LOD support interval of a previously reported PCa linkage signal on chromosome 17 [9,10] were included. A total of 695 candidate genes were included in the analysis (see Supplementary Table). All SNVs in candidate genes were identified. Nonsense variants, missense variants that were shared by all three family members in each family in which they were observed, and missense variants that were predicted to be deleterious/conserved by all five prediction programs and were shared by two of three family members were selected for further analysis.

### Variant Verification

All predicted candidate gene nonsense and shared missense variants were sequenced for verification using BigDye Terminator v1.1 chemistries on a 3100 Genetic Analyzer (Life Technologies). Confirmed variants were then sequenced in all family members with DNA available to assess co-segregation with PCa status.

## RESULTS

Eleven hereditary PCa families were selected for this analysis. Clinical characteristics of families and individuals sequenced are presented in Table I. All families described themselves as having European ancestry and all had between four and eleven confirmed PCa cases, with a median of seven PCa cases per pedigree. Age at PCa diagnosis of the men that were selected for exome sequencing ranged from 48 to 76 years with a median age of 58 years. Since PCa can be very clinically heterogeneous, we used a clinical definition of aggressive PCa (Table I) to assess the cases. Ten of 33% or 30.3% of sequenced individuals were classified as clinically aggressive.

Exome sequencing of all 33 men with PCa resulted in the identification of an average of over 1.4 million total SNVs per sample (Table II). Within the target regions, 22,481 coding SNVs including 10,830 missense and 122 nonsense variants were called on average per sample.

**TABLE I. Clinical Characteristics of Exome-Sequenced Families and Individuals**

| | N (%) or median [range] |
|---|---|
| Families (N = 11) | |
| Number of confirmed affecteds | 7 [4–12] |
| Average age of diagnosis | 65.0 [59.4–67.5] |
| Individuals (N = 33) | |
| Age of diagnosis (years) | 58 [48–76] |
| Pre-diagnosis PSA (ng/ml) | 5.6 [0.8–38.42] |
| Gleason grade[a] | |
| <7 | 12 (41.4%) |
| 7 | 14 (48.3%) |
| >7 | 3 (10.3%) |
| Stage[a,b] | |
| Local | 22 (75.9%) |
| Locally advanced | 6 (20.7%) |
| Metastatic | 1 (3.4%) |
| Clinically aggressive PCa[c] | 10 (30.3%) |

[a]Numbers do not add up to 33 due to missing data.
[b]Localized = T1 or T2, N0 and M0 or Pre-Dx PSA <20 ng/ml; locally advanced = T3 or T4, N0 and M0 or Pre-Dx PSA >20 ng/ml but <100 ng/ml; metastatic = N1 or M1 or Pre-Dx PSA >100 ng/ml.
[c]Clinically Aggressive PCa = (1) Gleason sum >7 or (2) Stage T3b or T4 or (3) N1 or (4) M1 or (5) pre-diagnosis PSA >15 ng/ml or (6) Gleason sum = 7 and positive margin or (7) Gleason sum = 7 and pre-diagnosis PSA >10 ng/ml.

**TABLE II. Summary of SNVs Called From Exome Sequencing**

| | Mean (range) |
|---|---|
| All called SNVs | 1,469,117 (377,621–2,245,788) |
| SNVs in target region | 58,829 (47,205–65,697) |
| Coding synonymous | 11,529 (10,877–11,854) |
| Missense | 10,830 (10,217–11,128) |
| Nonsense | 122 (106–140) |
| | **Total N** |
| SNVs in candidate genes | 4,856 |
| Synonymous | 1,035 |
| Nonsense | 10 |
| Missense | 1,052 |
| Shared[a] missense | 77 |
| Rare shared missense | 20 |
| Deleterious[b] | 59 |
| Shared[a] deleterious | 2 |
| Partially shared[c] deleterious | 15 |

[a]To be considered shared, a variant had to be present in all three family members in each family in which it was observed.
[b]To be considered deleterious, a variant had to be predicted to be damaging or conserved by five of five algorithms used.
[c]To be considered partially shared, a variant had to be present in exactly two family members in each family in which it was observed.

