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Research Article

Pharmacogenomic analysis of individual variation in prostate cancer

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ABSTRACT

Single Nucleotide Polymorphisms (SNPs) are the most common genetic variation among individuals. The work aims at identifying the SNPs associated with prostate cancer. In the present work, pharmacogenomic analysis has been carried out to analyze the impact of functional SNPs in prostate cancer. 11 genes involved in signal transduction in prostate cancer have been subjected to genomic analysis. The genomic analysis protocol includes microsatellite analysis, restriction fragment length polymorphism (RFLP) analysis, silent mutation analysis, GC content Analysis and deleterious SNP analysis. From the deleterious SNP analysis, it has been found that the mutations rs28571178 in IL16 (5' UTR) and rs17854206 in JAZF1 (3' UTR) cause functional effects on the specific genes. Upon stability analysis of native and mutated proteins, it has been concluded that the above deleterious mutations are supported by nature due to their increased stability. These SNPs have been identified as the most deleterious in causing prostate cancer.

Keywords: Comparative modeling; Individual variation; pharmacogenomic; prostate cancer; SNPs

INTRODUCTION

Prostate cancer that develops in the prostate gland in male reproductive system usually affects males above the age of 50. It is one of the major causes of death in males. Prostate cancer may occur due to mutations or dysregulation of genes involved in signal transduction and cell cycle (Lee J T *et al*., 2008). SNPs are one of the major causes of individual variation in humans. The functional effect of a SNP and its association with specific diseases can be predicted using several computational analyses (P K Krishnan Namboori *et al*., 2011). In the present study, eleven genes (LMTK2, KLK3, CTBP2, JAZF1, CPNE3, IL16, CDH13, EHBP1, NUDT10, and NUDT11) involved in signal transduction in prostate cancer have been taken up.

MATERIALS AND METHODS

Analysis of SNP

Microsatellites are short sequence repeats of di, tri or tetra nucleotides in the genome. They are one of the sources of genetic variation due to their highly polymorphic nature. Mutations occurring in the microsatellites located adjacent to specific genes are found to be associated with certain neurodegenerative disorders (Tautz and Schlotterer, 1994). Eleven genes were subjected to the analysis using Imperfect Microsatellite Extractor (IMEx). Six genes have been identified to be highly mutable were selected to proceed with further analysis. RFLPs are gene fragments resulting from digestion with restriction endonucleases. They are used as genetic markers due to the length variation in individuals. Restriction site mutations give rise to RFLP variation among individuals. Five genes having the comparatively higher number of fragments were selected from the six genes subjected to the analysis. Further silent mutation analysis was performed on the oligos of the selected genes obtained from RFLP analysis to examine the possible restriction site mutations that may be harmful in near future. All the gene fragment showed are found to be keeping a high number of base changes. Mutation effect depends on mutability of the gene and the effect of mutagen. In order to analyze the mutability of genes, GC content analysis has been performed. GC content determines the stability of the genes. Therefore, three genes with comparatively low GC content have been selected to proceed with further analysis.

SNPs in the coding non-synonymous region and the untranslated region (UTR) have been selected for the analysis as they are more likely to cause functional variation in genes. The coding non- synonymous SNPs (nsSNPs) and the 3' and 5' UTR SNPs were collected from dbSNP database of NCBI (National Centre for Biotechnology Information). Further deleterious SNP analysis was performed using the computational tools,

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Table 1: Deleterious SNP analysis

SNP Id	Gene Name	Mutated Position	Amino Acid Change	Functional Effects
rs28571178	Il16	195	R/w	Splicing regulation
rs17854206	Jazf1	133	D/g	Splicing regulation

Table 2: Stability analysis

SNP Id	Gene Name	Native protein Energy (kcal/mol)	Mutated protein energy (kcal/mol)
rs28571178	IL16	-7387.17000	-6580.36070
rs17854206	JAZF1	-15045.09211	-13297.51125

sorting intolerant from tolerant (SIFT) and Polyphen 2 for nsSNPs and FastSNP and UTRscan for UTR SNPs.

SIFT works on the assumption that significant amino acids in protein families will be conserved and any changes in the conserved regions are likely to be predicted as deleterious. The algorithm searches the protein repository for similar proteins for an input sequence followed by multiple sequence alignment. The calculated probability score indicates whether the specific amino acid substitution is within the tolerance level or deleterious, based upon the cut off value. The cut off score is set as 0.05. If the probability value is less than the cut off score then the substitution is predicted to be deleterious.

The deleterious predictions are subjected to Polyphen 2 analysis. Polyphen2 predicts all the probable conformational and functional effects of amino acid replacement in human proteins. The components such as the number of sequential features, functional and evolutionary information are the major criteria for prediction using Polyphen. For an amino acid replacement, the algorithm analyzes all the sequential and structural features of the particular replacement site and provides it to the probabilistic classifier. Polyphen recognizes the input protein as an UniProt/Swiss-prot entry and uses the feature table for recognition. The sequential features thus include the specific site at which replacement took place in the active site, transmembrane or non-globular region. For extraction of the structural features, Polyphen plots the amino acid replacement position to recognized protein structures using some conformational parameters from the database of secondary structure in proteins. Using all the parameters, the position-specific independent count (PSIC) score is computed. If the PSIC score difference between the variations is ≥0.9 it is predicted to be probably damaging.

FASTSNP (Fast Analysis and Selection Tool for Single Nucleotide Polymorphism) web server analyzes the phenotypic risk and recognized functional consequences of the input SNP and helps in identifications and characterization of the risk factor of SNPs. The tool is associated with eleven web servers information from which updated functional effects and risk analysis for a query is provided, proving to be very efficient in association studies.

UTRscan program analyzes for functional elements in the untranslated region of the input sequence. UTRsite is a collection of recognized patterns that represent different functional elements present in the 5' and 3' untranslated regions of the sequences. UTRscan, the untranslated regions analysis tool checks for patterns in the sequence based on the recognized patterns in UTRsite database. The result disclosed two SNPs to be damaging and are presented in Table 1.

Available native protein structure was collected from PDB repository and was modeled by comparative modeling using Modeler 9.10. Mutated proteins structures of the native protein were generated by making the amino acid substitution in the position corresponding to the specific SNP. Molecular modeling within the molecular mechanics level of computation was carried out for the two native and the mutated protein structures using CharmM forcefield of Accelrys Discovery Studio 2.1 and the results are presented in Table 2.

CONCLUSION

Insilco method of analyzing SNP has been effectively performed with several computational tools. It has been concluded with evidence that two mutations rs28571178 (arginine replaced with tryptophan) and rs17854206 (alanine replaced with glycine) are the most deleterious to the genes IL16 and JAZF1 involved in signal transduction in prostate cancer. Presence of these SNPs predicts a high susceptibility of prostate cancer.

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