Vitamin D Deficiency in Uygurs and Kazaks Is Associated with Polymorphisms in CYP2R1 and DHCR7/NADSYN1 Genes

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Background: Our study is aimed to 1) clarify the vitamin D status in Uygur and Kazak ethnic populations and 2) elucidate the relationship between 14 SNPs (in 5 vitamin D-related genes) and vitamin D deficiency in these 2 ethnic populations.

Material/Methods: A multistage-cluster sampling survey was carried out for residents with Uygur or Kazak ethnicity in Xinjiang, China. Anthropometric measurements were taken and the concentrations of 25OHD were measured. Fourteen common variants in VDR, GC, CYP2R1, CYP27B1, and DHCR7/NADSYN1 were genotyped by using multiple SNpShot assay. Logistic regression analysis was performed to identify the possible risk factors for vitamin D deficiency, after adjusting for several environmental and biological factors. The pattern of SNP associations was distinct between Uygurs and Kazaks.

Results: Anthropometric measurements and the concentrations of 25OHD were obtained from 1873 participants (945 Uygur ethnic and 928 Kazak ethnic). The genotypes of 14 SNPs were measured for 300 Uygurs and 300 Kazaks. The median 25OHD concentration was as low as 10.4 ng/ml in Uygurs and 16.2ng/ml in Kazaks. In Uygurs, the prevalence of vitamin D deficiency, in-sufficiency, and sufficiency was 91.2%, 5.8%, and 3.0%, respectively. CYP2R1-rs10766197 was significantly associated with the presence of vitamin D deficiency in the Uygur ethnic population (P=0.019, OR=6.533, 95%C.I.: 361–31.357), while DHCR7/NADSYN1-rs12785878 was significantly associated with the presence of vitamin D deficiency in the Kazak ethnic population (P=0.011, OR=2.442, 95%C.I.: 1.224–4.873). Of 10 SNPs in VDR and GC genes, none was associated with vitamin D status in these 2 ethnic populations.

Conclusions: Vitamin D insufficiency is highly prevalent in Uygurs and Kazaks living in Xinjiang, China. Polymorphisms in CYP2R1-rs10766197 and DHCR7/NADSYN1-rs12785878 are associated with vitamin D deficiency in Uygur and Kazak ethnic populations.

MeSH Keywords: Calcifediol • Ethnic Groups • Polymorphism, Genetic

Abbreviations: 25OHD – 25-hydroxy vitamin D; BMI – body mass index; CI – confidence interval; BP – blood pressure; HDL – high-density lipoprotein; LDL – low-density lipoprotein

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Background

Vitamin D status, measured by 25-hydroxyvitamin D (25(OH)D), was long been viewed as a hormone acting chiefly to regulate calcium-phosphate metabolism and bone mineralization [1]. Over the last decade, however, basic science and clinical researchers have produced a bewildering amount of information on the extra-skeletal effects of vitamin D. Consequently, vitamin D insufficiency is considered as a risk factor for a number of common chronic diseases, such as cancer, cardiovascular disease, metabolic syndrome, and type 2 diabetes [2,3].

Recently, attention has turned to gene-environment interactions that could influence various vitamin D-related disorders [4–6]. Twin- and family-based studies have confirmed that heritable factors have an appreciable influence on 25OHD concentrations. Genetic association studies, including genome-wide association studies (GWAS), have identified that a majority of SNPs are associated with circulating levels of 25OHD and vitamin D deficiency [7,8]. Various studies have demonstrated that race/ethnicity is an important predictor of serum circulating 25OHD levels [9,10]. However, these genetic association studies were mainly conducted in Western populations and in Han Chinese, and it remains unclear whether these genetic variants have similar effects in different ethnic groups.

Xinjiang Province, located in Northwestern China, is located at latitude N34° to 48° and has a long winter. There are 1.46 million Kazaks and 10.52 million Uygurs living in Xinjiang province. Although these 2 ethnic populations live in the same environment and both have low vitamin D levels, they have different genetic backgrounds; therefore, they present an interesting sample in which to investigate the relationship between vitamin D status and genetic variants. In this cross-sectional study, we aimed to clarify vitamin D status in these 2 ethnic populations, and to assess the association between 14 SNPs genetic variants and vitamin D status in Uygur and Kazak ethnic populations.

