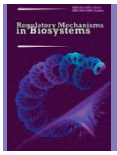

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The role of *GNAS1* gene polymorphisms in development of bladder and prostate cancer: A case–control study

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GNAS gene encodes G-protein alpha subunit (Gαs) in humans. T393C polymorphism of *GNAS1* is associated with Gαs production and augmented cAMP synthesis. Heterotrimeric G protein can stimulate the adenylyl cyclase, then leading to cAMP production in cells, which is crucial in many transduction pathways that are associated to proapoptotic mechanisms in cancer cells. The objective of our research was to clarify a potential correlation between T393C SNP of the *GNAS1* gene and bladder cancer (BC) and prostate cancer (PC). *GNAS1* genotyping in one hundred and twenty-four cases and one hundred controls was examined by PCR. We examined the distribution of *GNAS1* gene alteration in BC patients and compared it with the control group, which showed the risk for BC was elevated two fold in the patients with TC and was elevated nine fold in the patients with CC allele (95% CI – 1.094–4.028 and 1.000–88.054 respectively). The risk factor increased about 2 fold with PC patients with the TC allele (95% CI – 1.445–5.535), and about 6 fold in the patients with the CC allele (95% CI – 0.501–67.400). This paper exhibited a significant relationship of GNAS1 gene alterations with BC and PC, in particular TT and CT alleles may be a sign of propensity for BC and PC.

Keywords: *GNAS* gene; bladder cancer; prostate cancer; PCR.

Introduction

The *GNAS1* gene in humans, found on chromosomal 20q13.32, encodes the Gas protein and has 13 exons (Arjumand et al., 2012). Heterotrimeric G proteins serve as signal transducers and include Ga, Gb, and Gg, each produced by single genes. Ga operates as a GTPase, whereas Gb and Gg act as permanent components of the inactive G protein (Wilson et al., 2010). Ga interacts with adenylate cyclase to regulate cAMP stages (Peeters et al., 2006). Ligand-bound G-protein coupled receptors induce release of GDP, which is substituted through GTP to gas, leading to detachment from the receptor's surface and βγ complex. The gaseous component communicates with the enzyme adenylate cyclase for promoting synthesis of cAMP. The swift cessation of the cellular reaction is essential after the reduction in activating ligand concentration. GTP connected to Ga is swiftly converted to GDP; Ga is transformed into inactive form and re-associates with the bg subunits, reverting to the "off" state to initiate a another cycle (Weinstein et al., 2004).

Mutations in the *GNAS1* gene are often identified in various tumor types, occurring in around 5% of all sequencing malignant tumors (Steffen et al., 2017). Mutations in the *GNAS1* gene have been identified in 3.21% of all malignancies (Zauber et al., 2016). For years, significant progress has been made in determining the tumur epigenetics such as methylation of the aberrant DNA, since gene aberrations or mutations are well-established as critical factors in cancer progression (Afolabi et al., 2022). A significant drawback of cancer sequencing study is the constrained statistical capacity to effectively identify mutant genes with a moderate or lower mutation frequency (e.g., 5%) (Fadaka et al., 2020). The *GNAS1* gene encodes the G(alpha)s (Gas). *In vitro* studies indicate that elevated Gas expression correlates with greater apoptosis, and that cAMP, operating downstream of G proteins, is pivotal in pro-apoptotic mechanisms (Otterbach et al., 2007). The genotypes of the frequent T393C SNP in the *GNAS1* gene are correlated with bladder tumor, T-allele of T393C SNP correlates with Gas mRNA expression in tumor tissue (Frey et al., 2005).

Bladder tumor is the sixth prevalent malignancy in the United States, is seldom diagnosed in persons under forty years of age, the typical age upon diagnosis is 73 years, and concomitant medical disorders are often a significant influence in patient care (Flaig et al., 2024). Risk factors for bladder cancer encompass male gender, Caucasian ethnicity, tobacco use, personal or familial history of bladder cancer, pelvic radiotherapy, occupational or environmental exposures, certain medication exposures, chronic urinary tract irritation or infection, and specific medical conditions such as diabetes and obesity (DeGeorge et al., 2017). In 2024, around 83,190 new cases of urinary bladder cancer are projected to be identified in the United States, including 63,070 men and 20,120 females. Concurrently, around 16,840 fatalities are anticipated, including 12,290 males and 4,550 females (Siegel et al., 2024). Prostate cancer is the majority prevalent cancer among males in 114 nations (as of 2020), constituting one in every 14 cancer diagnoses worldwide and 15% of wholly male malignancies. It ranks second to lung cancer in cancer-related mortality in men (Bray et al., 2023). The prostate gland is located at the base of the bladder, primarily functioning to produce seminal fluid that feeds and encourages spermatozoa during ejaculation. Cancers originate in the epithelial lining of the prostate gland, varying from low-grade tumors that necessitate no treatment to aggressive, highly lethal cancers (Sandhu et al., 2021).