Six hundred ninety-seven candidate genes were identified based on function, location near a previously reported, chromosome 17 linkage signal, and/or previous association with PCa or other cancers (see Supplementary Table). Overall, 4,856 unique SNVs were identified in candidate genes, including 1,052 missense and 10 nonsense variants (Table II). Seventy-seven missense variants were shared by all three family members in each family in which they were observed; 57 of these were observed in all 33 cases and were presumed to be rare variants in the reference sequence and excluded from further analysis. Additionally, 59 variants were predicted to be deleterious by each of five in silico bioinformatics tools. Of the 59 deleterious variants, 17 were shared by at least two family members in each family in which they were observed; two of these were shared by three of three family members and were included in the shared, missense variants. The remaining 15 shared, deleterious variants were observed in two of three family members. All eleven families sequenced carried at least one nonsense; shared, missense; or partially shared, deleterious variant.

The 20 shared, missense variants, the 15 partially shared, deleterious variants and all 10 nonsense variants (Table III) were sequenced using Sanger methodology for verification. All 10 nonsense variants and 33 of 35 missense variants were confirmed. Two missense variants (*ATM* Arg189Lys and *ATM* Ile190-Lys) were determined to be false positives. Of the 43 confirmed variants, 36 were present in the dbSNP database, and 7 SNVs were novel. Twenty-six SNVs were observed in the 1000 genomes European ancestry samples; those had minor allele frequencies ranging from 0.1% to 42.1%.

Four missense variants, *PARP2* Arg283Gln, *BLM* Gln123Arg, *LRCC46* Ala295Thr, and *KIF2B* Pro91Leu, and 1 nonsense variant, *CYP3A43* Arg441Ter, showed complete co-segregation with PCa status (Fig. 1). The *PARP2* Arg283Gln, *BLM* Gln123Arg, *LRCC46* Ala295Thr, and *KIF2B* Pro91Leu minor alleles were observed in the same pedigree, and were carried by the proband, his affected brother, father and paternal uncle; none of the minor alleles was carried by the proband's mother while the *LRCC46* Ala295Thr minor allele was observed in the proband's paternal aunt. The *CYP3A43* Arg441Ter minor allele was observed in a different pedigree where it was carried by two affected brothers as well as their affected father and paternal uncle; it was not carried by an unaffected brother. Twelve additional variants, *RAD18* Glu453Ter, *IGHMBP2* Val293Ile, *POLE* Ala31Ser, *RAD51D* Asn138Ser, *ASB16* Glu223Gly, *HOXB13* Gly84Glu, *B4GALNT2* Tyr274Asn, *CHAD* Ala342Asp, *ANKFN1* Ile443Thr, *EGFR* Arg962Cys, and *POLG* Ala467Thr, exhibited partial co-segregation with PCa status with

