Haplotype of CaSR gene is associated with risk of Renal Stone Disease in West Indian Population

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Abstract

Purpose

Calcium is the most abundant metabolite involved in the stone matrix. The *CaSR* gene controls calcium homeostasis, and genetic variation in the *CaSR* gene could lead to the development of renal stone disease. Therefore, the current study has been designed to assess the association of genetic variants of *CaSR* gene polymorphisms with renal stone disease.

Method

A single-centric prospective study has been carried out on a total of 300 participants (150 cases and 150 controls). Serum levels of calcium, creatinine, parathyroid hormone, and 24 Hour urine metabolites were measured. Two polymorphisms, rs1801725 and rs1042636, of the *CaSR* gene, have been genotyped for each participant. T-test, Chi-square, and Receiving Operative Curve (ROC) curve analysis were used for statistical analysis.

Result

Renal stone patients had significantly higher levels of serum parathyroid hormone, creatinine, and 24hr urine metabolites in comparison to the controls. *CaSR* gene variants rs1801725 (GG) and rs1042636 (AA) both have shown significant association with renal stone disease. In addition, individuals having specific genotypes along with metabolic abnormalities such as hypercalcemia, and hyperparathyroidism are found to be at a higher significant risk of developing the renal stone disease. Further, ROC analysis also showed a higher risk (54%) for individuals carrying the GG/AA haplotype.

Conclusion

In the present study, the haplotype of the *CaSR* gene has shown an association with renal stone disease. Individuals with hyperparathyroidism and hypercalcemia and risk genotype have a higher susceptibility to developing the renal stone disease.

1. Introduction

Renal stone disease is among the most prevalent renal disease. Humanity has suffered kidney stones for centuries dating back to 4000 B.C.[1] Preventing the recurrence of kidney stones is still a major issue in human health. [2] Chronic kidney disease[3], end-stage renal disease[3], cardiovascular disease[4], diabetes, and hypertension[5] have all been linked to renal stone disease. As a result, stone recurrence avoidance has become a major responsibility, and doing so necessitates greater knowledge of the mechanisms underlying stone formation. The renal stone disease affects 12% of the world's population and causes a tremendous economic burden that developed countries like America spend 2–3% of their health budget on renal stone disease. [5]

The prevalence of the renal stone disease is the highest among all renal diseases and affects almost 20 million new cases that are reported in the Indian population every year. In the absence of meta-phylaxis, the recurrence rate of renal stone formation is greater, accounting for 10–23 percent per year, 50 percent in 5–10 years, and more than 75 percent in 20 years of the first episode.[6]

Since the beginning, it has been believed that nutrition and environment are the most prominent cause of urinary stone formation. Indeed, with the advancement in medical science and technology, different possibilities and areas have been explored; genetic polymorphisms and mutation could be the potential factor that may be involved in the pathology of renal stones.

Renal stone disease is a multifactorial idiopathic disease that includes very complex mechanisms which could be linked to metabolic abnormalities. Calcium is the major component of 80% of renal stones. So, any change in any mechanism or pathways of Calcium ion homeostasis may lead to renal stone formation directly or indirectly.

The calcium-sensing receptor (*CaSR*) gene encodes the calcium-sensing receptor, which regulates parathyroid hormone (PTH) secretion and calcium reabsorption, making it the primary controller of extracellular calcium homeostasis. CaSR receptor activation would decrease the PTH secretion and reabsorption of calcium from the thick ascending limb (TAL). *CaSR* receptor activation thereby enhances urine calcium excretion via a direct and PTH-mediated action on renal tubular cells. While *CaSR* activation, particularly in the TAL, causes hypercalciuria, which is the primary risk factor for renal stone formation. [7] So, any mutation or polymorphism in the *CaSR* gene can cause major changes in this series of events and lead to the formation of stone.

Genetic variants of *CaSR* “rs1801725” and “rs1042636” both SNPs are being studied in various populations for many of the severe diseases, including cancer[8, 9], hyperparathyroidism[10–12], and renal stone disease[13], which has shown a potential association with disease pathophysiology. Also, Guha et al. studied these two SNP in the east Indian population (from Kolkata) and found them to be associated with the risk of development of renal stone disease[14], indeed no study has been carried out on the west Indian population, so considering the facts we have included only this two SNPs for the study.