Material and methods

The study was approved by the Research Ethics Committee at The Al-Sader Teaching Hospital. Based on the patients' verbal consent, blood samples were drawn.

The Al-Sader Teaching Hospital- for Oncology and Hematological Disease provided sixty blood samples from patients with prostate cancer (PC) and sixty blood samples from patients with bladder cancer (BC). These people were between the ages of 29 and 94. To create a control group, blood samples were taken from 100 people who were cancer-free. They were between the ages of 27 and 90. Sterile syringes were used to draw 2 mL of blood sample from the study groups. The samples were stored in tubes with ethylenediaminetetra acetic acid to make the complete DNA extraction. A gDNA Kit (Geneaid, Taiwan) was used to extract the genomic DNA, which was then exposed to ultraviolet light and underwent electrophoresis with ethidium bromide (Sambrook & Russell, 2001) Using percentages obtained from statistical analysis, a descriptive statistic describes the characteristics of the patient.

For detection the *GNAS1* gene polymorphisms, the PCR was done by two primers (F), 5-CTCCTAACTGACATGGTGCAA-3′; (R),

5-TAAGGCCACACAAGTCGGGGT-3 (Frey et al., 2005). The PCR parameters performed at denaturation stage at 94 °C for 5 min, followed by 35 cycles including the denaturation stage at 94 °C for half a minute, followed by the annealing stage performed at 57 °C for half min, then the extension stage performed at 72 °C for 35 seconds. The reaction was based on the manufacturer's directions for PCR Pre-Mix (Bioneer, Korea). The last extension took place for 5 min and was performed at 72 °C. The Macrogen Company "http://dna.macrogen.com received 20 mL of PCR products for sequencing. Basic Local Alignment Search Tool (BLAST) was used to analyse and analyze the sequences in order to look for homologous sequences in NCBI database (www.blast.ncbi.nlm.nih.gov).

By utilizing percentages, the statistics characterized the features of the patients. The SPSS application was used to determine ORs and the 95% CLs. According to Sheskin (2004) OR were deemed significant if OR was more than 1.5.

Results

The PCR products were detected by 1.5% agarose gel electrophoresis (Fig. 1). One of the most important tools for detecting gene polymorphisms TT, TC, CC in patients and controls is the sequencing of the *GNAS1* gene. Comparing genes across alleles was also important. Our sequences matched the NCBI GenBank reference sequence for GNAS1 (Fig. 2–4). Distribution of *GNAS1* gene alteration between BC patients and the healthy controls group revealed the risk for BC was increased about 2 fold in the patients who have TC and was increased about 9 fold in the patients with the CC allele (95% CI – 1.094–4.028 and 1.000–88.054, respectively as shown in Table 1).

The risk factor increased about 3 fold with PC patients with TC allele (95% CI – 1.445–5.535), and about 6 fold in patients with CC allele (95% CI – 0.501–67.400, Table 2).

This study points out that the risk factor increased about 22.5 fold with BC stages in patients with TC allele (95% CI – 5.584–90.659), and about 9.0 fold in patients with CC allele (95% CI – 0.781–103.724, Table 3). Also the risk factor increased about 10.7 fold with PC stages in patients with TC allele (95% CI – 3.057–37.556), and about 2.5 fold in patients with CC allele (95% CI – 0.134–46.774, Table 4). The risk factor increased about 80 fold with BC grade TC allele (95% CI – 13.397–477.702), and about 5 fold with CC allele (95% CI – 0.535–46.719, Table 5).

Also the risk factor increased about 165 fold with PC grade of TC allele (95% CI – 17.207–1582.163), and about 20 fold with CC allele (95% CI – 0.652–613.186, Table 6).

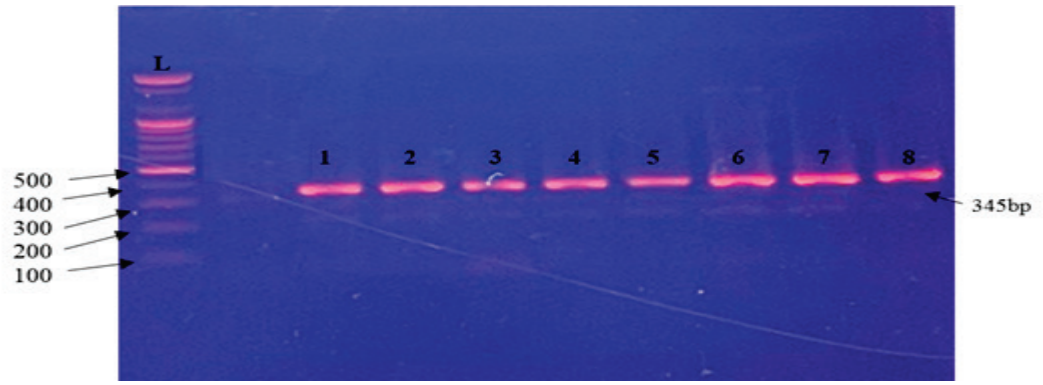

Fig. 1. Agarose gel electrophoresis (1.5%) of *GNAS1* gene fragment (345 bp): *L* – DNA ladder 100 bp; *1–4* – controls, *5–8* – patients

