



Research Article

Genotype and Allele Frequencies of Calcium-Sensing Receptor Gene a986s (rs1801725) Polymorphism in Saudi Adults

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Abstract

Objectives To determine the genotype and allele frequencies of the calcium sensing receptor (CaSR) gene A986S (rs1801725) polymorphism in healthy Saudi adults of both sexes and compare these frequencies with those in various ethnic groups.

Methods The study included 113 subjects, including 50 males and 63 females. The genotypes of polymorphic position A986S were determined by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

Results The genotype frequency in males was 26% (n=13) normal (AA), 56% (n=28) heterozygous (AS) and 18% (n=9) homozygous (SS), and the allele frequencies for the A and S alleles were 54% and 46%, respectively. The genotype distribution was out of Hardy-Weinberg equilibrium (goodness of fit $X^2=0.80$, $df=1$, $P=0.00001$). The genotype frequency in females was 49.21% (n=31) normal (AA), 36.51% (n=23) heterozygous (AS), and 14.29% (n=9) homozygous (SS), and the allele frequencies were 67% and 32.54% for the A and S alleles, respectively. The genotype distribution was out of Hardy-Weinberg equilibrium (goodness of fit $X^2=1.78$, $df=1$, $P=0.07$).

Conclusions The genotype frequencies of the CaSR polymorphism rs1801725 for the normal genotype AA were 75.21%, for the heterozygous genotype AS were 92.51% and for the homozygous genotype SS were 32.29%. The frequencies of the A and S alleles were 121.46 and 78.54%, respectively. The heterozygous genotype was higher in males, whereas the normal genotype was higher in females.

Keywords

Calcium sensing receptor; Polymorphism; Genotypes; Frequencies; SNPs; A986S; Allele

Introduction

The CaSR is a G-protein coupled receptor that is a member of a family of surface receptors. CaSR plays an important function in the regulation of extracellular fluid calcium levels [1,2]. The increased extracellular ionized calcium activates CaSR, inhibits parathyroid

hormone (PTH) secretion, and promotes urinary calcium excretion [3]. The CaSR gene expresses a protein that consists of 1078 amino acids [1]. In humans, the CaSR gene maps to 3q13.3-21 [4]. Polymorphisms in the CaSR gene are associated with a range of diseases related to calcium regulation [5], and the CaSR gene may have more than 450 single nucleotide polymorphisms (SNPs) [6]. The following three polymorphisms have been described in exon 7: 986Ala/Ser (A986S), 990Arg/Gly (R990G) and 1011Gln/Glu (Q1011E) [7]. Ala986Ser is the most common SNP in Caucasians [5,8]. A986S and R990G SNPs have been identified as regulators of the CaSR gene [9,10] and a number of studies have investigated the A986S SNP polymorphism of CaSR and its effects on bone and mineral diseases [11]. It has been shown that the A986S polymorphism may be a genetic determinant of serum calcium concentrations and responsible for a genetic predisposition to bone and mineral metabolism diseases. Therefore, this locus may be a useful therapeutic and diagnostic target in clinical investigations [11].

Parathyroid principal cells and renal tubular cells express CaSR, and other tissues and cells, such as lung, terminal ileum, colon, adrenal gland and thyroid tissue and osteoclasts, also contain CaSR.

The two chief calcium regulatory mechanisms occurring in the parathyroid glands and renal tubules are increased PTH secretion by the parathyroid glands and calcium reabsorption in the renal tubules [12]. Certain monogenic diseases, such as familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT), can be triggered by mutations in human CaSR [13]. Other inactivating mutations have been found in parathyroid lipohyperplasia [14], although evidence is not available that CaSR point mutations are present in familial idiopathic hypercalcemia. Several single nucleotide polymorphisms in exon 7 of the CaSR gene were evaluated for their role in calcium stone formation [15]. There are three polymorphisms in the intracellular tail encoded by exon 7: 986Ala/Ser (A986S), 990Arg/Gly (R990G) and 1011Gln/Glu (Q1011E) [4,7]. The CaSR gene polymorphism has a significant effect on extracellular calcium, and the presence of allele S is associated with increased serum calcium levels [11] related to a decrease in urinary calcium excretion [16].