Material and Methods

Study population

Our data originated from a cross-sectional comprehensive health examination for Uygur and Kazak ethnic populations living in Xinjiang, China. The details of the study design have been reported previously elsewhere [11]. In brief, a multi-stage-cluster sampling survey was conducted between October 2013 and December 2013. Uygurs were recruited from 3 cities – Kashgar (N39.2°, n=633), Tacheng (N43.2°, n=232), and Urumqi (N42.5°, n=135) and Kazaks were recruited from 3 cities – Altay (N47.5°, n=486), Fukang (N43.9°, n=368), and Urumqi (N42.5°, n=146) according to the regional distribution of these 2 ethnic populations. Of the 2000 participants, 127 were excluded because of inadequate blood sampling to test 25OHD (n=54), or lack of demographic data (n=73). Thus, a total of 1873 participants were included in the vitamin D status analysis. The study was conducted in agreement with the 1990 Declaration of Helsinki and subsequent amendments. The study protocol was approved by the Ethics Committee Board in the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from all participants. In each ethnic population, 14 SNPs were determined in 300 participants. For 300 Uygur ethnic participants, 217 persons were randomly selected from 862 participants with vitamin D deficiency and 83 participants were without vitamin D deficiency; and 300 Kazak ethnic participants, including 150 persons with vitamin D deficiency and 150 without vitamin D deficiency, were randomly selected from 672 vitamin D deficiency participants and 256 without vitamin D deficiency participants.

Measurement of vitamin D levels

Blood samples were obtained after an overnight fasting and serum was stored in aliquots at −80°C until analysis. Total serum 25OHD, including D₃ and D₇, was measured by ROCHE MODULAR ANALYTICS E170 with commercially available kits. The measurable range was 3.0–70.0 ng/ml, with an inter- and intra-assay variable coefficients were 9–15% and <10%, respectively. Biochemical parameters – fasting blood glucose, calcium, phosphate, alkaline phosphatase, kidney function, and lipid profiles (including triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol) – were measured by an auto-analyzer (ADVIDA1650; Siemens, NY, USA) with commercially available kits. Other measurements – age, sex, weight, height, and ethnicity – were also obtained. Body mass index (BMI) was calculated using the standard BMI formula as body weight (in kilograms) divided by height (in meters) squared.

DNA collection, SNP selection, and genotyping

VDR, maps on the chromosome 12q12-q14, is a candidate gene of vitamin D deficiency [12]. Previous studies have related genetic variants of VDR with circulating vitamin D levels [13–15]. We selected 14 SNPs in 5 candidate genes known to have a biological impact on vitamin D metabolism [8,16,20]. These genes and SNPs were VDR (vitamin D receptor; rs7975232, rs731236, rs2239179, rs1544410, rs2228570, rs12717991, rs11168275), GC (“group-specific components”; rs4588, rs7041, rs2282679), DHR7/NADSYN1 (7-Dehydrocholesterol reductase/NAD synthetase; rs12785878), CYP27B1 (1α-hydroxylase gene; rs10877012), and CYP2R1 (cytochrome, P450, family 2, subfamily R, polypeptide 1; rs10741657, rs10766197).

Blood samples were taken from consenting participants in 4-ml tubes containing EDTA anticoagulant. Genomic DNA was...
isolated from peripheral blood leukocytes using the conventional phenol-chloroform extraction method. DNA stock aliquots were diluted to a concentration of 50 ng/ul. Genotyping was performed using the multiplex SNaPshot assay with the ABI 3130XL Genetic Analyzer (Applied Biosystem, CA, U.S.A). The genotyping success rates for the 14 SNPs were all >99% and the concordance rates were >99% based on 10% duplicate samples.

Table 1. Characteristics of participants in two ethnic populations.