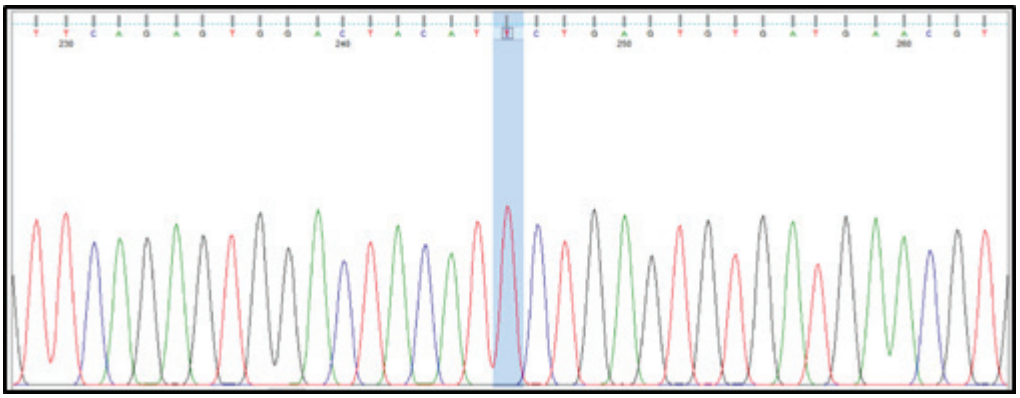

Fig. 2. Sequence of *GNAS1* gene of Sample TT

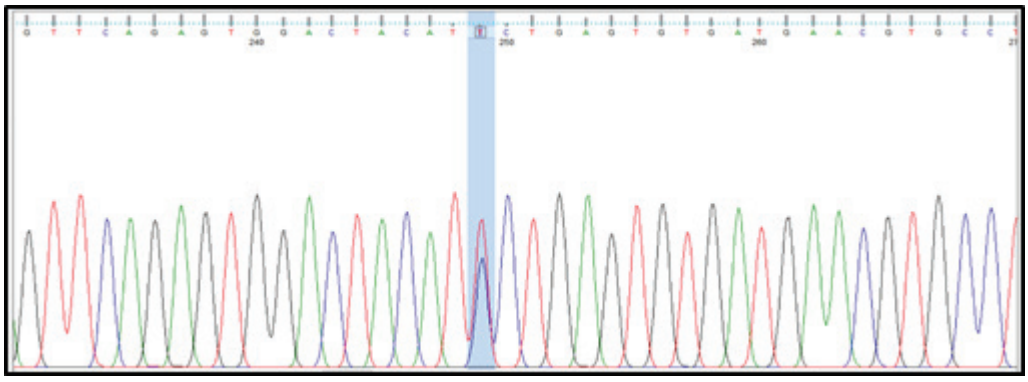

Fig. 3. Sequence of *GNAS1* gene of Sample TC

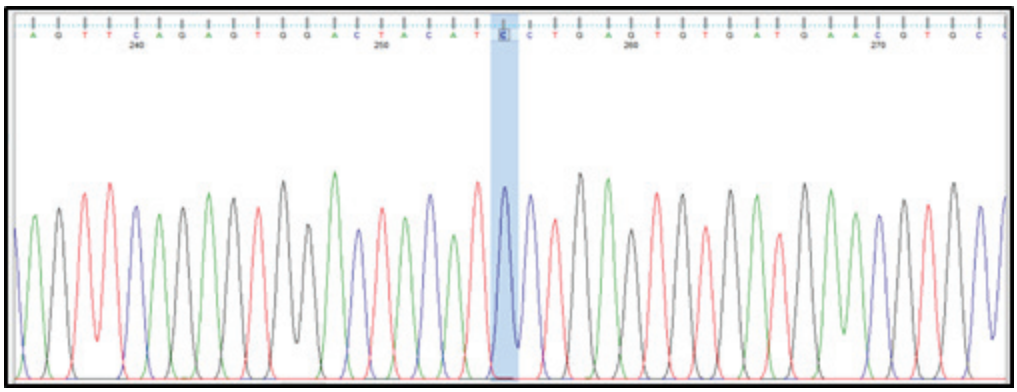

Fig. 4. Sequence of *GNAS1* gene of Sample CC

Table 1
Distribution of *GNAS1* gene polymorphism between BC patients and the healthy controls group