**TABLE III. Nonsense and Shared Missense Variants in Exome Sequencing Data**

| Gene | Chromosome | Protein substitution | dbSNP ID | No. of individuals | No. of families | 1000 Genomes frequency |
|---|---|---|---|---|---|---|
| Nonsense variants | | | | | | |
| RNASEL | 1 | Glu265Ter | rs74315364 | 1 | 1 | 0.003 |
| RAD18 | 3 | Glu453Ter | N/A | 3 | 1 | N/A |
| PARP3 | 3 | Glu373Ter | N/A | 1 | 1 | N/A |
| CYP3A43 | 7 | Arg441Ter | rs149091175 | 3 | 1 | N/A |
| RAD52 | 12 | Ser346Ter | rs4987207 | 3 | 3 | 0.016 |
| RAD52 | 12 | Tyr415Ter | rs4987208 | 1 | 1 | 0.011 |
| PARP4 | 13 | Leu245Ter | N/A | 1 | 1 | N/A |
| RFC3 | 13 | Arg105Ter | rs142666536 | 1 | 1 | N/A |
| FANCA | 16 | Gln271Ter | rs372163487 | 1 | 1 | N/A |
| C17orf57 | 17 | Art211Ter | rs71377306 | 3 | 2 | 0.090 |
| Missense variants | | | | | | |
| GSTM1 | 1 | Lys173Asn | rs74837985 | 6 | 2 | 0.421 |
| MSH2 | 2 | Gly322Asp | rs4987188 | 3 | 1 | 0.020 |
| PDGFRA | 4 | Leu221Phe | rs139913632 | 3 | 1 | 0.007 |
| UGT2B15 | 4 | Ser172Arg | rs200638397 | 3 | 1 | 0.000 |
| IGHMBP2 | 11 | Val293Ile | N/A | 3 | 1 | N/A |
| POLE | 12 | Ala31Ser | rs34047482 | 3 | 1 | 0.015 |
| PARP4 | 13 | Ala710Val | rs200991879 | 3 | 1 | 0.001 |
| PARP2 | 14 | Arg283Gln | rs3093926 | 3 | 1 | 0.073 |
| RAD51B | 14 | Leu172Trp | rs34094401 | 3 | 1 | 0.009 |
| BLM | 15 | Gln123Arg | rs371223446 | 3 | 1 | N/A |
| RAD51D | 17 | Asn138Ser | rs201676898 | 3 | 1 | 0.000 |
| ASB16 | 17 | Glu223Gly | rs200625323 | 3 | 1 | 0.007 |
| SLC4A1 | 17 | Glu40Lys | rs45562031 | 3 | 1 | 0.017 |
| LRRC46 | 17 | Ala295Thr | rs145648581 | 3 | 1 | 0.008 |
| HOXB13 | 17 | Gly84Glu | rs138213197 | 3 | 1 | 0.003 |
| B4GALNT2 | 17 | Tyr274Asn | N/A | 3 | 1 | N/A |
| CHAD | 17 | Ala342Asp | rs144030994 | 3 | 1 | 0.007 |
| KIF2B | 17 | Pro91Leu | rs145539514 | 3 | 1 | 0.003 |
| ANKFN1 | 17 | Ile443Thr | rs147516817 | 3 | 1 | N/A |
| Deleterious variants | | | | | | |
| MLH1 | 3 | Lys618Glu | rs35001569 | 2 | 1 | 0.005 |
| MLH1 | 3 | Lys618Thr | rs63750449 | 2 | 1 | 0.005 |
| PLXNB1 | 3 | Val503Met | rs140699424 | 2 | 1 | N/A |
| VEGFC | 4 | Arg61Gln | rs41278571 | 2 | 1 | 0.018 |
| EGFR | 7 | Arg962Cys | N/A | 2 | 1 | N/A |
| FANCI | 15 | Leu605Phe | rs117125761 | 2 | 1 | 0.005 |
| POLG | 15 | Ala467Thr | rs113994095 | 2 | 1 | 0.001 |
| RECQL5 | 17 | Arg943His | rs200535477 | 2 | 1 | 0.004 |
| XRCC1 | 19 | Ser485Tyr | rs2307184 | 2 | 1 | 0.001 |
| WNK4 | 17 | Pro1025Leu | rs56099549 | 4 | 2 | 0.013 |
| SLC4A1 | 17 | Val245Met | rs148170067 | 2 | 1 | 0.004 |
| SP2 | 17 | Gly497Arg | rs143694248 | 2 | 1 | N/A |
| KAT7 | 17 | Arg234Thr | rs200465294 | 2 | 1 | N/A |
| UTP18 | 17 | Tyr535Cys | N/A | 2 | 1 | N/A |

each pedigree containing either one affected non-carrier or one unaffected carrier of the minor allele.

## DISCUSSION

PCa has a well-described heritable component; genes that are recognized to influence PCa susceptibility include *BRCA1* and *BRCA2* [11–18], *HOXB13* [5] and possibly genes associated with the mismatch repair phenotype [19–21]. In this report, we describe a candidate gene approach to analyzing whole exome sequencing data to identify additional hereditary PCa gene mutations. Our modest sample size (33 PCa cases from 11 hereditary PCa families) and large number of

