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Identification and characterization of novel SNPs in *CHEK2* in Ashkenazi Jewish men with prostate cancer

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Abstract

Checkpoint kinase 2 (CHEK2) is a protein involved in arresting cell cycle in response to DNA damage. To investigate whether it plays an important role in the development of prostate cancer (PRCA) in the Ashkenazi Jewish (AJ) population, we sequenced *CHEK2* in 75 AJ individuals with prostate, breast, or no cancer ($n = 25$ each). We identified seven coding SNPs (five are novel) that changed the amino-acid sequence, resulting in R3W, E394F, Y424H, S428F, D438Y, P509S, and P509L. We determined the frequency of each variant in 76 AJ families collected by members of the International Consortium for Prostate Cancer Genetics (ICPCG) where $\geqslant 2$ men were affected by PRCA. Only one variant, Y424H in exon 11, was identified in more than two families. Exon 11 was then screened in nine additional AJ ICPCG families (a total of 85 families). The Y424H variant occurred in nine affected cases from four different families; however, it did not completely segregate with the disease. We performed bioinformatics analysis, which showed that Y424H is a non-conservative missense substitution that falls at a position that is invariant in vertebrate *CHEK2* orthologs. Both SIFT and Align-GVGD

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predict that Y424H is a loss of function mutation. However, the frequency of Y424H was not significantly different between unselected AJ cases from Montreal/Memorial Sloan Kettering Cancer Centre (MSKCC) and AJ controls from Israel/MSKCC (OR 1.18, 95%CI: 0.34–4.61, $p = .99$). Moreover, functional assays using *Saccharomyces cerevisiae* revealed that the Y424H substitution did not alter function of CHEK2 protein. Although we cannot rule out a subtle influence of the *CHEK2* variants on PRCA risk, these results suggest that germline *CHEK2* mutations have a minor role in, if any, PRCA susceptibility in AJ men.

Keywords: Checkpoint kinase 2; Prostate cancer; CHEK2; Single-nucleotide polymorphism; Ashkenazi Jewish; Budding yeast

1. Introduction

Prostate cancer (PRCA) is a leading cause of morbidity and mortality in men. It is diagnosed in almost one-fifth of US men during their lifetime. Although many etiological factors have been implicated, genetic predisposition and age remain as the two major factors in the development of PRCA [1]. Epidemiological studies suggest that up to 5% of all cases may be due to autosomal dominant genes [2–5] and twin studies suggest that approximately 42% of PRCA cases diagnosed under the age of 70 years are likely due to heritable factors [6]. Men with an affected father or brother are twice as likely to develop PRCA as men with no affected relatives [7]. In addition, the relative risk of developing PRCA rises considerably as the number of cases in a family cluster increases and the average age at diagnosis in the cluster decreases [8]. A recent meta-analysis on the risk of PRCA among men with a positive family history found a 1.8- to 2.1-fold increased risk if a second degree relative is affected and 2.9-fold increased risk if the father or a brother is affected [9].

The *CHEK2* gene, located on chromosome 22q, encodes a checkpoint kinase that acts to prevent cellular entry into mitosis in response to DNA damage, presumably to gain time needed for DNA repair [10,11]. Activated CHEK2 phosphorylates BRCA1 and TP53 proteins, regulating tumor suppressor function of these proteins [12–14]. Mutations in *CHEK2* were originally described in Li–Fraumeni syndrome and Li–Fraumeni-like families [15,16], and the 1100delC variant was later found to be a moderate risk breast cancer susceptibility allele [17–19].

Several studies suggest that the *CHEK2* locus or its variants maybe important in PRCA susceptibility. In a linkage study of 1233 PRCA families, analysis of the 269 families with at least 5 affected members identified a LOD score of 3.57 at 22q12 [20], which is near the *CHEK2* locus at 22q12.1 and this has recently been further refined [21]. Additionally, four independent studies have investigated the association between *CHEK2* mutations and PRCA risk with some conflicting results [22–26]. In this study, we investigated whether germline *CHEK2* mutations play an important role in the development of PRCA in the Ashkenazi Jewish population. This ethnic group has founder mutations in other cancer-predisposing genes such as *BRCA2* 6174delT that have been shown to be more frequent in AJ men with prostate cancer [27] making it a good study population.

2. Materials and methods

2.1. SNP discovery and frequency estimate

In the first step in the identification of *CHEK2* variants ($q > 0.01$), *CHEK2* was sequenced in 75 AJ individuals with prostate, breast, or no cancer ($n = 25$ each). DNA was extracted from blood lymphocytes using standard methods. Since a portion of the *CHEK2* gene (exons 10–14) shares high homology with regions on other chromosomes [28], all primers were tested by in silico PCR and BLAT (UCSC Genome Bioinformatics Website) to confirm the specificity of each primer pair. Sequencing was done in both forward and reverse directions using an ABI 3730XL DNA Sequencer (Applied Biosystems, Foster, CA). Sequences were analyzed using Chromas 2.3 (Technelysium Pty, Tewantin, Qld, Australia). Long range PCR using previously described methods [29] was used to confirm that all identified variants were located within the functional copy of *CHEK2*, on chromosome 22q12.

The 150 prostate cancer cases used for SNP discovery, including the 25 samples used for the preliminary frequency analysis, were obtained from an on-going recruitment effort among McGill University-affiliated hospitals as described previously [30]. Twenty-five of the 150 prostate cancer cases were chosen for SNP discovery based on the presence of a family history and/or a high Gleason score (mean score = 7.4; mean age at diagnosis = 67.5 years). The 25 healthy AJ controls were recruited as part