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ORIGINAL ARTICLE

Distribution of vitamin D-binding protein/group-specific component gene subtypes in Kuwaiti population

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Abstract

Background: Vitamin D-binding protein or group-specific component (Gc) is the major plasma carrier protein of Vitamin D. Two single nucleotide polymorphisms, rs7041 (NM_000583.3:c.1296G>T;NP_000574.2:p.Asp432Glu) and rs4588 (c.1307C>A; p.Thr436Lys), in the *GC* gene result in three major genotypes, that is, *GC1F* (c.1296T, c.1307C), *GC1S* (c.1296G, c.1307C), *GC2* (c.1296T, c.1307A), and phenotypes such as Gc1F (p.432Asp, p.436Thr), Gc1S (p.432Glu, p.436Thr), and Gc2 (p.432Asp, p.436Lys). Significant variations in the frequencies of *GC* subtypes (genotypes/phenotypes) are reported in different populations living in different geographical locations, for example, *GC1S*/Gc1S (c.1296G, c.1307C/p.432Glu, p.436Thr) and *GC2*/Gc2 (c.1296T, c.1307A/p.432Asp, p.436Lys) are predominant in Caucasians and people living in the northern hemisphere, and *GC1F*/Gc1F (c.1296T, c.1307C/p.432Asp, p.436Thr) is predominant in Africans. However, frequencies of major *GC* subtypes are not known in the Kuwaiti population. In this study, we investigated 512 alleles to identify the major *GC* subtypes in Kuwaiti nationals.

Methods: Genomic DNA was isolated from blood samples of 128 healthy subjects. DNA regions covering the targeted mutations were amplified by PCR. Amplified DNAs were sequenced by the Sanger method and analyzed for specific mutations to determine the *GC* genotypes and phenotypes.

Results: The results identified the presence of four *GC* genotypes/phenotypes namely *GC1F*/Gc1F (c.1296T, c.1307C/p.432Asp, p.436Thr), *GC1S*/Gc1S (c.1296G, c.1307C/p.432Glu, p.436Thr), *GC2*/Gc2 (c.1296T, c.1307A/p.432Asp, p.436Lys), and *GC3*/Gc3 (c.1296G;c.1307A/p.432Glu, p.436Lys). Among the allelic subtypes ($n = 512$), *GC1S* (c.1296G; c.1307C) ($n = 270$, 52.7%) was predominant, followed by *GC1F* (c.1296T; c.1307C) ($n = 138$, 27%), *GC2* (c.1296T; c.1307A) ($n = 72$, 14%), and *GC3* (c.1296G; c.1307A) ($n = 32$, 6.3%). Three common subtypes, that is, *GC1F* (c.1296T; c.1307C), *GC1S* (c.1296G; c.1307C), and *GC2* (c.1296T; c.1307A) are well documented in the literature, but *GC3* (c.1296T; c.1307A) is an uncommon variant found in our study subjects.

Conclusion: We found that *GC* subtype distribution was unique in the Kuwaiti population, with some affinity to Caucasians. Several factors including ancestral origin, migration history, and environmental forces such as solar intensity may be responsible for the unique distribution of *GC* subtypes in this population.

KEYWORDS

DNA sequencing, *GC* subtypes, Kuwaiti population, rs4588, vitamin D-binding protein gene polymorphism

1 | INTRODUCTION

The vitamin D-binding protein, also known as the group-specific component (Gc), is the major plasma carrier protein of vitamin D and its metabolites. In humans, Gc is an abundant protein present in major organs, namely the brain, heart, lungs, kidneys, etc., and in different body fluids including serum, cerebrospinal fluid, saliva, urine, breast milk, etc. (Delanghe et al., 2015). The main function of Gc is binding, solubilizing, and transporting vitamin D and its metabolites (Delanghe et al., 2015). In addition to its sterol-binding capacity, the Gc plays several key biological roles including the transport of fatty acids and activation of macrophages as part of immune response (Bouillon & Pauwels, 2018; Speeckaert et al., 2006). Gc is also responsible for the chemotaxis of macrophages and may be involved in maintaining bone density (Delanghe et al., 2015; Speeckaert et al., 2006). The isoelectric focusing technique (IEF) has identified Gc as one of the most polymorphic proteins belonging to the albumin/α-fetoprotein family (Cleve & Constans, 1988). In humans, the *GC* gene (OMIM: *139200) is localized on the long arm of chromosome 4 (4q12–q13) (Speeckaert et al., 2006). It extends over 35 kb DNA, contains 13 exons and 12 introns, and encodes a protein containing 474 amino acids (Malik et al., 2013). Two single nucleotide polymorphisms (SNPs) have been reported in the *GC* gene due to missense mutations in exon 11; they are known as rs7041 (c.1296T>G) and rs4588 (c.1307C>A) (Malik et al., 2013). rs7041 (c.1296T>G) encodes glutamic acid instead of aspartic acid at position 432, that is, p.Asp432Glu, and rs4588 (c.1307C>A) encodes lysine instead of threonine at position 436, that is, p.Thr436Lys (Bouillon & Pauwels, 2018). These polymorphisms lead to three major Gc isoforms namely Gc1S (p.432Glu, p.436Thr), Gc1F (p.432Asp, p.436Thr), and Gc2 (p.432Asp, p.436Lys). These were identified by differences in their electrophoretic mobilities (Bouillon, 2017; Kamboh & Ferrell, 1986). Moreover, more than 120 rare variants of Gc have also been identified (Cleve & Constans, 1988). It has been reported that more than 160 populations have been tested for Gc polymorphism using the IEF technique (Bouillon, 2017). However, genotyping studies are less common. In reviewing the literature, it was noted that the frequency of Gc1S (p.432Glu, p.436Thr) increases while that of Gc1F (p.432Asp, p.436Thr) decreases steadily toward the Northern Hemisphere. Concluding that populations living in northern climates expressed more Gc1S (p.432Glu, p.436Thr) and less Gc1F (p.432Asp, p.436Thr) isoform (Bouillon, 2017; Malik et al., 2013). The frequency of Gc2 (p.432Asp, p.436Lys) is abundant in Caucasians and rare in Africans (Bouillon, 2017; Braithwaite et al., 2015; Kamboh & Ferrell, 1986). Proteomic studies previously reported almost similar Gc2 (p.432Asp, p.436Lys) frequency among European and Mongoloid populations (Kamboh & Ferrell, 1986). Recently, a genotyping study of a healthy Korean population reported that *GC1F* (c.1296T; c.1307C) was predominantly present (49%) followed by *GC2* (c.1296T; c.1307A) (33%) (Kim et al., 2019). Though *GC1F* (c.1296T; c.1307C) was found to be predominant in Africans, some exceptions were reported in North-East African populations, where frequencies of *GC1S* (c.1296G; c.1307C) (>0.50) were higher than frequencies of the *GC1F* (c.1296T; c.1307C) subtype (<0.50) (Constans et al., 1980; Langer-Gould et al., 2018; Lefranc et al., 1981; Navas-Nazario et al., 2014). Furthermore, the frequency of *GC2* (c.1296T; c.1307A) was reported to be sporadic in Africa from a complete absence in the Tuareg Kel Kummer population in Mali (Constans et al., 1980) to a frequency of 0.21 within the Tunisian population (Lefranc et al., 1981).