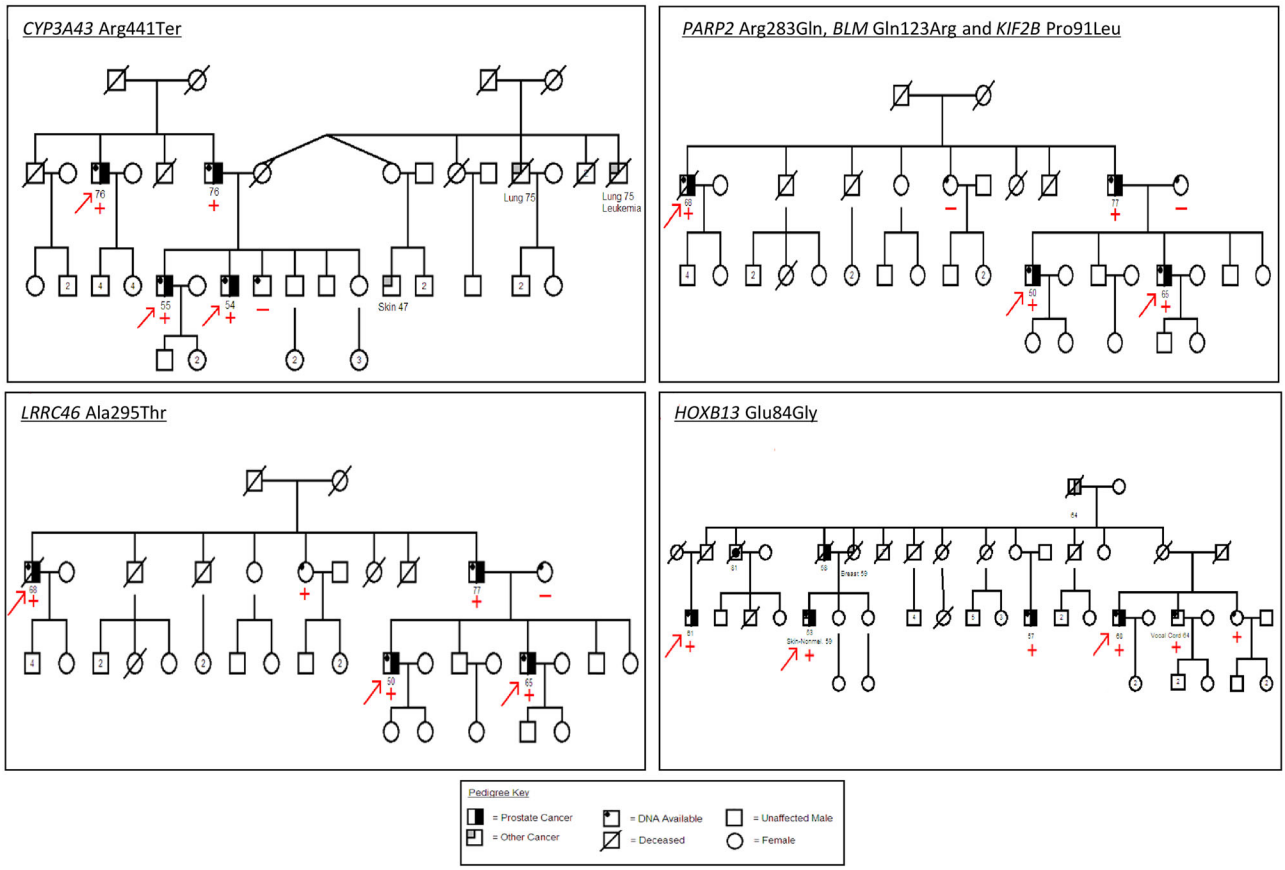


**Fig. 1.** Pedigrees of families with complete co-segregation of a candidate gene variant and PCa status. Red arrows indicated individuals with exome sequencing data. Plus signs indicate carriers of the variant allele, and minus signs indicate individuals who are wild-type for the specified variant

variants identified led us to focus first on candidate genes. Examination of SNVs in 697 candidate genes identified 10 nonsense, 19 shared, and 14 partially shared, deleterious missense variants in these 11 families. Five variants, *CYP3A43* Arg441Ter, *PARP2* Arg283Gln, *BLM* Gln123Arg, *LRCC46* Ala295Thr, and *KIF2B* Pro91Leu, were shown to completely co-segregate with PCa status. However, given the late onset nature of PCa and likely incomplete penetrance of any given PCa gene mutation, all 43 confirmed mutations are in genes that should be studied in larger data sets.