Although a study has been conducted in the eastern Indian population to evaluate the association between *CaSR* gene polymorphism and renal stone disease[14] though, they did not evaluate the role of metabolic abnormalities and their susceptibility towards the *CaSR* gene polymorphisms. To the best of our knowledge, no study has been conducted in the western part of India, and therefore present study was carried out to determine the association between *CaSR* gene polymorphism and renal stone disease and its influence on the major metabolic abnormalities in the west Indian population.

2. Materials And Method

2.1 Ethical

This study was approved by Human Ethics Committee held at Muljibhai Patel Society for Research in Nephro-Urology, Muljibhai Patel Urological Hospital, Nadiad 387002, Gujarat, India. (Ref. Number: EC/575/2019 & EC/697/2020).

2.2 Participants

A total of 150 patients (male and female both) aged between 18 to 65 having a current or history of renal stone(s) supported by conforming renal ultrasound or CT scan and admitted to the department of urology at Muljibhai Patel Urological Hospital (MPUH), Nadiad, Gujarat, India was enrolled into the case group. Patients with a history of renal disorders other than renal stone, known metabolic disorder, pregnancy, or on drugs like diuretics and calcium supplements, which may affect the stone formation, have been excluded from the study. Age and sex-matched 150 healthy individuals were enrolled in the control group who belonged to the same geographical region and had no known history of renal stones. Renal Ultrasound sonography was performed for control participants to avoid asymptomatic renal stones and calcification; individuals diagnosed with renal stone(s) or calcification were excluded from the control group. Detailed socio-demographic information about age, gender, drinking water, food habit (Vegetarian or Non-vegetarian), salt intake, and family history for renal stones were collected with the help of paper case record forms from the participants of both groups.

2.3 Blood and Urine Analysis

Once the written consent was taken from the participant, a total of 6 ml of peripheral blood was withdrawn, from which 2 ml of blood was withdrawn into the ethylene diamine tetra-acetic acid (EDTA) vacutainer stored at -20º C for further genetic analysis and remaining 4 ml of blood sample was used to perform biochemistry parameters like serum creatinine, serum calcium, and parathyroid hormone levels. The 24-hr urine sample was collected to perform 24-hour metabolite levels, including creatinine, calcium, magnesium, phosphorus, oxalate, citrate, and uric acid. All the biochemistry parameters from blood and urine samples were performed on the Fully automated analyzer of Beckman AU480. Parathyroid hormone level was performed by radioimmunoassay (RIA) on Maglumi 800 CLIA analyzer. A Colorimetry method developed by MPUH was used to perform 24-hour citrate levels.

2.4 Genomic DNA extraction

Genomic DNA was extracted by GeneJET mini Whole Blood Genomic DNA Purification Kit (K0781), Thermo Scientific, USA, as per the manufacturer's instruction. Isolated DNA was checked for quality using QIAxpert, QIAGEN, Germany.

2.5 Genotyping

Two different genetic variants rs1801725 and 1042636 of the *CaSR* gene were genotyped using real-time polymerase chain reaction (PCR) by the TaqMan SNP Genotyping Assay method. Probes for the TaqMan SNPs genotyping were acquired from Thermo Scientific, USA. Real-time PCR amplification is done in a 10µL volume reaction containing 50 ng of genomic DNA, 5 µL genotyping master mix (TaqMan™ Genotyping Master Mix, Catalogue number: 4371355), 2.5 µL SNPs specific TaqMan assay probes

(TaqMan™ SNP Genotyping Assay; rs1801725- C___7504853_20, rs1042636- C___7504854_20) and 1.5 µL nuclease-free water (AccuGENE™ Molecular Biology Water, Catalogue number: 51200) using Rotor-Gene Q (QIAGEN, Germany) instrument.

2.6 Statistical Analysis

A binary logistic regression test was used to, investigate the association of *CaSR* gene variants rs1801725 and rs1042636 with renal stone disease by applying various genetic models (Odds ratios were adjusted for the age and gender distribution among case and control group). Student t-test was applied to calculate statistically significant differences in continuous independent variables like age, gender, food habit, drinking water, family history, serum creatinine, serum calcium, parathyroid hormone level, and 24-hour urine metabolites between the case and control groups. All the biochemical parameters and genotyping data were statistically analyzed using SPSS 29.0.0, IBM software.