Though *GC* polymorphism has been extensively studied in different populations according to their geographical locations (Braithwaite et al., 2015; Kamboh & Ferrell, 1986; Kim et al., 2019), however, there are minimal reports available covering the Middle Eastern populations (; Constans et al., 1980; Kamboh & Ferrell, 1986). In a review article, Kamboh and Ferrell (1986) cited that European, Asiatic Indian, and Middle Eastern populations share comparable frequencies of Gc major variants. Moreover, a previous report on an Arab Muslim population in Israel reported the following frequencies of Gc subtypes: Gc1S (p.432Glu, p.436Thr) = 0.6023, Gc1F (p.432Asp, p.436Thr) = 0.2120, and Gc2 (p.432Asp, p.436Lys) = 0.1857 (Nevo & Cleve, 1983). Almost similar frequencies of Gc subtypes (Gc1S (p.432Glu,

p.436Thr) = 0.59, Gc1F (p.432Asp, p.436Thr) = 0.27, and Gc2 (p.432Asp, p.436Lys) = 0.14 were also reported in a Bedouin population from North Yemen (Malik et al., 2013). Additionally, in a Saudi Arabian population, the frequencies of Gc subtypes were 0.236 (Gc1F [p.432Asp, p.436Thr]), 0.610 (Gc1S [p.432Glu, p.436Thr]), 0.150 (Gc2, [p.432Asp, p.436Lys]), and 0.004 for rare alleles (Degheishem et al., 1991). In different Arab Muslim populations, Gc1S (p.432Glu, p.436Thr) was reported to be the predominant subtype followed by Gc1F (p.432Asp, p.436Thr) and Gc2 (p.432Asp, p.436Lys) (Degheishem et al., 1991; Elkum et al., 2014; Nevo & Cleve, 1983). However, the distribution of major Gc subtypes in the Kuwaiti population is not yet known.

Geographically, Kuwait is situated in Southwest Asia, bordering the Arabian Gulf, between Iraq and Saudi Arabia. It is located in North-East of the Arabian Peninsula at the Northern end of the Arabian Gulf (latitude 28–30°N). The Kuwaiti population encompasses early migrators from the tribes of nearby Arabian and Persian countries (specifically Saudi Arabia and Iran). It also comprises the nomadic Arabs of the desert, also known as the Bedouins, who lived around the borders of the Arabian Peninsula (Alsmadi et al., 2013). Although Kuwait has always served as a residence and a workplace for noncitizens originating from many Arabic, East Asian, and European populations, there is minimal genetic exchange expected between Kuwaiti nationals and expatriates. This is due to the presence of a conservative Kuwaiti society and culture. In this study, the participants recruited were Kuwaiti nationals and non-Kuwaiti residents were excluded from the study to prevent any confounding effects. Although the majority of Kuwaiti citizens can trace their ancestry back to Arabian or Persian roots, the Middle East (including Kuwait) is a transcontinental region centered between Western Asia, Turkey (comprises of both Asian and European region), and Egypt (which is mostly in North Africa). Consequently, this formerly served as a route for human migration among these regions. Hence, it was considered interesting to find the *GC* alleles frequency in Kuwait. Moreover, a genotyping study of *GC* polymorphism may help to describe genetic stock, trace human migration, and gene exchange (Bouillon, 2017). It also serves as a tool to identify genetic isolation. In addition, differences in the allele frequency of *GC* subtypes are known to be associated with several common diseases, namely diabetes, pulmonary disease, and obesity (Speeckaert et al., 2006). Furthermore, *GC* variants are identified as a risk factor for many chronic diseases of immunological etiologies, for example, inflammatory bowel disease, multiple sclerosis, and asthma (Langer-Gould et al., 2018; Navas-Nazario et al., 2014; Speeckaert et al., 2006). Hence, exploring major Gc subtypes in Kuwaiti populations may be deemed valuable.

This study aimed to determine the frequency of major *GC* genotypes within the Kuwaiti population. The information generated by this study was expected to add valuable information about the genetic stock and may shed some light on the origin of the Kuwaiti population.

2 | MATERIALS AND METHODS

2.1 | Study population

In this cross-sectional study, we investigated 512 alleles to identify major *GC* subtypes in Kuwaiti nationals. The participants were 128 healthy Kuwaiti adults aged 18 or over, who were recruited randomly among Kuwaiti nationals. Healthy Kuwaiti adults (above age 18 years) not taking any medication regularly and having no comorbidities including common lifestyle diseases such as hypertension, diabetes, dyslipidemia, etc. were included in this study. The study subjects were adjusted for age and gender. Informed consent was obtained. The study protocol was approved by the Institutional Research Ethics Committee. Each subject was interviewed and requested to fill in a questionnaire that was designed to collect information about anthropometric parameters, specifically height, weight, and sociocultural information, such as ancestry. Other data collected included the presence or the absence of any comorbidities and whether or not the subjects were on any regular medications. Moreover, clinical evaluations were done for all subjects.

