International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

SIRT1 gene analysis by bioinformatics and genotyping in breast cancer patients

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Abstract

Sirtuin (Silent mating type information regulation 2 homolog)1(SIRT1) protein plays a vital role in many disorders such as diabetes, cancer, obesity, inflammation, and neurodegenerative and cardiovascular diseases. The objective of the study was to analyse in SIRT1's functional SNPs by bioinformatics and also to study the polymorphism of the gene(rs3740051) in breast cancer patients. nsSNPs of SIRT1 protein were analysed by various bioinformatic tools such as SIFT, Provean, and I- mutant to find the most deleterious ones. PCR-Sequencing method was used to study the pattern of polymorphisms of rs3740051 in 104 Indian breast cancer patients in a cross-sectional study. Out of 252 nsSNPs, SIFT analysis showed that 94 were deleterious, Provean listed 77 dangerous, and I mutant found 66 nsSNPs resulting in lowered stability of proteins. genotype frequencies for rs3740051 in the SIRT1 gene among the breast cancer patients do not significantly deviate from the expected frequencies. The Chi-square value of 0.765 is relatively small, and the p-value of 0.68 is greater than the commonly used significance threshold of 0.05. Bioinformatics study could give the details of all the damaging SNPs of SIRT1.The genotyping of the gene cancer patients suggested predominant wild pattern of the allele among breast cancer patients.

Keywords: SIRT1, ns SNP, bioinformatics, breast cancer, Sequencing.

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1. Introduction

Sirtuins represent a class of NAD+-dependent deacetylases that govern intracellular transcriptional activities and are distributed across various tissues, including adipose, kidney, brain, liver, and muscle [1,2]. Among these, SIRT1 (Silent mating type information regulation 2 homolog 1) stands out for its role in modulating an array of cellular processes such as lipid and glucose metabolism, stress tolerance, autophagy, circadian rhythms, and mitochondrial biogenesis [3-5]. Notably, SIRT1 gene expression intricately regulates downstream pathways impacting conditions like diabetes, cancer, obesity, inflammation, and neurodegenerative and cardiovascular diseases through interactions with key cellular proteins, including nuclear factor- kappa B (NF-κB), endothelial nitric oxide synthase (eNOS), forkhead transcriptional factors (FoxOs), AMP-activated protein kinase (AMPK), and protein tyrosine phosphatase (PTP). However, emerging evidence indicates SIRT1's upregulation in various malignant cell types, and research has shown that SIRT1 antagonists can impede the growth of cancer cells [6-8]. Single nucleotide polymorphisms (SNPs) refer to genetic variations stemming from alterations in a single nucleotide base (A, T, C, or G) within the DNA sequence. These SNPs hold significant value in biomedical research, pharmaceutical development, enhanced medical diagnostics, and personalized medicine applications [9]. SNPs are accountable for distinct phenotypes, underscoring the importance of their identification. Yet, this task poses substantial challenges, involving the rigorous assessment of numerous candidate gene SNPs. In such complex scenarios, bioinformatics tools play a pivotal role in distinguishing between neutral and functional SNPs, shedding light on their structural implications and functional significance. These bioinformatics applications serve as valuable resources for evaluating SNPs in terms of their functional impact, and the independent validation of SNP functionality through prediction tools serves as an additional means to discern genuine associations from false positives.

Studying SIRT1 gene polymorphisms in Indian breast cancer patients holds significant importance due to its potential implications for both cancer risk assessment and personalized treatment strategies. Genetic variations in the SIRT1 gene can influence its activity and function,

potentially impacting critical cellular processes related to cancer development and progression. By investigating SIRT1 polymorphisms in the Indian population, we can gain insights into the genetic factors that may contribute to breast cancer susceptibility and prognosis among Indian individuals. This knowledge can aid in identifying individuals at higher risk, facilitating early detection, and guiding tailored treatment approaches based on the specific genetic profiles of patients. Additionally, understanding how SIRT1 gene polymorphisms interact with environmental factors in the Indian context can provide valuable information for developing more targeted and effective interventions for breast cancer prevention and management in this population. rs3740051 is the least studies SNP among breast cancer patients in our population. It is expected that research would be done to know the SIRT1 gene and its structural and functional changes and genetic study will let us know the prevalence of SIRT1 gene mutations in Indian breast cancer patients. Objectives of the study were to find the SIRT1 protein's most dangerous nsSNPs by bioinformatics tools as well as to assess the genetic pattern of SIRT1 gene (rs3740051) by PCR-Sanger sequencing methos in breast cancer patients to investigate the prevalence of mutation in Indian patients.