3. Result

Socio-demographic characteristics of stone patients and controls are presented in table 1. The mean age of stone patients was 44.15±11.71 years; out of 150 stone patients, the renal stone disease was significantly observed in males accounting for 74% incidence, while females were less affected (26%) compared to the males. Family history of stone has been significantly observed in 58.66% of stone patients. Though there is no significant role of drinking water observed in stone formation, non-vegetarian food has remarkably impacted stone formation.

Biochemical determinants of stone patients and healthy controls are manifested in table 2. Serum creatinine, parathyroid hormone levels, and all the 24-hour urine metabolites showed a significant difference between stone patients and healthy controls, though no significant difference was found in serum calcium levels. In addition, some metabolic abnormalities, such as hyperparathyroidism (HPT), hypercalciuria, and hyperoxaluria, are diagnosed in 51.33%, 52%, and 67.33% of stone patients, respectively.

In the current study, *CaSR* gene variants rs1801725 and 1042636 have been analyzed for the frequency distribution among the West Indian population, and frequencies have been found to differ from the various populations around the world. (rs1801725; G allele-63%, T allele-37% and rs1042636; A allele-58%, G allele- 42%). (figure 1.)

3.1 *CaSR* gene rs1801725 polymorphism and renal stone disease

Homozygous genotype GG is present in a higher percentage of stone patients as compared to healthy controls (31.33 vs. 20.66). Heterozygous co-dominant (GG vs GT) and dominant (GG vs GT/TT) models showed significant association OR =1.785, p=0.0300 95% CI=1.033-3.083 and OR=1.785, p=0.0360 95% CI=1.039-3.067, respectively. (table 3.) Hypercalcemia has been found to be significantly associated with rs1801725 polymorphism. The heterozygous co-dominant (GG vs GT) model has shown a significantly increased risk of hypercalcemia (OR=2.13, p=0.0105, 95% CI=1.21-3.88) in renal stone patients. While

there was no significant association found between hyperparathyroidism and hypercalciuria and rs1801725 polymorphism. (table 5.)

3.2 *CaSR* gene rs1042636 polymorphism and renal stone disease

Homozygous genotype AA is present in a higher percentage of stone patients compared to healthy controls (32.66 vs. 20.66). Heterozygous co-dominant (AA vs AG) and dominant (AA vs AG/GG) models showed significant association OR=2.010, p=0.0140, 95% CI=1.153-3.505 and OR=1.992, p=0.0170, 95% CI=1.122-3.295), respectively. (table 4.) Hyperparathyroidism and hypercalcemia have been found to be significantly associated with rs1042636 polymorphism. The heterozygous co-dominant model (AA vs AG) showed a significant association with hyperparathyroidism (OR =2.0, p=0.0303, 95% CI =1.09-3.78). The heterozygous co-dominant (AA vs AG) and dominant (AA vs AG/GG) models have shown significant association with hypercalcemia (OR=2.60, p=0.0069, 95% CI=1.30-5.03; OR=2.41, p=0.0090, 95% CI=1.24-4.74, respectively). while the homozygous co-dominant model (GG vs AA) has shown a significant association with hypercalciuria (OR =2.76, p=0.0217, 95% CI =1.17 to 6.62). (table 6.)

3.3 Combine effect of rs1801725 and rs1042636 polymorphisms on renal stone disease

We analyzed combined all the genotypes of rs1801725 and rs1042636 as we expected that haplotypes could show a significant additive or synergistic effect on renal stone disease. Homozygous GG/AA dominant genotype is present in 32% of stone patients compared to 20.66% in healthy controls, suggesting a significant association with renal stone disease. (table 7.)