Overnight fasting peripheral blood samples were withdrawn from all subjects in 4 ml tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The complete blood samples in the EDTA tubes were frozen at −80°C. Genomic DNA was isolated from the thawed blood samples using the DNA Blood Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The quantity and the quality of the isolated genomic DNA were determined by measuring optical densities at 260 and 280 nm in a low volume (2 µl sample) spectrophotometer (Epoch, BioTek). The DNA concentrations were adjusted to 1 mg/ml.

2.2 | Oligonucleotide primers for amplification of the VDBP target DNA

The forward (5′-gatctcgaagaggcatgtttc-3′) and reverse (5′-gttgcctgtgttcacagactc-3′) primers were designed based on the genomic DNA sequence of the human *GC* gene (GenBank: L10641.1: OMIM: *139200, gene

length = 55,136 nucleotides, Witke et al., 1993) using Primer Design Software of the National Centre for Biotechnology Information (NCBI). These primers spanned the mutation sites in the *GC* gene as given below: 35,476-gatctcg aagaggcatgtttcactttctgatctcaaattgactattctataccacaggtatag aattttcttgagacaggcaagtatttctattttcatttttattgtaaaagatctgaaa tggctattattttgcattagaaatttgtataaaataaatacatgtagtaagacct-tacatttaaatggtttttcagactggcagagcgactaaaagcaaaattgcctga**g**-gccacaccca**c**ggaactggcaaagctggttaacaagcgctcagactttgcctc-caactgctgttccataaactcacctcctctttactgtgattcagaggtaggaaaat-gtaaccctccacttaacatggcagaatcttttaagaacgtatgcactccaatc-tactcatttctttcctgttattgagatgccattatgtgacaggcttttcctggtgttatt-gtaacttggctgtctttgcaatgaaagtaagaaacataactgatttcatgctatgct-catttaaaagcaagttgaggtagttgtaaaacttaggaaattttatacttttttta-gaaacaaaag agtctgtgaacacaggcaac-36,072.

Here, the primer-annealing sites (underlined) and the sites of mutations (g and c, given in bold and underlined) in the *GC* gene are marked. The numbers are given at the beginning (35476) and end (36072) of the target sequence to be amplified by the primers mentioned, and the nucleotide sequences in between are according to GenBank: L10641.1. Furthermore, the protein sequence of Gc (Protein ID = AAA61704.1) and the mutation sites (432 [E, Glutamic acid] and 436 [T, Threonine]) are given below in bold and underlined.

1 MKRVLVLLLAVAFGHALERGRDYEKNKVCKE
FSHLGKEDFTSLSLVLYSRKFPSGTFEQVSQLVKEV
VSLTEACCAEGADPDCYDTRTSALSAKSCESNSPF
PVHPGTAECCTKEGLERKLCMAALKHQPQEFPT
YVEPTNDEICEAFR KDPKEYANQ FMWEYSTNYG
QAPLSLLVSYTKSYLSMVGSCCTSASPTVCFL
KERLQLKHLSLLTTLSNRVCSQYAAYGEKKSRLSN
LIKLAQKVPTADLEDVLPLAEDITNILSKCCESAS
EDCMAKELPEHTVKLCDNLSTKNSKFEDCCQEK
TAMDVFVCTYFMPAAQLPELPDVELPTNKDVCDPGN
TKVMDKYTFELSRRTHLPEVFLSKVLEPTL
KSLGECCDVEDSTTCFNAKGPLLKKELSSFIDKGQELC
ADYSENTFTEYKKKLAERLKAKLP**E**ATP**T**ELAK
LVNKRSDFASNCCSINSPPLYCDSEIDAELKNIL-474

2.3 | PCR amplification of target region

The forward (F) and reverse (R) primers were used in PCR according to standard methods (Mustafa et al., 1999). In brief, PCRs were performed in a microtube with a total volume of 50-µl containing 100 ng of genomic DNA, 250 µM of each dNTP, 10 mM Tris–HCl (pH 8·3), 50 Mm KCl, 2 mM $MgCl_2$, 2·5 units of *AmpliTaq* Gold® DNA polymerase (Thermo Fisher Scientific), and 25 pmol of each F and R primer. PCR cycles were performed with an initial denaturation step of 10 min at 95°C for the activation of *AmpliTaq* Gold, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s with a final extension step of 72°C for 5 min. The expected size of the PCR product (596 bp) was confirmed by agarose gel electrophoresis using 1.5% agarose gels as described previously (Shaban et al., 2013). A preliminary experiment with DNA from five healthy subjects showed that these primers amplified a band of expected size at all the tested annealing temperatures ranging from 57 to 63°C (Figure 1).

2.4 | Sequencing of PCR products and analysis of data

The PCR products were sequenced according to standard procedures, as described previously (Shaban et al., 2013). In brief, the PCR products were purified by addition of 2 µl of ExoSAP-IT® to 5 µl of the PCR product and incubated for 15 min at 37°C, followed by heating at 80°C for 15 min to make the enzyme inactive. The purified DNA fragments (10 ng) were used in a cycle-sequencing reaction using a BigDye Terminator v1.3 Cycle Sequencing Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The generated DNA fragments were purified using BigDye XTerminator® Purification Kits and analyzed using a 3130xl Genetic Analyzer (Thermo Fisher Scientific). The sequence data were analyzed for specific mutations (G–T or C–A) using the software in the Genetic Analyzer. Theoretical possibilities for *GC* genotypes and phenotypes based on rs7041 and rs4588 alleles are represented in Table 1. The resultant changes in amino acids expected due to mutations based on rs7041 and rs4588 alleles are given in Table 2.