Data were not available on the allele and genotype frequency distribution of the CaSR gene polymorphism in the Saudi population. Therefore, the aim of this study was to determine the genotype and allele frequencies of the CaSR gene A986S (rs1801725) polymorphism in healthy Saudi adults of both sexes and compare these frequencies with those established in various ethnic groups.

Materials and Methods

Subjects and study protocol

This quantitative cross-sectional exploratory study was conducted at the Center of Excellence for Osteoporosis Research (CEOR) in KAU, Jeddah, Saudi Arabia. Fifty Saudi males and 63 Saudi females participated in this study and were conveniently selected from subjects attending the CEOR, and their ages ranged between 20 and 60 years old. Subjects with renal or liver disease, thyroid disorders, diabetes mellitus, or pregnancy were excluded. Subjects taking any

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medications that could potentially affect bone metabolism (e.g., steroids and antiepileptic medications) were also excluded. All of the participants underwent a complete physical examination and routine blood biochemical analyses, and the allele and genotype frequencies for the CaSR gene A986S polymorphism were determined. The study protocol was in agreement with CEOR ethical standards. This study was approved by the Human Ethics Research Committee at the CEOR, and informed written consents were obtained from all subjects who participated in this study.

Blood samples

Venous blood samples (7 ml) were obtained from the antecubital vein after 9 hours of overnight fasting and collected into plain vacutainer tubes for serum preparation. Whole blood was used for DNA extraction (3 ml) and collected into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. The blood was stored at -80°C.

Genomic DNA extraction and genotyping

Genomic DNA was extracted from whole blood (freshly collected or stored at -80°C) using a Gentra Puregene Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The concentration of DNA was determined by reading the absorbance at a wavelength of 260 nm using an UV spectrophotometer (Gene Quant 1300, Model No. 80-2120-02, England).

Genotyping of A986S SNP

The region containing the polymorphism was amplified by polymerase chain reaction (PCR) using a previously described primer pair [17]. The forward primer for SNP A986S (rs1801725) was 5'-CTT TGA TGA GCC TCA GAA GAG C -3' (the oligo number: 706B3-9642C01), the reverse primer was 5'-ACA ACT CTT CAG GGT CCT CC -3' (the oligo number: 10706B3-9642D01) (Metabointernational AG, Germany). The final concentration of the primers was 100 µM. The PCR reactions (Promega Go Taq® Hot Star Green Master Mix Kit, USA) included genomic DNA, 25 µl HotStar Taq Master Mix, 2 µl of each primer and 15 µl RNase free water in a thermal cycler (BIO-RAD, DNA Engine Tetrad2 Peltier Thermal Cycler, serial number: AL116086, USA). The reaction profiles consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 30 sec, and a final extension at 72°C for 7 min. The PCR products were resolved on 2% agarose gels and visualized by SYBR green staining. The genotypes for the A986S SNP were determined by a restriction fragment length polymorphism (RFLP) procedure. The PCR products (0.2 µg/µl) were digested with endonuclease *hin6I* (*HinP1I*) at 80°C for 10 min and the restriction enzyme *SacI* at 65°C for 5 min (Fermentas FastDigest Restriction Enzymes, Thermo Scientific, USA). Thereafter, the genotypes were resolved after running it on 2% agarose gels electrophoresis.

Statistical Analysis

The statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 21 (SPSS Inc., Chicago, IL, USA). Descriptive data were reported as the mean ± standard deviation (SD). Categorical variables are expressed as frequency and percentage. Levene's test used to determine equality of variances ($P > 0.05$). Hardy-Weinberg equilibrium was used to determine the allele and genotype frequencies in a population.