4. Discussion

Renal stone disease is among the most prevalent renal disorders, affecting a large population of the world and putting 50% of patients at risk of developing other severe renal diseases. Renal stone disease is an idiopathic multifactorial disease, and environmental factors such as temperature, dietary habits, and drinking water play a critical role in stone formation. [15, 16] The considerable regional and ethnic heterogeneity in renal stone incidence implies that it is influenced by genetic and environmental factors. Though we hypothesized that genetics influence the occurrence of kidney disease investigated the role of *CaSR* genetic variants in renal stone disease.

In the present study, we have analyzed socio-demographic parameters, blood parameters, 24-h urine metabolite profiles, and the *CaSR* gene polymorphisms rs1801725 and rs1042636, their genotype distribution in case and control groups, and their association with renal stone disease and other metabolic disorders that can play a significant role in stone formation. Most patients were from the 31 years – 58 years age group accounting for 70%. Males have a higher incidence rate (77%) with a male-to-female ratio of 3:1, similar to other studies conducted on different populations.[17, 18]

Parathyroid hormone, calcium, and urine metabolites like calcium, oxalate, and uric acid have a significant presence in the stone matrix or promote stone formation. The present study found that

parathyroid hormone, calcium, urine-calcium, oxalate, and uric acid showed a remarkable difference between stone patients and healthy controls. However, citrate and magnesium are associated with the inhibition of stone formation. In our study, we observed significant protection with a high level of citrate in urine samples of healthy individuals. An increased level of magnesium in urine suggests an increase in filter load and reduced reabsorption from the loop of Henle. [19] This reabsorption mechanism can be affected by renal stone disease. Altered reabsorption of urine mechanism may explain the high level of urine magnesium in our study.

Although, the presence of calcium in the stone matrix among 80% of all stones signifies its importance in studying the complex mechanism of calcium homeostasis of calcium ions. However, PTH and *CaSR* gene has a central role.

Studies have reported polymorphism in *CaSR* gene variants rs1801725, and rs1042636 are associated with various diseases, i.e., renal stone disease[10, 14, 20], prostate cancer[21], colorectal cancer[22], and primary HPT[23]. In our study, we have observed that individuals carrying GG genotype (rs1801725) and AA genotype (rs1042636) are associated with a high risk of developing renal stone disease. On the contrary, a study from east India has observed a significant association between the GT genotype (rs1801725) and AG genotype (rs1042636) of the *CaSR* gene with renal stone disease. [14] European ancestry population has shown a strong association of *CaSR* gene polymorphism of GG genotype (rs1801725) with a high level of serum phosphate, and AA genotype (rs1042636) has been shown to be associated with a higher level of serum PTH. [24]

Both SNPs of the *CaSR* gene were in linkage disequilibrium; therefore, we performed the haplotype analysis. We have observed that GG/AA haplotype increases the risk of renal stone disease by two-fold. The finding of ROC analysis also confirms that the presence of haplotype increases the risk of developing the renal stone disease.

In the present study, we found that allele frequencies of *CaSR* gene variants rs1801725 and rs1042636 differ from the previously reported allele frequencies among different populations around the world, including South Asian, European, African, and East Indian populations (figure 1.), which suggest that the west Indian population has a different genotype distribution compared with other population. It has been shown that genetic variants occur in all ethnic groups but with variable frequency. For example, genetic variants of NAT2-associated phenotypes are variable due to population frequency variability, East Asians (14%), Black Americans (34%), and Whites (54%). [25, 26]

Studies have reported that HPT, hypercalciuria, and hypercalcemia are the primary metabolic disorders and significantly increase the risk of the development of renal stone disease.[27–30] In the present study, we have observed these metabolic abnormalities in stone patients with a high percentage of HPT (51.53%) and hypercalciuria (52%). In comparison, the incidence of hypercalcemia has been observed in less number (18.66%) of stone patients. *CaSR* gene controls ionized calcium in the blood through PTH up and down-regulation and reabsorption of calcium ions from urine. In addition, many reports have suggested a positive association of gene polymorphisms with hypercalciuria and HPT.[31–33]

In the present study, we have examined the association of *CaSR* gene variants rs1801725 and rs1042636 with these significant metabolic abnormalities. Individuals with GG genotype of rs1801725 and hypercalcemia were found to be associated with a high risk of developing the renal stone disease. In addition, the individuals with HPT and AA genotype of rs1042636 were associated with a high risk of developing the renal stone disease. Results suggest the remarkable association of *CaSR* gene polymorphisms rs1801725 and rs1042636 with significant metabolic abnormalities that contribute to the development of renal stone disease.