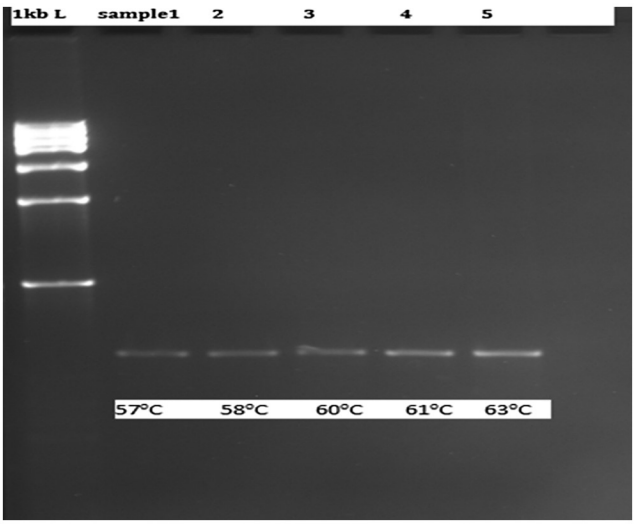

FIGURE 1 PCR amplification of target DNA by the primer pair used in this study. Genomic DNAs isolated from the peripheral blood of five donors were PCR amplified using primers given in the Materials and Methods. The amplified DNAs were analyzed by agarose gel electrophoresis

TABLE 1 Theoretical possibilities for *GC* genotypes and phenotypes based on rs7041 and rs4588 allelic mutations

Alleles of rs7041	Alleles of rs4588	*GC* genotype	*GC* phenotype
G/G	C/C	G-C/G-C	Gc1S/Gc1S
T/T	C/C	T-C/T-C	Gc1F/Gc1F
T/T	A/A	T-A/T-A	Gc2/Gc2
G/G	A/A	G-A/G-A	Gc3/Gc3
G/T	C/C	G-C/T-C	Gc1S/Gc1F
G/G	C/A	G-C/G-A	Gc1S/Gc3
T/T	C/A	T-C/T-A	Gc1F/Gc2
G/T	A/A	G-A/T-A	Gc3/Gc2
G/T	C/A	G-C/T-A or G-A/T-C	Gc1S/Gc2 or Gc1F/Gc3

Representative DNA-sequencing results of four donors (A, B, C, and D) showed the wild-type and mutated sequences for rs4588 and rs7041 (Figure 2a–d).

2.5 | Statistical analyses

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) (IBM Corporation, Armonk, NY, USA 2013). Qualitative variables were described using numbers and percentages. Differences between qualitative variables were tested with the Fisher Exact test and chi-square test, as applicable. A Spearman's correlation was done to test the association between categorical variables.

3 | RESULTS

3.1 | Demographic characteristics of sample

A total of 512 alleles were investigated to identify major *GC* subtypes in Kuwaiti nationals. For this purpose, we recruited 128 healthy Kuwaiti adults (46 males and 82 females). Our subjects were young adults (mean age: 33.7 ± 9.3 years); with no reported comorbidities or are on any regular medications. The descriptive characteristics of the cohort are presented in Table 3. The study population of Kuwaiti nationals comprised of three ethnicities, that is, non-Bedouin Arabs (44.5%,) Bedouin Arabs (42.2%), and Persians (13.3%) (Table 3).

3.2 | *GC* allelic subtypes

As per the possibilities of mutations and the resulting genotypes and phenotypes explained in Table 1, we have identified the existence of four different allelic subtypes of *GC*, namely, *GC1F* (c.1296T, c.1307C), *GC1S* (c.1296G, c.1307C), *GC2* (c.1296T, c.1307A), and *GC3* (c.1296T; c.1307A) in the study population (Table 4). Figure 2a–d shows the representative DNA-sequencing results with wild-type and mutated sequences for rs4588 and rs7041.

TABLE 2 Amino acid changes in Gc protein subtypes due to the mutations rs7041 and rs4588

	Mutations	
Gc subtype	rs7041Glu432Asp	rs4588Thr436Lys
Gc1F	Asp432	Thr436
Gc1S	Glu432	Thr436
Gc2	Asp432	Lys436
Gc3	Glu432	Lys436

Among the allelic subtypes ($n = 512$), *GC1S* (c.1296G, c.1307C) ($n = 270$, 52.73%) was predominant, followed by *GC1F* (c.1296T, c.1307C) ($n = 138$, 26.95%), *GC2* (c.1296T, c.1307A) ($n = 72$, 14.06%), and *GC3* (c.1296T; c.1307A) ($n = 32$, 6.26%) (Table 4).

There were no significant associations in the distribution of different alleles among people belonging to different ethnicities or gender (Table 5). However, it was noted that *GC3* (c.1296T; c.1307A) was rarely present in people with Persian ancestry, and *GC2* (c.1296T, c.1307A) was more frequent in females (Table 5). A Spearman's correlation was run to determine the relationship between different *GC* allelic subtypes. We found that *GC1F* (c.1296T, c.1307C) showed positive correlation with *GC2* (c.1296T, c.1307A) (rs = 0.486, $p < 0.001$) and *GC3* (c.1296T; c.1307A) (rs = 0.398, $p < 0.001$). Though there was a negative correlation between *GC1F* (c.1296T, c.1307C) and *GC1S* (c.1296G, c.1307C), it was not significant (rs = −166, $p > 0.05$). Hereby, it is more likely that *GC1F* (c.1296T, c.1307C) will be present in combination with *GC1S* (c.1296G, c.1307C), *GC2* (c.1296T, c.1307A), and *GC3* (c.1296T; c.1307A) to form heterozygous phenotypes