Results

All of the study subjects (113) were of Saudi origin without any known ancestors from other ethnic groups.

Physical and biochemical characteristics

The mean and standard deviations for the anthropometric parameters of the males and females are listed in Table 1.

Performing Levene's test for homogeneity of variance indicated homogeneity of the population (P value was > 0.05).

Analysis of the CaSR Gene A986S polymorphism

PCR was used to amplify the region that contained rs1801725 SNP for all of the collected samples ($n=113$), and amplification with the specific primers produced 218 bp long fragments. After digestion with the *SacI* restriction enzyme, the normal (AA) genotype remained uncut (218 bp), the heterozygous (AS) genotype produced two bands (218 and 198 bp), and the homozygous (SS) genotype produced one band (198 bp). Subsequent to digestion with the restriction enzyme *Hin6I*, the normal (AA) genotype produced one band (195 bp), the heterozygous (AS) genotype produced two bands (218 and 195 bp), and the homozygous (SS) genotype produced one band (218 bp). The genotypes and allele frequencies of rs1801725 in the male and female participants are presented in Table 2. The genotype AA (normal) was found in 13 males (26%). Additionally, the genotype AS (heterozygous) was found in 28 males (56%), and the genotype SS (homozygous) was found in 9 males (18%). The allele frequencies for A and S were 54% and 46%, respectively. The genotype distribution was out of Hardy-Weinberg equilibrium (goodness of fit $X^2=0.80$, $df=1$, $P=0.00001$).

The genotype frequencies of the female participants were 49.29% ($n=31$) normal (AA), 36.51% ($n=23$) heterozygous (AS), and 14.29% ($n=9$) homozygous (SS), and the allele frequencies for A and S were 67.46% and 32.54%, respectively. The genotype distribution was out of Hardy-Weinberg equilibrium (goodness of fit $X^2=1.78$, $df=1$, $P=0.07$).

Table 1: Summary of the physical and biochemical characteristics of the study participants.

Groups	Males (n=50)	Females (n=63)	Levene's test for equality of variances	
	Mean± SD	Mean± SD	F	Sig.
Age (years)	43.32 ± 13.87	44.40 ± 13.22	0.39	0.53
Weight (kg)	83.15 ± 18.59	73.28 ± 15.36	1.96	0.16
Height (cm)	167.35 ± 7.24	154.58 ± 6.91	0.02	0.88

SD: standard deviation. Data are presented as mean ± SD.

Table 2: Genotype and allele frequencies of rs1801725 in the male and female participants.

Genotype	Frequency %	
	Male (n=50)	Female (n=63)
AA	26.00 (n=13)	49.21 (n=31)
AS	56.00 (n=28)	36.51 (n=23)
SS	18.00 (n=9)	14.29 (n=9)
AS+SS	74.00 (n=37)	50.79 (n=32)
Alleles		
A	54.00	67.5
S	46.00	32.5

Discussion

CaSR variants such as A986S, R990G or Q1011E have been identified in many association studies as indicators of several common diseases, such as hypertension [18,19], cancer [20,21] and diabetes [19,22]. In addition, the relationship between CaSR, colon cancer [19,23,24], prostate cancer [25] and diabetes mellitus [19,26] has been emphasized by many scientists worldwide. Furthermore, inactivating mutations of the CaSR gene can lead to conditions such as familial hypocalciuric hypercalcemia (FHH) [19,27] and neonatal severe hyperparathyroidism (NSHPT) [28,29]. NSHPT results in life-threatening conditions, including severe hypercalcemia, metabolic bone disease and potential neurodevelopmental abnormalities [30]. Prompt medical and/or surgical treatment is necessary in cases of severe hypercalcemia [31]. Infants with NSHPT who present symptoms of hypercalcemia and hyperparathyroidism are treated with intravenous fluid hydration and loop diuretic therapy as well as calcitonin, bisphosphonates and calcimimetic drugs [32,33,34,35,36]. Primary hyperparathyroidism [37] and severe neonatal hypercalcemia [38] have also been documented in Saudi Arabia.