In summary, the present results indicate that the GG genotype of the rs1801725 variant and AA genotypes of the rs1042636 variant of the *CaSR* gene could be associated with a significantly increased risk of development of renal stone disease. The renal stone disease results from convoluted reciprocity between environmental and genetic variables. Gene polymorphisms in *CaSR* gene variants could be partly responsible for an individual's susceptibility in the presence of an appropriate environmental condition. The current study only examined a small number of populations; therefore, larger-scale genetic epidemiology investigations are needed to validate the study.

Statements And Declarations

There are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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Disclosure

Conflict of Interest

The authors declare no conflict of interest.

Human Studies:

The protocol for this research project has been approved by the Muljibhai Patel Society for Research in Nephro-Urology at Muljibhai Patel Urological Hospital, Nadiad, Gujarat, India, on 29th July 2019, and the approval number is “EC/575/2019 & EC/697/2020”.

Informed Consent

All informed consent forms were signed and obtained from the participants.

Animal studies

Not Applicable

Clinical Trials Registry:

Not Applicable

Author's Contribution

Yash Patel: Conceptualization, Data Curation, Investigation, Resources, Visualization, Writing-Original Draft Preparation.

Sachchida Nand Pandey: Conceptualization, Methodology, Resources, Software, Supervision, Validation, Writing-Review & Editing.

Sandip B Patel: Conceptualization, Visualization, Writing-Review & Editing.

Aditya Parikh: Investigation

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Nitiraj Shete: Formal Analysis

Ratika Srivastava: Writing-Review & Editing.

Manan A Raval: Funding Acquisition.

Arvind Ganpule: Conceptualization, Project Administration, Supervision, Writing-Review & Editing.

Samir G Patel: Conceptualization, Project Administration, Resources, Supervision, Visualization, Writing-Review & Editing.

Mahesh R Desai: Funding Acquisition, Supervision.

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Tables

Table 1. Socio-demographic characteristics of participants in this study

	Case (mean+SD)	Controls (mean+SD)	p-value
Age (years)	44.15±11.71	41.03±13.73	0.3587
Gender (male/female)	111/39	109/41	0.1056
Family history	88	25	0.0001
Food habit (vegetarian/non-vegetarian)	85/65	110/40	0.0008
Water grade (tap /filtered/RO)	25/74/51	24/87/39	0.09

Table 2. Biochemical determinants of participants

	Case (mean+SD)	Controls (mean+SD)	p-value
Serum creatinine	0.94±0.38 mg/dl	0.77±0.17 mg/dl	<0.0001
Serum calcium	9.57±0.90 mg/dl	9.60±0.38 mg/dl	0.7247
Parathyroid hormone	93.37±52.78 pg/ml	64.67±31.50 pg/ml	<0.0001
24-hr creatinine	901.10±234.90 mg/day	817.50±284.59 mg/day	0.0058
24-hr urine phosphorus	500.90±164.74 mg/day	304.70±94.57 mg/day	<0.0001
24-hr urine oxalate	46.67±10.75 mg/day	31.05±10.38 mg/day	<0.0001
24-hr urine uric acid	609.60+196.60 mg/day	472.40+164.62 mg/day	<0.0001
24-hr urine calcium	156.10±67.12 mg/day	70.32±32 mg/day	<0.0001
24-hr urine citrate	243.40±79.24 mg/day	273.8±91.87 mg/day	0.0024
24-h urine magnesium	76.66+28.54 mg/day	61.64±17.51 mg/day	<0.0001

Table 3. Association of *CaSR* gene variant rs1801725 with renal stone disease risk according to the genetic association models

Genotype	Case (n)	Control (n)	Model	* OR (95% CI)	*p*-value
GG	47	31	Homozygous Co-dominant (GG vs TT)	1.789 (0.588-5.440)	0.3060
GT	95	111	Heterozygous Co-dominant (GG vs GT)	1.785 (1.033-3.083)	0.0300
TT	08	08	Dominant (GG vs GT/TT)	1.785 (1.039-3.067)	0.0360
			Recessive (GG/GT vs TT)	1.172 (0.416-3.305)	0.7640