| Characteristics                  | Uygur ethnic (n=945) | Kazak ethnic (n=928) | P*  |
|----------------------------------|----------------------|----------------------|-----|
|                                  | M (P25, P75)         | M (P25, P75)         |     |
| Clinical and anthropometric measures |                      |                      |     |
| Age (years)                      | 44.0 (40.0, 55.0)    | 47.0 (39.0, 56.4)    | 0.059 |
| Female (%)                       | 38.6                 | 59.1                 | 0.001 |
| BMI (Kg/m²)                      | 24.0 (21.9, 27.3)    | 27.5 (24.4, 30.6)    | <0.001 |
| Waist circumference (cm)         | 86.1 (77.0, 96.2)    | 99 (88.0, 107.0)     | <0.001 |
| Systolic BP (mmHg)               | 130.0 (116.0, 140.0) | 139 (125.0, 160.0)   | <0.001 |
| Diastolic BP (mmHg)              | 75.0 (67.0, 82.0)    | 80 (71.0, 90.0)      | <0.001 |
| Hypertension (%)                 | 33.0                 | 56.1                 | 0.001 |
| Obesity (%)                      | 12.1                 | 28.9                 | 0.001 |
| Biochemical measures             |                      |                      |     |
| 25OHD (ng/ml)                    | 10.4 (6.5, 15.2)     | 16.2 (11.8, 20.5)    | <0.001 |
| 25OHD deficiency (%)             | 91.2                 | 72.4                 | 0.001 |
| 25OHD insufficiency (%)          | 5.8                  | 22.7                 | 0.001 |
| 25OHD sufficiency (%)            | 3.0                  | 4.8                  | 0.001 |
| Calcium (mmol/L)                 | 2.3 (2.2, 2.4)       | 2.3 (2.2, 2.4)       | 0.010 |
| Phosphate (mmol/L)               | 1.2 (1.0, 1.3)       | 1.4 (1.2, 1.5)       | <0.001 |
| Alkaline phosphatase (U/L)       | 81.5 (70.5, 94.0)    | 86 (71.0, 105.0)     | 0.075 |
| Fasting glucose (mmol/L)         | 5.3 (5.1, 5.7)       | 4.9 (4.5, 5.3)       | <0.001 |
| Total cholesterol (mmol/L)       | 4.1 (3.4, 4.7)       | 5.1 (4.5, 5.8)       | <0.001 |
| HDL cholesterol (mmol/L)         | 1.0 (0.8, 1.2)       | 1.2 (1.0, 1.5)       | <0.001 |
| LDL cholesterol (mmol/L)         | 2.6 (2.2, 3.0)       | 2.1 (1.7, 2.5)       | <0.001 |
| Triglycerides (mmol/L)           | 1.4 (0.9, 2.2)       | 1.1 (0.7, 1.5)       | <0.001 |
| Urea nitrogen (mmol/L)           | 4.7 (4.0, 5.9)       | 4.1 (3.5, 5.0)       | <0.001 |
| Serum creatinine (umol/L)        | 62.1 (51.0, 75.0)    | 64 (56.0, 74.0)      | 0.045 |

* P values were calculated from ANCOVA for continuous variables and Fisher’s exact test for categorical variables. For definitions of obesity, hypertension and vitamin D status, see Methods.

Definitions

According to the recommendation of the Institute of Medicine, vitamin D deficiency was defined as serum 25OHD <20 ng/ml, vitamin D insufficiency as serum 25OHD level 20–30 ng/ml, and vitamin D sufficiency as serum 25OHD ≥30 ng/ml [17].

Obesity was defined by the World Health Organization [18] as Normal: 18.5 ≤BMI <25 kg/m²; overweight: 25 ≤BMI <30 Kg/m²; and obesity: BMI ≥30 kg/m².

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Table 2. Allele frequencies in 14 SNPs by ethnicity.

| SNP          | chr | Gene   | Alleles | Uygur n=300 | Kazak n=300 | P  |
|--------------|-----|--------|---------|-------------|-------------|----|
| rs7975232    | 12  | VDR    | C/A     | 0.42        | 0.42        |    |
| rs731236     | 12  | VDR    | G/A     | 0.19        | 0.20        |    |
| rs2239179    | 12  | VDR    | C/T     | 0.32        | 0.34        |    |
| rs1544410    | 12  | VDR    | G/A     | 0.20        | 0.23        |    |
| rs2228570    | 12  | VDR    | G/A     | 0.32        | 0.34        |    |
| rs12717991   | 12  | VDR    | C/T     | 0.45        | 0.47        |    |
| rs11168275   | 12  | VDR    | C/T     | 0.39        | 0.42        |    |
| rs4588       | 4   | GC     | G/T     | 0.22        | 0.26        |    |
| rs2282679    | 4   | GC     | G/T     | 0.23        | 0.26        |    |
| rs7041       | 4   | GC     | C/A     | 0.34        | 0.43        |    |
| rs10741657   | 11  | CYP2R1 | G/A     | 0.45        | 0.40        |    |
| rs10766197   | 11  | CYP2R1 | G/A     | 0.41        | 0.36        |    |
| rs10877012   | 12  | CYP2B1 | G/T     | 0.37        | 0.47        |    |
| rs12785878   | 11  | DHCR7/NADSYN1 | G/T | 0.46 | 0.46 | 0.057 |