Mutations in the CaSR gene are also associated with many benign diseases that result in mild or non-specific symptoms that do not require any particular management. However, conditions associated with these mutations can also be life threatening and require surgery. Thus, early screening is preferable to late treatment because progressing or late stages of a disease could lead to major health problems. Therefore, determining the allele frequencies of the CaSR gene polymorphism in Saudi adults will help to determine the population's susceptibility to the above-mentioned diseases.

In the present study, we found that the frequency distribution of the CaSR gene A986S polymorphism among Saudi healthy subjects is higher than that of other nationalities, and this significant difference persisted when the studies were stratified by gender. Extensive research has revealed that the individual studies included in a meta-analysis of CaSR A986S gene polymorphism between 1999 and 2009 primarily included pre- or post- menopausal women and a number of healthy adolescent girls, whereas few studies included healthy men and women. In addition, the study populations were mostly Caucasian, with one Chinese and one Finnish group. The AS+SS alleles were less common variants compared with the wild type AA [39]. Moreover, in these studies, comparisons were not performed between males and females, and most of the studies included either females alone or males and females combined into one group. In the only study conducted in 2013 that compared genders, the genotype frequency of the CaSR gene polymorphism for A/A was 0% for boys (7-12 y) and 0.3% for girls (7-12y) and the allele frequency for A was 9.3% for boys and 8.6% for girls [40]. The allele frequencies reported in our study are consistent with the results of Hanks et al. [40].

We found that in females, the frequency distribution of the CaSR heterozygous genotype AS (36.51%) was higher than that of the homozygous genotype SS (14.29%) but lower than that of the wild type AA (49.21%) and the allele frequencies for A (67.46%) were higher than that of S (32.54%), and these results are consistent with that of Lorentzon et al.; Bollerslev et al.; Wang et al. [1,41,42,43]. Lorentzon et al. reported similar findings for an analysis performed in healthy adolescent Caucasian girls [11] and noted that the frequency distribution of the wild type genotype of the CaSR A986S gene was higher than that of the homozygous genotype [11]. Furthermore, research conducted in an Australian elderly population that included

randomly selected women between the age of 70 and 85 years reported comparable results. In this latter study, the CaSR A986S gene was analyzed in 1252 samples, and the genotype frequencies were 74.9%, 23.7% and 1.4% for AA, AS and SS, respectively [41]. These results are also consistent with our data.

We investigated the mutation in postmenopausal women with mild to moderate hypertension, and the frequency distribution of the wild type allele of the calcium sensing receptor gene was higher than that of the heterogeneous and homogenous alleles, with a prevalence of 82% for allele A and 18% for allele S [42]. The results obtained by Perez-Castrillón et al. [17] are consistent with our findings. We also compared our data to that obtained by a study in Beijing that included healthy young women of Han nationality and found similar results, although the homogenous allele SS allele of the calcium sensing receptor gene was absent [43]. Compared with our data, the frequency distribution of the CaSR gene A986S variant in healthy Chinese males and females was higher for the A allele and lower for the S allele [44].

Our study provides novel findings of the frequency distribution of CaSR gene polymorphism in adult males because most similar studies have been conducted with females. We also found that the frequency distribution percentage of the CaSR gene polymorphism was higher in males than in females, although this difference may be related to genetics or lifestyle. Genes are influenced by multifactorial genetics as well as environmental factors, and differences in the genetic backgrounds of diverse populations may change the results of association studies. However, our study population was homogeneously of Saudi origin, which suggests that there may be potential clinical implications resulting from this polymorphism. Therefore, we propose to further investigate the genetic variations in the CaSR gene in a patient cohort to better characterize the function of this gene and establish a correlation between CaSR variants.

Furthermore, we intend to investigate gene-gene interactions as well as gene- environment interactions.

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