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Mutational Landscape of Candidate Genes in Familial Prostate Cancer

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Abstract

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, 4856 candidate gene SNVs were identified, including 1052 missense and 10 nonsense variants. Twenty missense variants were shared by all 3 family members in each family in which they were observed. Additionally, 15 missense variants were shared by 2 of 3 family members and predicted to be deleterious by 5 different algorithms. Four missense variants, *BLM* Gln123Arg, *PARP2* Arg283Gln, *LRCC46* Ala295Thr and *KIF2B* Pro91Leu, and 1 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.

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 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 prostate cancer susceptibility gene, *HOXB13* with the G84E mutation resulting in a 10–20 fold increased risk of prostate cancer[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 prostate cancer 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 prostate cancer 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 5 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 697 candidate genes were included in the analysis (see Supplementary Table). All single nucleotide variants (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 5 prediction programs and were shared by 2 of 3 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 1. 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 prostate cancer (Table 1) 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 2). Within the target regions, 22,481 coding SNVs including 10,830 missense and 122 nonsense variants were called on average per sample.

697 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, 4856 unique SNVs were identified in candidate genes, including 1052 missense and 10 nonsense variants (Table 2). 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 5 *in silico* bioinformatics tools. Of the 59 deleterious

variants, 17 were shared by at least 2 family members in each family in which they were observed; 2 of these were shared by 3 of 3 family members and were included in the shared, missense variants. The remaining 15 shared, deleterious variants were observed in 2 of 3 family members. All eleven families sequenced carried at least 1 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 3) 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* Ile190Lys) 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 (Figure 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 2 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 each pedigree containing either 1 affected non-carrier or 1 unaffected carrier of the minor allele.

Discussion

Prostate cancer has a well described heritable component; genes that are recognized to influence prostate cancer 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 prostate cancer gene mutations. Our modest sample size (33 PCa cases from 11 hereditary PCa families) and large number of 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 prostate cancer 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 prostate cancer[5]. Most notably, a recurrent nonconservative substitution of glutamic acid for glycine (Gly84Glu), was found more commonly in men with early-onset, hereditary PCa (3.1%) compared to men with late onset-nonfamilial 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 3).

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 truncating 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 prostate cancer is warranted.

We identified mutations in 3 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 3 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 PhyloP but benign by SIFT, PolyPhen and Mutation Taster.

A rare, shared missense mutation in the *BLM* gene (Gln123Arg) was identified in 1 of eleven 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 5 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 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

Table 1

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)	58 [48–76]
Age of Diagnosis (years)	5.6 [0.8–38.42]
Pre-Diagnosis PSA (ng/mL)	
Gleason Grade *	12 (41.4%)
<7	14 (48.3%)
7	3 (10.3%)
>7	
Stage *†	22 (75.9%)
Local	6 (20.7%)
Locally Advanced	1 (3.4%)
Metastatic	10 (30.3%)
Clinically Aggressive PCa ‡	

* Numbers do not add up to 33 due to missing data

† Localized = T1 or T2, N0 and M0 or Pre-Dx PSA <20ng/ml

Locally Advanced = T3 or T4, N0 and M0 or Pre-Dx PSA >20ng/ml but <100ng/ml

Metastatic = N1 or M1 or Pre-Dx PSA >100ng/ml

‡ Clinically Aggressive PCa = 1) Gleason Sum > 7 or 2) Stage T3b or T4 or 3) N1 or 4) M1 or 5) Pre-diagnosis PSA > 15ng/ml or 6) Gleason Sum = 7 and positive margin or 7) Gleason Sum = 7 and Pre-diagnosis PSA > 10 ng/ml

Table 2

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	4856
Synonymous	1035
Nonsense	10
Missense	1052
Shared* Missense	77
Rare Shared Missense	20
Deleterious**	59
Shared* Deleterious	2
Partially Shared*** Deleterious	15

* To be considered shared, a variant had to be present in all 3 family members in each family in which it was observed.

** To be considered deleterious, a variant had to be predicted to be damaging or conserved by 5 of 5 algorithms used.

*** To be considered partially shared, a variant had to be present in exactly 2 family members in each family in which it was observed.

Table 3

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	Arg211Ter	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

GENE	CHROMOSOME	PROTEIN SUBSTITUTION	dbSNP ID	NO. OF INDIVIDUALS	NO. OF FAMILIES	1000 GENOMES FREQUENCY
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