Alleles – Allele major allele/minor allele; MAF – minor allele frequency. HWE P-values for Hardy-Weinberg Equilibrium test. P chi-square test of MAF between Uygur and Kazak ethnic group. Bold numbers represent significant P-values.

Hypertension was identified from a self-reported questionnaire and the clinical data measured by the investigators. The diagnosis meets at least 1 of 3 criteria: systolic BP (SBP) ≥140 mmHg; diastolic BP (DBP) ≥90mmHg, or using anti-hypertension medications [19].

Statistical analysis

For database management and statistical analyses, we used the IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY). Data are given as median (interquartile range) for continuous variables or percentages (%) for categorical variables. Median and proportions were compared with ANCOVA and Fisher’s exact test, respectively. Post-hoc Bonferroni correction was used for multiple comparisons. Logistic regression models were used to investigate the risk factors associated with vitamin D deficiency (<20 ng/ml). We performed separate analyses for Uygurs and Kazaks because of differences in linkage disequilibrium (LD), allele frequencies, and biological and environmental factors contributing to serum 25OHD levels. For logistic regression models, we adjusted for age, sex, BMI, and study site. Deviation from Hardy-Weinberg equilibrium was assessed by the Pearson χ² test. A 2-sided probability value ≤0.05 was considered statistically significant.

Results

The study population (1873 in total) comprised 945 Uygurs and 928 Kazaks. The descriptive characteristics of all participants are summarized in Table 1. There were substantial differences in BMI, systolic BP, diastolic BP, hypertension, obesity, fasting glucose, and lipid profiles (all p<0.05) between these 2 ethnic groups; Kazaks had higher BMI and hypertension prevalence than Uygurs. Median serum 25OHD levels was significantly higher in Kazaks than in Uygurs (16.2 ng/ml vs. 10.4 ng/ml, P<0.001). Vitamin D deficiency were more common in Uygurs than in Kazaks (91.2% vs. 72.4%, P=0.001). The majority (97.0%) of Uygur participants were vitamin D insufficient or deficient, with 25OHD levels <20 ng/ml.

The distribution of minor alleles frequencies of each SNP in Uygurs and Kazaks are shown in Table 2. The minor allele frequencies in GC-rs7041 and CYP2B1-rs10877012 were significantly different between these 2 ethnic populations (P<0.05). Hardy-Weinberg equilibrium (HWE) was not met for some SNPs across the Uygur ethnic population (P<0.05). CYP2R1-rs10741657 and CYP2R1-rs10766197 in the Uygur population had HWE<0.05 (Table 2).

The allele distributions in 14 SNPs were similar according to the presence (n=217) and absence of vitamin D deficiency (n=83)
in Uygurs (P>0.05, Table 3). The allele distribution in 14 SNPs were also similar in Kazaks with different vitamin D status. Association between genotypes and 25OHD deficiency are presented in Table 4. In each ethnic population, the genotype frequencies of 7 SNPs in VDR and 3 SNPs in GC were similar in the presence and absence of vitamin D deficiency (P³0.05, Table 4). Multivariable adjusted logistic regression analysis showed that CYP2R1-rs10766197 (A/G) was a significant risk factor for the presence of vitamin D deficiency in Uygurs (P=0.011, OR=2.442, 95%C.I.: 1.224–4.873) and DHCR7/NADSYN1-rs12785878 (T/G) was a significant risk factor for the presence of vitamin D deficiency in Kazaks (P=0.011, OR=6.533, 95%C.I.: 1.361–31.357).