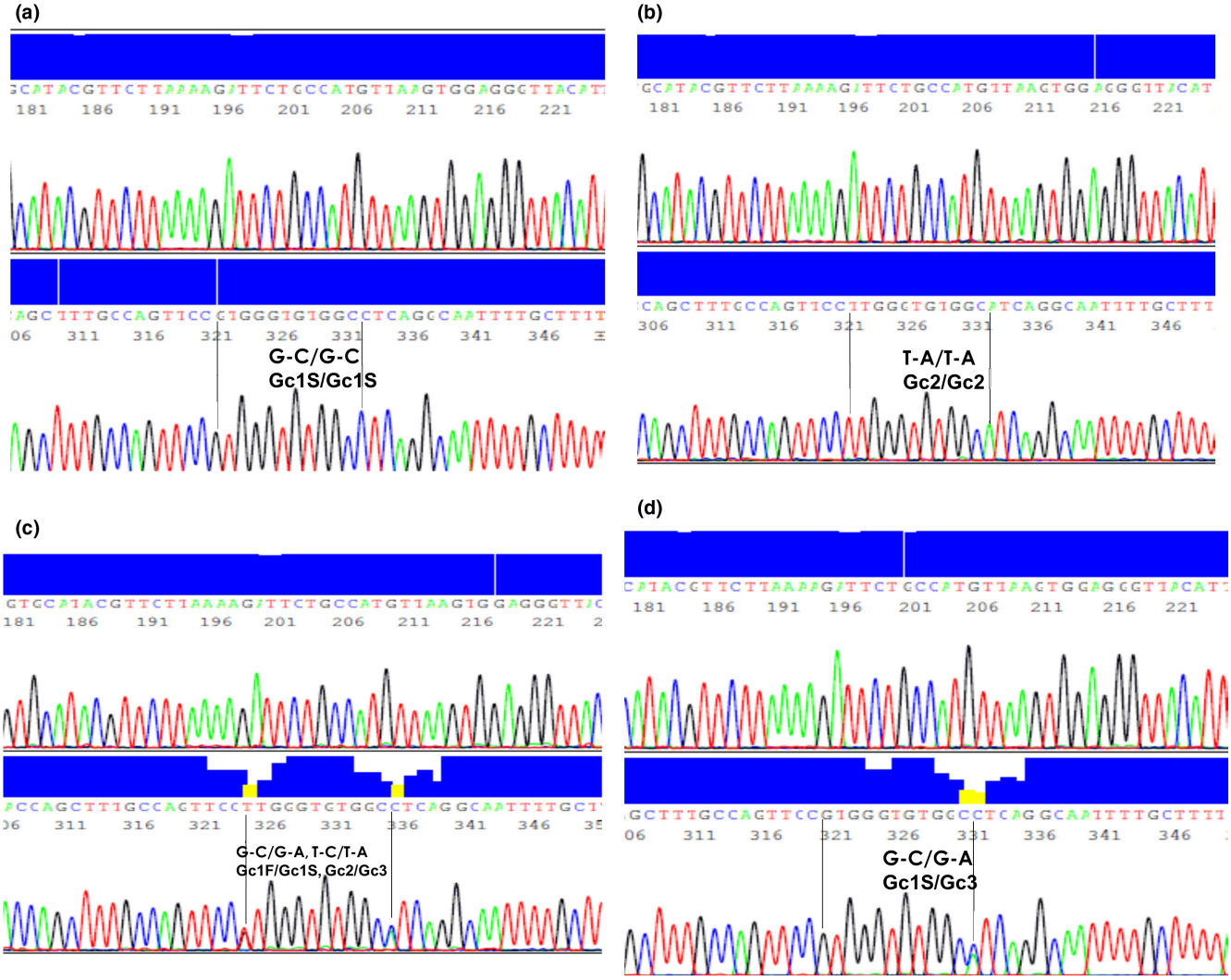

FIGURE 2 (a) Representative DNA-sequencing results of PCR-amplified DNA from a donor (a) included in the study. The PCR-amplified DNA from the donor was sequenced using the Sanger method. The nucleotide sequences obtained are given as colored peaks and corresponding letters (red for T, green for A, Black for G, and blue for C). The mutation sites are marked by arrows and the resulting genotype and phenotype are shown. (b) Representative DNA-sequencing result of PCR-amplified DNA from a donor (b) was included in the study. The PCR-amplified DNA from the donor was sequenced using the Sanger method. The nucleotide sequences obtained are given as colored peaks and corresponding letters (red for T, green for A, Black for G, and blue for C). The mutation sites are marked by arrows and the resulting genotype and phenotype are shown. (c) Representative DNA-sequencing results of PCR-amplified DNA from a donor (c) included in the study. The PCR-amplified DNA from the donor was sequenced using the Sanger method. The nucleotide sequences obtained are given as colored peaks and corresponding letters (red for T, green for A, Black for G, and blue for C). The mutation sites are marked by arrows and the resulting genotype and phenotype are shown. (d) Representative DNA sequencing result of PCR amplified DNA from a donor (d) was included in the study. The PCR amplified DNA from the donor was sequenced using Sanger method. The nucleotide sequences obtained are given as colored peaks and corresponding letters (red for T, green for A, Black for G, and blue for C). The mutation sites are marked by arrows and the resulting genotype and phenotype are shown

GC1F (c.1296T, c.1307C)/*GC1S* (c.1296G, c.1307C), *GC1F* (c.1296T, c.1307C)/*GC2* (c.1296T, c.1307A), or *GC1F* (c.1296T, c.1307C)/*GC3* (c.1296T; c.1307A). *GC1S* (c.1296G, c.1307C) was positively correlated with *GC3* (c.1296T; c.1307A) (rs = 0.204, p <0.05) and negatively correlated with *GC2* (c.1296T, c.1307A) (rs = −405, p <0.001). Therefore, suggesting that it was unlikely to find a *GC1S* (c.1296G, c.1307C)/*GC2* (c.1296T, c.1307A) genotype. Moreover, *GC2* (c.1296T, c.1307A) showed a positive correlation with all the other alleles, except *GC1S* (c.1296G, c.1307C).

3.3 | Gc phenotypes

In our study, the four *GC* allelic subtypes resulted in different Gc phenotypes (Table 6). Among those, Gc1S/Gc1S

TABLE 3 Demographic characteristics of the studied subjects

Variables	$n = 128$
Gender	*n* (%)
Male	46 (35.4)
Female	82 (64.6)
Ethnicity	*n* (%)
Bedouins	54 (42.2)
Non-Bedouins	57 (44.5)
Persians	17 (13.3)
Age in years expressed as mean ± SD	33.7 ± 9.3

TABLE 4 Frequency of different *GC* genotypes in the studied subjects

GC allele	*n* (%)
GC1S (c.1296G, c.1307C)	270 (52.7)
GC1F (c.1296T, c.1307C)	138 (27)
GC2 (c.1296T, c.1307A)	72 (14)
GC3 (c.1296T, c.1307A)	32 (6.3)
Total number of alleles	512 (100)