2. Methods

2.1 Part A: Bioinformatics analysis

The study involved multiple steps to assess the impact of non-synonymous single nucleotide polymorphisms (nsSNPs) on the SIRT1 gene. Firstly, the NCBI database was used to obtain information on the SIRT1 gene, including its nsSNPs. Amino acid sequences for the SIRT1 gene were retrieved and filtered nsSNPs from the dbSNP database were analyzed. Next, the study focused on identifying potentially damaging nsSNPs. Three bioinformatics tools were employed for this purpose: SIFT (Sorting Intolerant from Tolerant) Server, which predicts the effect of nucleotide substitutions and frame shifts on protein function based on amino acid residue conservation; Provean, which assesses the impact of amino acid variations on protein function and uses a threshold score for interpretation; and I-Mutant 2.0, which analyzes protein stability changes due to mutations. Each tool provided insights into the potential functional impact of the nsSNPs. Overall, these steps were integral to understanding the potential consequences of nsSNPs in the SIRT1 gene on protein function and stability. The study conducted a comprehensive assessment of non-synonymous single nucleotide polymorphisms (nsSNPs) in the SIRT1 gene, focusing on their structural and functional effects. Three highly deleterious nsSNPs were selected from each of the SIRT1 gene's isoforms and analyzed using three distinct software tools: Polyphen, HOPE, and I-Tasser. Polyphen, or Polymorphism Phenotyping v2, employed physical and comparative considerations to predict the potential impact of amino acid substitutions on the structure and function of the SIRT1 protein. HOPE, an automated server, analyzed mutants to reveal structural effects by gathering data from various sources, including UniProt annotations and 3D protein coordinates. I-Tasser, a software package for protein modeling, used Template modeling scores to compare wild-type and mutant models, determining structural similarity with RMSD and TM values. Additionally, the study explored gene-gene interactions of the SIRT1 gene using STRING to understand the broader context of protein interactions crucial for system homeostasis. This multi-faceted approach provided insights into the effects of nsSNPs on the SIRT1 protein, contributing to a comprehensive understanding of their potential implications.

2.2 Part B- Genetic analysis

The study was conducted at the Medical Oncology Department of Justice KS Hegde Charitable Hospital in Deralakatte and the Central Research Laboratory of KSHEMA, following a cross-sectional research design. The study was initiated after obtaining approval from Central Ethics Committee, NITTE Deemed to be University (Ref: No: NU/CEC/2021/212). It involved 104 individuals diagnosed with breast cancer who were recruited as participants. Inclusion criteria required that these individuals be female patients with primary breast carcinoma, confirmed through histopathological analysis, and undergoing treatment with anticancer medications. Exclusion criteria ensured that patients with comorbid conditions such as Diabetes Mellitus, congestive heart disease, inflammatory and autoimmune disorders, as well as renal stone disease, were not included in the study. This approach aimed to provide a focused and representative sample for the research investigation. Two ml of blood sample was collected in EDTA tubes for genetic analysis. The SIRT1 rs3740051 was analysed by Sanger sequencing after amplifying by PCR (conditions given in table 1).

3. Results

In the study, a total of 15,865 single nucleotide polymorphisms (SNPs) were initially extracted from the SIRT1 gene database at NCBI. Three isoforms of the SIRT1 gene (isoform a, isoform b, and isoform c) were identified, each containing different numbers of non-synonymous SNPs (nsSNPs): isoform a had 597 nsSNPs, isoform b had 330 nsSNPs, and isoform c had 331 nsSNPs. After eliminating duplicate nsSNPs, a total of 252 unique nsSNPs were identified. To assess the potential impact of these nsSNPs on the SIRT1 gene, several bioinformatics tools were employed. SIFT analysis revealed that 94 nsSNPs were predicted to be intolerant based on a Tolerance Index threshold of 0.05. Additionally, I-Mutant was used to predict the stability of the SIRT1 protein with different amino acid substitutions, resulting in 216 nsSNPs showing a decrease in stability. Furthermore, PROVEAN identified 77 nsSNPs with a negative impact, as their scores fell below the specified threshold of -2.5 (Table 2). These analyses collectively provided insights into the potentially damaging effects of specific nsSNPs on the structure and function of the SIRT1 gene. Based on the HWE results, it appears that the genotype frequencies for rs3740051 in the SIRT1 gene among the breast cancer patients do not significantly deviate from the expected frequencies. The Chi-square value of 0.765 is relatively small, and the p-value of 0.68 is greater than the commonly used significance threshold of 0.05 (Table 3). Therefore, there is no strong evidence to suggest a departure from Hardy-Weinberg Equilibrium for this genetic variant among patients. This suggests that the genotype distribution for rs3740051 in this population is consistent with what would be expected under standard population genetics assumptions.