When selecting the candidate genes for this analysis, we elected to include all genes within a previously identified candidate linkage interval at 17q21-22. This linkage region was first identified by the UM PCGP [9,10] and later confirmed by several consortium [22]. In 2012, we discovered that mutations in the *HOXB13* gene, a member of the homeobox superfamily of transcription factors, were associated with the occurrence of early onset and hereditary PCa [5]. Most notably, a recurrent non-conservative substitution of glutamic acid for glycine (Gly84Glu), was found more commonly in men with early-onset, hereditary PCa (3.1%) compared to me with late onset-non-familial PCa (0.6%, $P = 2.0 \times 10^{-6}$). In our discovery work, four of 94 unrelated families harbored the HOXB13 Gly84Glu mutation. When these families were removed and the remaining 90 families were analyzed together, evidence for linkage to the chromosome 17q21-22 linkage region persisted leading us to include all 202 genes in the linkage region in this current analysis. As proof of principle supporting the design of our candidate gene analysis, we identified a single family from the 11 in which three affected family members shared the *HOXB13* Gly84Glu mutation (Table III).

We identified the *CYP3A43* Arg441Ter variant in four affected members of a single hereditary PCa family. Overall, this mutation appears to be rare; it was not observed in the 1000 Genomes data. Although it was not seen in the Exome Sequencing Project (ESP) European ancestry (EA) sample; it was observed in 2/4406 chromosomes of African ancestry in the ESP database [23]. *CYP3A43* Arg441Ter is a protein trun-

cating mutation resulting in the loss of the final 62 residues of the protein. Although the functional significance of the variant is unknown, it was predicted to be damaging by PolyPhen, MutationTaster and LRT but benign/non-conserved by SIFT and PhyloP. *CYP3A43* is a member of the cytochrome p450 superfamily whose members play roles in drug and steroid hormone metabolism. The CYP3A43 enzyme, like other members of this gene family, is involved in testosterone metabolism [24]. Variants in *CYP3A43* have been previously associated with PCa risk and mortality [25–29], and investigation of the role of *CYP3A43* Arg441Ter in PCa is warranted.

We identified mutations in three PARP genes (PARP2, PARP3, and PARP4) in hereditary PCa families. *PARP2* is one of several poly-ADP ribose polymerase genes. The *PARP* gene products are involved in the repair of single strand breaks. Additionally, *PARP1* and *PARP2* germline variants have been associated with risk of several cancers [30–33], and PARP inhibitors have been studied as therapeutic agents in several cancers [34–36]. *PARP2* Arg283Gln was the most common of the three co-segregating variants we identified, with a minor allele frequency of 7% in the 1000 genomes database and 6.44% in the ESP EA sample [23]. *PARP2* Arg283Gln was predicted to be damaging by LRT and conserved by PhyolP but benign by SIFT, PolyPhen, and Mutation Taster.

A rare, shared missense mutation in the *BLM* gene (Gln123Arg) was identified in 1 of 11 PCa families. The *BLM* gene encodes a RecQ helicase involved in DNA damage repair in the homologous recombination (HR) pathway. Other HR genes, namely *BRCA1* and *BRCA2*, have previously been associated with PCa risk [11–18]. Mutations in *BLM* are known to cause Bloom syndrome, a recessive disorder with multiple phenotypic effects including the predisposition to various cancers [37,38]. Additionally, a *BLM* truncating mutation was recently identified as a possible breast cancer susceptibility allele [39]. *BLM* Gln123Arg was not observed in either the dbSNP or 1000 Genomes databases and was observed in 1 of 8596 EA chromosomes in the ESP database. It was predicted to be benign/non-conserved by five algorithms (SIFT, PolyPhen, MutationTaster, LRT, and PhyloP).

In summary, we have used a candidate gene approach to characterize nonsense, shared missense, and deleterious mutations shared by at least two affected family members in a set of hereditary PCa families. This approach would have led to the identification of the *HOXB13* Gly84Glu mutation in a hereditary PCa family serving as proof-of-principle for our study design. Further investigation is needed to determine the role of the entire set of candidate gene variants described here in PCa risk and progression.

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