(p.432Glu, p.436Thr/p.432Glu, p.436Thr) ($n = 43$, 33.6%) was the most common phenotype, followed by Gc1S/Gc1F (p.432Asp, p.436Thr/p.432Glu, p.436Thr) ($n = 33$, 25.8%), Gc1F/Gc1S/Gc2/Gc3 (p.432Asp, p.436Thr/p.432Glu, p.436Thr/p.432Asp, p.436Lys/p.432Glu, p.436Lys) ($n = 32$, 25%). The lowest frequencies were found for phenotypes Gc2/Gc2 (p.432Asp, p.436Lys/p.432Asp, p.436Lys) ($n = 3$, 2.3%) and Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr) ($n = 3$, 2.3%). However, none of the subjects had Gc3/Gc3 (p.432Glu, p.436Lys/p.432Glu, p.436Lys) phenotype. Genderwise distributions of different phenotypes were nearly similar, except for Gc2/Gc1F (p.432Asp, p.436Lys/p.432Glu, p.436Thr), which were positively associated with the female gender ($p < 0.05$) (Figure 3). Though some differences in Gc phenotype distribution were noted among people having a different ethnic origin, like subjects with Persian ethnicity did not possess Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr) and Gc2/Gc2 (p.432Asp, p.436Lys/p.432Asp, p.436Lys) phenotypes, but these differences were not significant ($p > 0.05$) (Figure 4).

4 | DISCUSSION AND CONCLUSION

This study was aimed to determine the frequency of major *GC* genotypes and phenotypes in the Kuwaiti population. We have observed that *GC1S* (c.1296G, c.1307C) was the predominant genotype followed by *GC1F* (c.1296T, c.1307C), *GC2* (c.1296T, c.1307A), and *GC3* (c.1296T, c.1307A). Moreover, we found that in the studied Kuwaiti subjects, the most common phenotype was Gc1S/Gc1S (p.432Glu, p.436Thr/p.432Glu, p.436Thr), followed by Gc1S/Gc1F (p.432Asp, p.436Thr/p.432Glu, p.436Thr), Gc1F/Gc1S/Gc2/Gc3 (p.432Asp, p.436Thr/p.432Glu, p.436Thr/p.432Asp, p.436Lys/p.432Glu, p.436Lys). The lowest frequencies were for phenotypes Gc2/Gc2 (p.432Asp, p.436Lys/p.432Asp, p.436Lys) and Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr). We could not find any significant association in the distribution of different alleles among people belonging to different ethnicities or genders. We noted some differences in *GC* phenotype distribution among people having a different ethnic origin, but these differences were not significant too. However, the Gc2/Gc1F (p.432Asp, p.436Lys/p.432Glu, p.436Thr) phenotype was significantly associated with the female gender. It is worth mentioning that including only healthy adults could influence the allele frequencies found (as certain phenotypes are associated with lower vitamin D levels and disease).

It has been found that the GC subtypes, *GC1F* (c.1296T, c.1307C) and *GC1S* (c.1296G, c.1307C), usually genetically separate two distinct populations namely Caucasians and Africans (Constans et al., 1980). In our population, *GC1S* (c.1296G, c.1307C) (52.7%) is found to be the predominant subtype, which may denote some affinity of the Kuwaiti population to the Caucasian population, as the presence of *GC1S* (c.1296G, c.1307C) allele was reported to be high in Europeans ranging from 50% to 60% (Kamboh & Ferrell, 1986). In the Kuwaiti cohort, *GC1S* (c.1296G, c.1307C) is followed by the presence of *GC1F* (c.1296T, c.1307C) (27%). The Kuwaiti population stands out as unique by *GC2* frequency which is almost half (14%) that of Europeans (25%) (Constans et al., 1980; Kamboh & Ferrell, 1986). Our finding is well supported by a study from Saudi Arabia which reported similar results with 61% *GC1S* (c.1296G, c.1307C), followed by *GC1F* (c.1296T, c.1307C) (23.6%) and Gc2 (c.1296T, c.1307A) (15%) (Degheishem et al., 1991). The authors also reported an increase of *GC1S* (c.1296G, c.1307C) and a decrease of *GC1F* (c.1296T, c.1307C) from South to North of Saudi Arabia (Degheishem et al., 1991). Classically, *GC2* (c.1296T, c.1307A) is also reported to be more frequent in Caucasians and closely related groups than in the colored or black population (Constans et al., 1980). However, it is difficult to explain the presence of a similar frequency of *GC2* (c.1296T, c.1307A) in both Europeans and Mongoloids (Kamboh & Ferrell, 1986) from the genetic point of view, rather it may indicate some association of *GC2* (c.1296T, c.1307A) with lighter skin color and exposure to solar intensity. In this regard, it is worth mentioning that a

TABLE 5 Frequency of different *GC* genotypes among 128 subjects belonging to different ancestries and genders

Ancestry	*n*	Total number of alleles	*GC1F* (c.1296T, c.1307C)	p^a	*GC1S* (c.1296G, c.1307C)	p^b	*GC2* c.1296T, c.1307A	p^c	*GC3* (c.1296T, c.1307A)	p^d
			Frequency*n* (%)		Frequency*n* (%)		Frequency*n* (%)		Frequency*n* (%)	
Bedouins	54	216	50 (23.15)		128 (59.26)		24 (11.11)		14 (6.48)	
Non-Bedouins	57	228	66 (28.95)		104 (45.61)		42 (18.42)		16 (7.02)	
Persians	17	68	22 (32.35)	0.297	38 (55.88)	0.215	6 (8.83)	0.099	2 (2.94)	0.387
Gender										
Male	46	184	50 (27.17)	0.672	100 (54.35)	0.442	22 (11.96)	0.216	12 (6.52)	0.832
Female	82	328	128 (39.02)		170 (51.83)		24 (7.32)		20 (6.09)	

Note: (*p* value generated by Pearson chi-square test). p^a- Difference in Gc1F frequency between different ancestries and genders. p^b- Difference in Gc1S frequency between different ancestries and genders. p^c-Difference in Gc2 frequency between different ancestries and genders. P^d-Difference in Gc3 frequency between different ancestries and genders.

positive correlation between low *GC2* (c.1296T, c.1307A) frequency and a level of ultraviolet light has been reported (Kirk et al., 1963; Mourant et al., 1976]. Hereby, lower *GC2* (c.1296T, c.1307A) frequency in the Kuwaiti population may be associated with some environmental factors such as intense solar radiation that Kuwait is exposed to throughout the year.