Genotype	Control group, % (n = 100)	Patients, % (n = 64)	OR	95% CI
TT	61.0	40.6	1.00	–
TC	38.0	53.1	2.09	1.094–4.028
CC	1.0	6.3	9.38	1.000–88.054

Table 2
Distribution of *GNAS1* gene polymorphism between PC patients and healthy controls group

Genotype	Control group, % (n = 100)	Patients, % (n = 60)	OR	95% CI
TT	61.0	35.0	1.00	–
TC	38.0	61.7	2.83	1.445–5.535
CC	1.0	3.3	5.80	0.501–67.400

Table 3
The distribution of alleles of *GNAS1* gene with BC stages

Genotype	Stage I, II	Stage III, IV	OR	95% CI
TT	18	6	1.0	–
TC	4	30	22.5	5.584–90.659
CC	1	3	9.0	0.781–103.724

Table 4
The distribution of alleles of *GNAS1* gene with PC stages

Genotype	Stage I, II	Stage III, IV	OR	95% CI
TT	15	6	1.0	–
TC	7	30	10.7	3.057–37.556
CC	1	1	2.5	0.134–46.774

Table 5
The distribution of alleles of *GNAS1* gene with grade BC

Genotype	Grade I, II	Grade III, IV	OR	95% CI
TT	20	4	1	–
TC	2	32	80	13.397–477.702
CC	2	2	5	0.535–46.719

Table 6
The distribution of alleles of *GNAS1* gene with grade of PC

Genotype	Grade I, II	Grade III, IV	OR	95% CI
TT	20	1	1	–
TC	4	33	165	17.207–1582.163
CC	1	1	20	0.652–613.186

Discussion

This research reveals a substantial link between the genetic makeup of the T393C SNP of *GNAS1* and bladder cancer in relation to prostate cancer. The *GNAS1* T393C polymorphism is located in the fifth exon inside a recombination hotspot associated with disequilibrium between two haplotype blocks, situated 5′ and 3′ of the T393C variant. The *GNAS1* TT genotype is linked with increased Gas mRNA levels in several tissues (Arjumand et al., 2012). Genotype-dependent variances in mRNA decay arise from altered secondary structures, resulting in disparities in Gas mRNA, with increased Gas expression corresponding with elevated apoptosis. cAMP is a secondary messenger generated upon the activation of Gas, which plays a role in this pro-apoptotic pathway. An increased level of cAMP triggers death in several cell types, including leukemic cells, ovarian cancer cells, and lymphoma cells, while simultaneously blocking Ras-dependent activation of Chen & Iyengar (1994), Myklebust et al. (1999) and Yan et al. (2000).

Weinstein et al. (2004) elucidated that mutations in *GNAS1* might result in either a gain or loss of function by eliminating or stimulating the transmission of signals, thereby leading to clinical phenotypes of hormone shortage or excess. Frey et al. (2005) demonstrated that the *GNAS1* T393C variant linked to varying G(alpha)s mRNA expression serves as a unique independent prognostic indicator for clinical outcomes, underscoring the functional involvement of G(alpha)s in tumor development. Frey et al. (2005) proposed that such nucleotide exchange might modify the structure of pre-mRNA, thereby influencing mRNA stability as well. This notion was corroborated by the observation that $G\alpha_s$ mRNA expression was markedly elevated in bladder cancer, adipose tissue, and cardiac tissue from individuals with 393 TT genotypes.

Huetter et al. (2016) investigated potential associations between *GNAS* C393T genotypes and many aspects of human empathy using genomes from a large cohort of previously described healthy individuals. The functional consequences of the c.393C>T polymorphism in bladder cancer patients, as well as in normal adipose and cardiac tissues. The T allele resulted in an increase in Gαs mRNA levels. A gene-dose effect was observed with the highest expression in TT genotypes, then in CT genotypes. Decreased expression of Gαs mRNA was noted in bearers of the CC genotype (Peitz et al., 2022). Future research must elucidate the proposed tumor type-dependent correlation between the various genotypes of T393C SNP of the *GNAS1* gene and the activation of certain signaling pathways that promote tumor proliferation. The study's limitation was a very restricted sample size.

Conclusion

GNAS1 (CC) polymorphism was related and associated to the prevalence of bladder cancer and prostate cancer in the Iraqi population. Further research with a broader population is necessary to clarify the impact of variations in *GNAS1* on cancer risk.

All authors declare that they have no conflict of interest.

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