* Adjusted for age and sex

Table 4. Association of *CaSR* gene variant rs1042636 with renal stone disease risk according to the genetic association models

Genotype	Case (n)	Control (n)	Model	* OR (95% CI)	*p*-value
AA	49	31	Homozygous Co-dominant (AA vs GG)	1.614 (0.752 - 3.465)	0.2200
AG	77	98	Heterozygous Co-dominant (AA vs AG)	2.010 (1.153 – 3.505)	0.0140
GG	24	21	Dominant (AA vs AG/GG)	1.992 (1.122 - 3.295)	0.0170
			Recessive (AA/AG vs GG)	0.999 (0.518 - 1.929)	0.9980

* Adjusted for age and sex

Table 5. Association of *CaSR* gene variant rs1801725 with metabolic abnormalities present with renal stone disease

Associated condition	Genotype	Present	Absent	Reference Genotype	OR (95% CI)	p-value
Hyperparathyroidism	GG	0.35	0.27	GT	1.45 (0.77 - 2.63)	0.2346
	GT	0.60	0.67	GG	0.69 (0.38 – 1.29)	0.2767
	TT	0.05	0.06	GG	1.56 (0.44 - 5.19)	0.4994
	GT/TT	0.65	0.73	GG	1.46 (0.79 - 2.61)	0.2213
Hypercalcemia	GG	0.50	0.30	GT	2.13 (1.21 - 3.88)	0.0105
	GT	0.50	0.64	GG	0.47 (0.26 – 0.83)	0.0131
	TT	0.0	0.06	GG	0.10 (0.01 – 0.69)	0.0130
	GT/TT	0.50	0.73	GG	0.41 (0.23 – 0.74)	0.0023
Hypercalciuria	GG	0.28	0.35	GT	0.66(0.37 - 1.23)	0.1810
	GT	0.69	0.57	GG	1.51 (0.81 – 2.73)	0.2173
	TT	0.02	0.08	GG	3.20 (0.66 - 15.72)	0.1444
	GT/TT	0.71	0.65	GG	0.73 (0.41 -1.34)	0.3084

Table 6. Association of *CaSR* gene variant rs1042636 with metabolic abnormalities present with renal stone disease

Associated condition	Genotype	Present	Absent	Reference Genotype	OR (95% CI)	p-value
Hyperparathyroidism	AA	0.37	0.28	AG	2.00 (1.09 - 3.78)	0.0303
	AG	0.41	0.62	AA	0.50 (0.26 – 0.92)	0.0389
	GG	0.22	0.10	AA	0.60 (0.24 - 1.46)	0.2619
	AG/GG	0.63	0.72	AA	0.66 (0.37 - 1.20)	0.1742
Hypercalcemia	AA	0.17	0.33	AG	0.38 (0.20 - 0.78)	0.0059
	AG	0.67	0.50	AA	2.60 (1.30 – 5.03)	0.0069
	GG	0.16	0.17	AA	0.55 (0.23 - 1.41)	0.1870
	AG/GG	0.83	0.67	AA	2.41 (1.24 - 4.74)	0.0090
Hypercalciuria	AA	0.27	0.39	AG	0.67 (0.36 - 1.21)	0.2015
	AG	0.52	0.50	AA	1.50 (0.82 – 2.81)	0.2103
	GG	0.21	0.11	AA	2.76 (1.17 - 6.62)	0.0217
	AG/GG	0.73	0.61	AA	1.73 (0.96 - 3.07)	0.0711

Table 7. Haplotype analysis of *CaSR* gene variants rs1801725 and rs1042636 in stone patients and controls

Haplotype	Patients (%)	Controls (%)	Reference haplotype	OR (95% CI)	p-value
GG+AA	96 (32)	62 (20.66)	GT+AG	1.88(1.28 - 2.75)	0.0010
GT+AG	172 (57.33)	209 (69.66)	GG+AA	0.53 (0.36 – 0.78)	0.0013
TT+GG	32 (10.66)	29 (9.66)	GG+AA	1.40 (0.76 - 2.56)	0.2638