We also identified the presence of an uncommon allele *GC3* (c.1296T; c.1307A) in Kuwaiti subjects. This allele is rarely reported in the literature and was only identified recently by a genotyping study in a Lebanese population by Medlej-Hashim et al. (2015). Phenotypic analysis of our data revealed that Gc3 (p.432Glu, p.436Lys) is always present as heterozygous with other alleles (Gc1S [p.432Asp, p.436Thr], Gc1F [p.432Glu, p.436Thr], Gc2 [p.432Asp, p.436Lys]) and no homozygous phenotype was found in the studied subjects. This finding was in contrast to the Lebanese study where both homozygous and heterozygous associations were found for Gc3 (p.432Glu, p.436Lys) (Medlej-Hashim et al., 2015). The occurrence of Gc1F (p.432Glu, p.436Thr) in the homozygous phenotypic association is also an uncommon phenomenon in the Kuwaiti subjects. These results are very uncertain and could be due to randomness.

Though within the scope of our study, we did not investigate the serum Vitamin D level of the study subjects. There are studies suggestive of the presence of endemic hypovitaminosis D in the Kuwaiti population (Al-Taiar et al., 2018; Zhang et al., 2016) and other Gulf countries (Muhairi et al., 2013). Other studies have reported the association between polymorphisms in genes involved in vitamin D metabolism and vitamin D concentrations in the blood of healthy nationals of Kuwait and other Gulf countries (Al-Temaimi et al. 2015; Anouti et al., 2017; Osman & Al Anouti, 2015; Safar et al., 2018), but this is the first study to determine the *GC* gene polymorphism in healthy Kuwaiti nationals.

The genetic heterogeneity of *GC* found in our study may shed some light on the reported Vitamin D deficiency in the Kuwaiti population. In this regard, it is worth mentioning that *GC* polymorphism results in affinity differences of the encoded protein for vitamin D compounds (Lauridsen et al., 2005). The bioavailability of the free vitamin D metabolites is regulated by their affinity to the Gc subtypes (Braithwaite et al., 2015; Lauridsen et al., 2005). Lauridsen et al. (2005) reported that the presence of a higher concentration of vitamin D metabolites in Gc1/Gc1 (irrespective of Gc1F [p.432Asp, p.436Thr], or Gc1S [p.432Glu, p.436Thr]) than in Gc2/Gc2 (p.432Asp, p.436Lys/p.432Asp, p.436Lys). In a West African population, Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr) was found to have the highest 25-hydroxy vitamin D concentration compared with other Gc variants such as Gc1S (p.432Glu,

TABLE 6 Frequencies of different Gc phenotypes in the study population

Gc phenotypes	Frequency, *n* (%)
Gc1S/Gc1S (p.432Glu, p.436Thr/p.432Glu, p.436Thr)	43 (33.6)
Gc1S/Gc1F (p.432Glu, p.436Thr/p.432Asp, p.436Thr)	33 (25.8)
Gc1F/Gc1S/Gc2/Gc3 (p.432Asp, p.436Thr/p.432Glu, p.436Thr/p.432Asp, p.436Lys p.432Glu, p.436Lys) p.432Asp, p.436Lys p.432Asp, p.436Lys p.432Asp, p.436Lys	32 (25)
Gc2/Gc1F (p.432Asp, p.436Lys/p.432Glu, p.436Thr)	14 (11)
Gc2/Gc2 (p.432Asp, p.436Lys/p.432Asp, p.436Lys)	3 (2.3)
Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr)	3 (2.3)
Gc3/Gc3 (p.432Glu, p.436Lys/p.432Glu, p.436Lys)	0 (0.0)
Total number of subjects	128 (100)

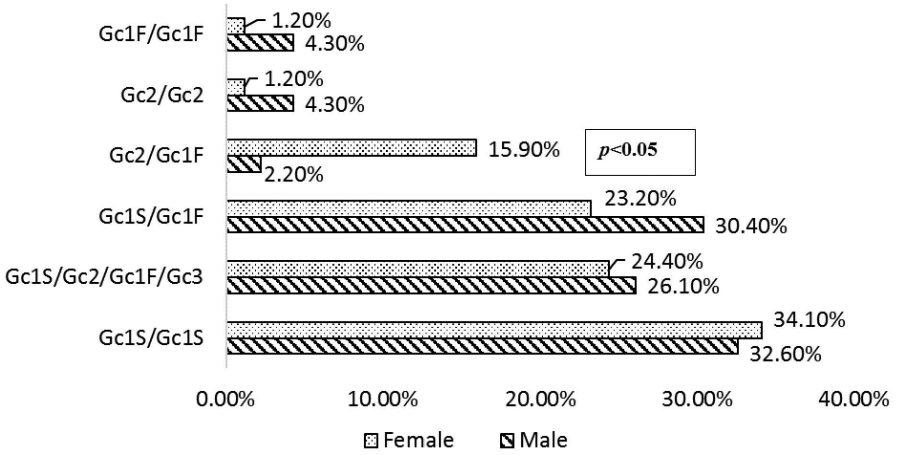

FIGURE 3 A comparative account of gender-wise distributions of Gc phenotypes. Statistical significance was tested by chi-square test. No statistically significant differences were noted in distributions of Gc phenotypes between genders except for Gc2/Gc1F, which was significantly higher in females ($p < 0.05$; generated by Fisher's exact test)

p.436Thr) (Braithwaite et al., 2015). In general, the concentration of vitamin D metabolites decreases in the order of being highest in Gc1F (p.432Asp, p.436Thr), intermediate in Gc1S (p.432Glu, p.436Thr), and lowest in Gc2 (p.432Asp, p.436Lys) (Braithwaite et al., 2015; Lauridsen et al., 2005). Gc3 (p.432Glu, p.436Lys) is associated with normal vitamin D levels (Medlej-Hashim et al., 2015). However, some variations have been reported in the literature which can be interpreted as drift across the population and ancestral groups (Arnaud and Constans, 1993). The predominant presence of Gc1S (p.432Glu, p.436Thr) and low presence of Gc3 (p. 432Glu, p.436Lys) in our study population is predictive of intermediate to low vitamin D concentration.