4. Discussion

It was evident from the study that homozygous dominant (AA) allele was the predominant allele (84%) in breast cancer patients whereas mutation was only 16% among the patients (table 3). SIRT1 gene polymorphisms are changes in the DNA sequence of the SIRT1 gene. These polymorphisms can affect the activity of SIRT1, which may have an impact on cancer development and progression. A number of studies have investigated the association between SIRT1 gene polymorphisms and breast cancer. Some studies have found that certain SIRT1 gene polymorphisms are associated with an increased risk of breast cancer, while other studies have found no association. A study published by El-Khodary *et al* found that two SIRT1 gene polymorphisms, rs3758391 and rs12778366, were associated with an increased risk of breast cancer in Egyptian women [10]. The study also found that these polymorphisms were associated with more aggressive breast cancer tumours. Another study, by Chen *et al*, found that a SIRT1 gene polymorphism, rs10830119, was associated with a decreased risk of breast cancer in Chinese women [11]. The study also found that this polymorphism was associated with more favourable breast cancer outcomes. However, the rs3740051 is not been reported so far. It is important to note that the results of studies on SIRT1 gene polymorphisms in breast cancer have been mixed. More research is needed to confirm the association between SIRT1 gene polymorphisms and breast cancer and to determine the impact of these polymorphisms on cancer development and progression.

In the context of cancer, the SIRT1 gene and its protein product Sirtuin 1 have been the subject of significant research due to their potential roles in both cancer development and cancer treatment. Here are some key aspects of SIRT1's involvement in cancer. SIRT1's role in cancer can be complex and context-dependent. In some cases, SIRT1 has been described as a tumor suppressor because it can help regulate cellular processes that prevent cancer, such as DNA repair, apoptosis (cell death), and the inhibition of excessive cell proliferation [12-14]. On the other hand, in certain contexts, SIRT1 has been implicated as an oncogene because it can promote cancer cell survival and resistance to therapy [15-17]. SIRT1 is involved in DNA repair mechanisms, which are critical for maintaining genomic stability and preventing the accumulation of DNA mutations that can lead to cancer. By deacetylating and activating proteins involved in DNA repair, SIRT1 can help protect against cancer-causing mutations. SIRT1 is also known to be involved in the cellular stress response pathways, including responses to oxidative stress and DNA damage. These responses can help prevent the development of cancer by removing damaged cells or repairing DNA lesions. SIRT1 plays a role in cellular metabolism and energy regulation. Dysregulation of metabolism can contribute to cancer development, and SIRT1's involvement in this process is an area of active research. SIRT1 can modify histone proteins and other epigenetic factors, influencing gene expression patterns. Epigenetic changes can contribute to the development and progression of cancer by altering the expression of genes involved in cell growth, differentiation, and apoptosis. Due to its various roles in cancer biology, SIRT1 has been explored as a potential target for cancer therapy [18-20]. Researchers have investigated the development of SIRT1 inhibitors or activators as potential treatments for different types of cancer.

It's important to note that the role of SIRT1 in cancer can vary depending on the specific cancer type, stage, and the genetic and environmental factors involved. Additionally, ongoing research continues to uncover the complexities of SIRT1's functions in cancer, and its precise role in different cancer contexts is still an area of active investigation. Mutations play a crucial role in protein stability, structure, and function, serving as the raw material for evolution. Negative selection predominantly eliminates protein mutations, limiting the potential for future adaptations, while only a fraction of mutations are positively selected for new functions. Neutral mutations may randomly become fixed in small populations due to neutral drift. The effects of mutations on organismal fitness are multifaceted and don't necessarily correlate with single gene or protein properties due to redundancy and resilience mechanisms. Protein stability is a key determinant of how proteins and organisms evolve, with destabilizing mutations hindering the acquisition of new functions. Recent computational advancements enable the prediction of mutation effects, such as decreased thermodynamic stability, on proteins. While SNPs in genes like leptin and leptin receptor have known effects, the functional implications of SNPs in some genes remain unexplored. Methods like SIFT and Provean aid in predicting mutation impacts, although they have associated error rates. Additionally, the I-Mutant approach examines protein stability in response to mutations. Overall, understanding mutation effects on proteins and their functional consequences is a complex challenge in evolutionary biology, with potential implications for disease and adaptation.