Figures

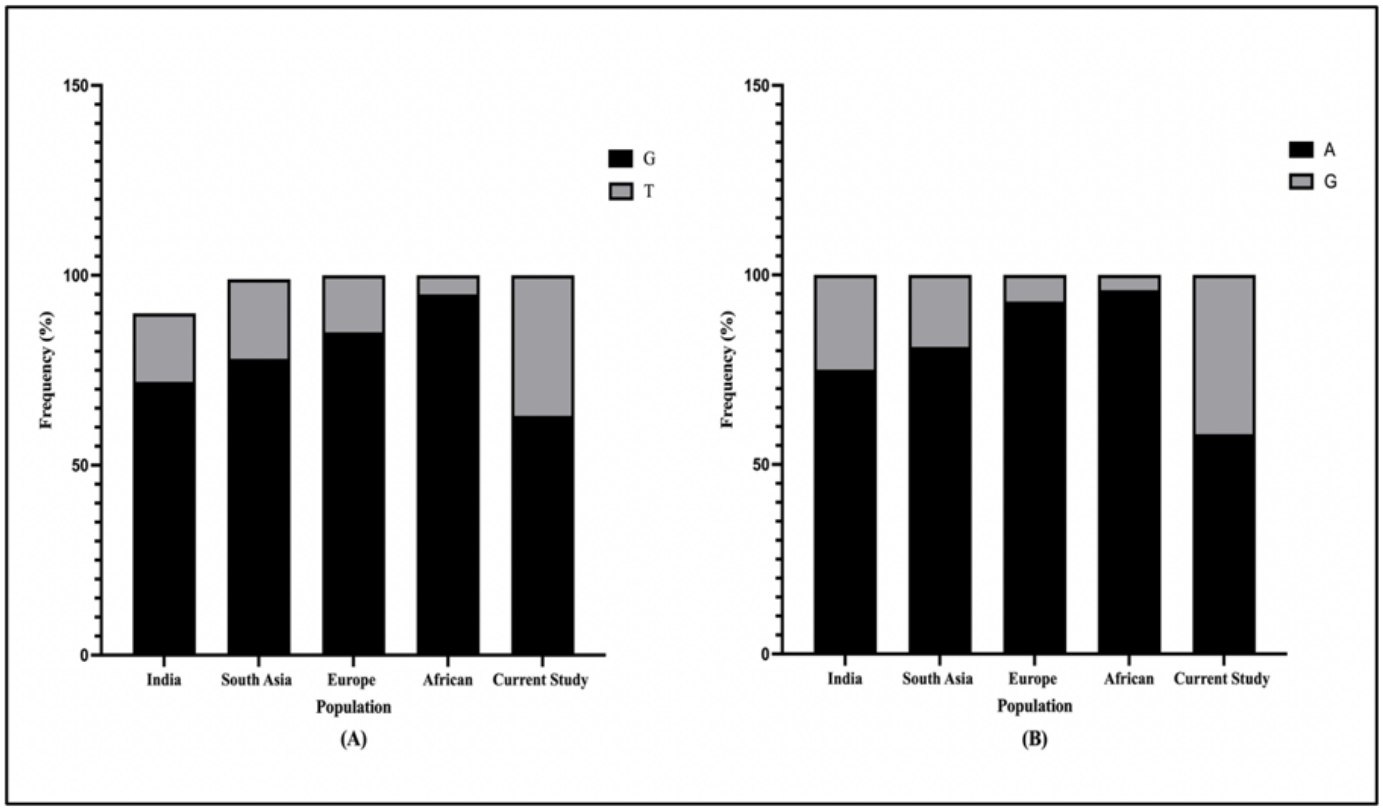

Figure 1

Frequency distribution of CaSR gene variants rs1801725 (A) and 1042636 (B) among various populations

*We have recorded the alleles frequency in healthy individuals of the Indian population for CaSR gene variants rs1801725 (A) and 1042636 (B) from IndiGenomes data (https://clingen.igib.res.in/indigen/). The allele frequencies for European, South African, and South Asian populations were recorded from National Center Biotechnology Information (https://www.ncbi.nlm.nih.gov).