Our finding is well supported by the prevalence of Vitamin D deficiency in Kuwaiti adults (Elkum et al., 2014). The low presence of homozygous Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr) in the Kuwaiti population is also suggestive of the prevalence of hypovitaminosis D in the Kuwaiti population as Gc1F/Gc1F (p.432Asp, p.436Thr/p.432Asp, p.436Thr) phenotypes are reported to have a high 25 (OHD)-Vitamin D3 concentration compared with other Gc variants (Braithwaite et al., 2015). Positive association of Gc2/Gc1F (p.432Asp, p.436Lys/p.432Glu, and p.436Thr) phenotype with female gender in our study population may result in lower Vitamin D in peripheral blood as Gc2 (p.432Asp, p.436Lys may counter the effect of Gc1F (p.432Asp, p.436Thr. This is in accordance with the previous finding that Kuwaiti females are more prone to have less serum Vitamin D concentrations possibly due to sociocultural practices (Al-Shammri et al., 2015).

Overall, the similar distribution of *GC1S* (c.1296G, c.1307C), *GC1F* (c.1296T, c.1307C), *GC2* (c.1296T, c.1307A), and *GC3* (c.1296T; c.1307A) genotypes among different ethnic ancestries (Bedouin, non-Bedouin, and Persian ancestry) of Kuwaitis may indicate that irrespective of their tribes, the Kuwaiti population experienced similar exposure to races such as Caucasians and Africans. In addition to the ancestral history, environmental factors may have played some roles in defining Gc phenotypes

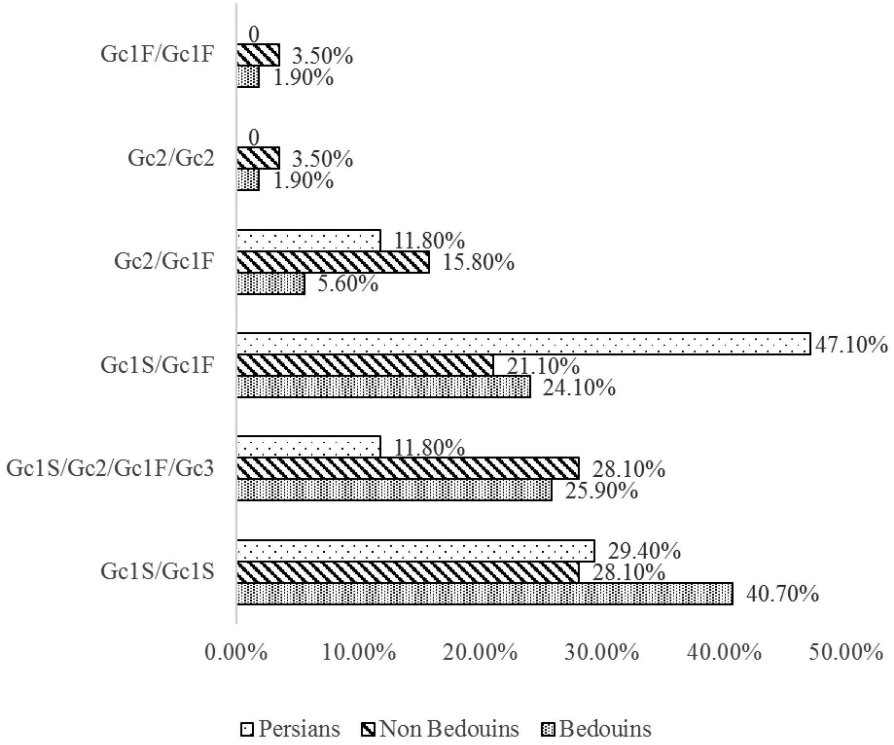

FIGURE 4 A comparative account of Ancestry-wise distributions of Gc phenotypes. Statistical significance was tested by chi-square test. No statistically significant differences were noted

in the Kuwaiti population. This is supported by previous findings showing that environmental factors played a major role in Vitamin D level and disease exposures in the Kuwaiti population (Al-Shammri et al., 2015; Mourant et al., 1976). Moreover, Kuwait's first national nutritional survey suggests a very high prevalence of Vitamin D deficiency in Kuwaiti adults (Zhang et al., 2016), which is well explained by our genotyping data which may be further modified by the natural selection driven by environmental forces (Al-Shammri et al., 2015; Mourant et al., 1976).

This study is the first attempt to identify the major *GC* genotypes in a population of Kuwaiti nationals. The major strengths of this study can be identified in gathering genetic data of a relatively homogenous population from a small country in the Middle East. These data may be helpful to trace the ancestry and migration of said population. Moreover, recent studies have shown that ethnic differences in the allele frequency of *GC* subtypes are associated with the onset and prognosis of different common diseases namely diabetes (Kirac et al., 2018), cardiovascular diseases (Kuliczkowska-Plaksej et al., 2018), and metabolic syndromes (Karuwanarint et al., 2018). The genetic data may further help to generate interest in the relationship of major *GC* genotypes with the susceptibility of the Kuwaiti population to different diseases.

However, this study had some limitations; we were able to recruit only a limited number of healthy Kuwaiti nationals; the sample size was relatively small in comparison with the total Kuwaiti population, which is around 1.4 million (http://stat.paci.gov.kw; 2020). Moreover, it will be interesting to assess the relationship of major *GC* subtypes and Vitamin D levels in the study population. The subject for our future investigation will be to assess the contribution of *GC* variants in hypovitaminosis D (if any) in these healthy Kuwaiti subjects.

ETHICAL COMPLIANCE

The study was approved by the Kuwait University Health Science Centre ethical committee.

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AUTHORS' CONTRIBUTION

S.N.A.S. conceived and designed the study, formulated research goals and aims, arranged funding, recruited subjects after medical review, reviewed and edited the initial manuscript, and contributed to the preparation of the final manuscript for publication. A.S.M. conceptualized the study design, developed the methodology, supervised the performance of the laboratory procedures, reviewed and edited the initial manuscript, and contributed to the preparation of the final manuscript. A.B. coordinated and contributed in the laboratory procedure,

collected demographic data, managed and computed data, analyzed data statistically, wrote the initial draft, and contributed to the preparation of the final manuscript for publication.

CONFLICT OF INTEREST

None of the authors admits any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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