Table 1: PCR Conditions

Target Gene	Primer sequence	PCR Program (35 cycles)	PCR Program (35 cycles)	PCR Product length
SIRT1 rs3740051	Forward	5’GCTCACGCTAGAAAGGAAGGA 3’	95^0C – 5’, 95^0C – 30’’, 60^0C - 30’’, 72^0C - 30’’, 72^0C – 5’	764bp
	Reverse	5’ GGGCCAGACCACAACACTA 3’		

Table 2: SIFT, I mutant analysis, Provean for the nsSNPs of SIRT1 Gene

	Damaging	Tolerant
SIFT	94	158
PROVEAN	77	175
I mutant	216(lowered stability)	36(stability high)

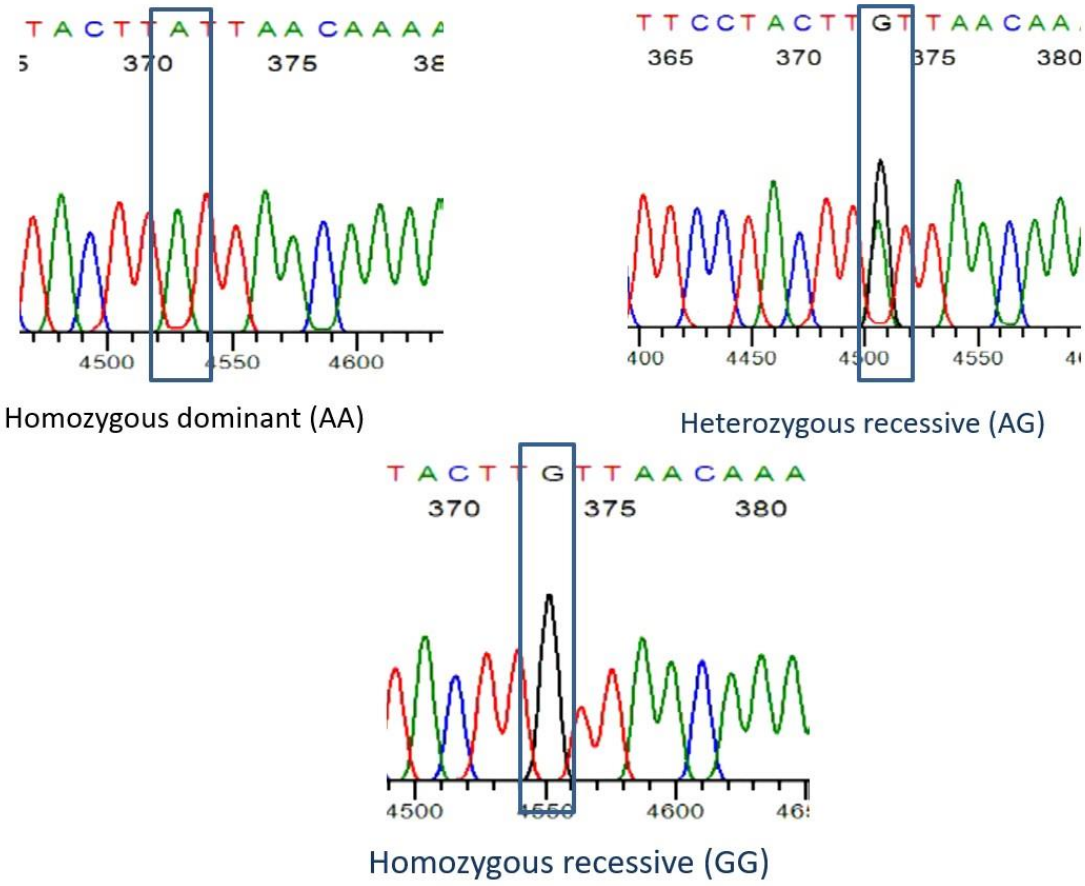

Fig 1: Sanger sequencing results of rs3740051

Table 3: HWE for the SIRT1 gene

Gene variant SIRT1			CASE (N=104) (%)	Chi-square Value	P value
rs3740051	AA	Observed	87 (83.65)	0.765	0.68
		Expected	87.62(84.25)		
	AG	Observed	17 (16.34)		
		Expected	14.76(14.19)		
	GG	Observed	0		
		Expected	0.62(0.59)		

5. Conclusion

The in-silico analysis of SIRT1's functional SNPs revealed significant insight into the potential harm that the ns-SNPs might do to the protein. Genotyping of the gene showed the predominance of wild variety in breast cancer patients in the Indian Population.

Conflicts of interest

None

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