### Table 3. Allele distributions according to the status of vitamin D in Uygur and Kazak ethnics.

| SNP       | Gene   | Alleles* (1/2) | group | Uygur  | Kazak  | p**  |
|-----------|--------|----------------|-------|--------|--------|------|
|           |        |                | 1     | 2      | 1      | 2    |      |
| rs7975232 | VDR    | C/A            | Case  | 0.58   | 0.42   | 0.77 | 0.59 | 0.41 | 0.40 |
|           |        |                | Control | 0.59 | 0.41 | 0.56 | 0.44 |
| rs731236  | VDR    | G/A            | Case  | 0.80   | 0.20   | 0.83 | 0.81 | 0.19 | 0.27 |
|           |        |                | Control | 0.78 | 0.22 | 0.72 | 0.27 |
| rs2239179 | VDR    | C/T            | Case  | 0.66   | 0.34   | 0.75 | 0.66 | 0.34 | 0.76 |
|           |        |                | Control | 0.69 | 0.31 | 0.74 | 0.29 |
| rs154410  | VDR    | G/A            | Case  | 0.77   | 0.23   | 1.00 | 0.79 | 0.21 | 0.39 |
|           |        |                | Control | 0.72 | 0.28 | 0.75 | 0.25 |
| rs2228570 | VDR    | G/A            | Case  | 0.66   | 0.34   | 0.35 | 0.69 | 0.31 | 0.53 |
|           |        |                | Control | 0.75 | 0.25 | 0.75 | 0.25 |
| rs12717991| VDR    | C/T            | Case  | 0.52   | 0.48   | 0.67 | 0.69 | 0.31 | 0.68 |
|           |        |                | Control | 0.56 | 0.44 | 0.56 | 0.44 |
| rs11168275| VDR    | C/T            | Case  | 0.58   | 0.47   | 0.66 | 0.64 | 0.36 | 0.56 |
|           |        |                | Control | 0.72 | 0.28 | 0.75 | 0.25 |
| rs4588    | GC     | G/T            | Case  | 0.73   | 0.27   | 0.27 | 0.76 | 0.24 | 0.85 |
|           |        |                | Control | 0.88 | 0.12 | 0.80 | 0.20 |
| rs2282679 | GC     | G/T            | Case  | 0.72   | 0.28   | 0.75 | 0.69 | 0.31 | 0.70 |
|           |        |                | Control | 0.88 | 0.12 | 0.80 | 0.20 |
| rs7041    | GC     | C/A            | Case  | 0.58   | 0.42   | 0.77 | 0.66 | 0.34 | 0.88 |
|           |        |                | Control | 0.53 | 0.47 | 0.56 | 0.44 |
| rs10741657| CYP2R1 | G/A            | Case  | 0.61   | 0.39   | 0.56 | 0.58 | 0.42 | 0.05 |
|           |        |                | Control | 0.53 | 0.47 | 0.56 | 0.44 |
| rs10766197| CYP2R1 | G/A            | Case  | 0.63   | 0.37   | 0.88 | 0.57 | 0.43 | 0.62 |
|           |        |                | Control | 0.67 | 0.33 | 0.62 | 0.38 |
| rs10877012| CYP27B1| G/T            | Case  | 0.52   | 0.48   | 0.77 | 0.64 | 0.30 | 0.88 |
|           |        |                | Control | 0.62 | 0.38 | 0.62 | 0.38 |
| rs12785878| DHCR7/ NADSYN1 | G/T  | Case  | 0.60   | 0.40   | 0.46 | 0.60 | 0.40 | 0.79 |
|           |        |                | Control | 0.66 | 0.34 | 0.61 | 0.39 |

* The major allele was referred to as allele 1 and the minor allele as allele 2. ** The p-value of alleles was calculated using Fisher’s exact test to compare the participants with vitamin D deficiency and those without in the same ethnic population. Vitamin D deficiency was defined as serum 25OHD <20 ng/ml. Case: participant with vitamin D deficiency, control: participant without vitamin D deficiency.

Discussion

Some recent studies have shown that vitamin D status has ethnic specificity, and the allele frequency in “vitamin D-associated SNPs” may influence the circulating 25OHD levels [9–11,20,21]. However, little information is available about the vitamin D status and the impact of these SNPs frequency on 25OHD levels in Uygur and Kazak ethnic populations. Our study found that...
| SNP       | Gene | Alleles* (1/2) | Group | Uygur | Kazak |       |       |
|-----------|------|----------------|-------|-------|-------|-------|-------|
| rs7975232 | VDR  | C/A            |       |       |       |       |       |
|           |      | Case           | 0.35  | 0.46  | 0.19  | 0.685 | 0.532 |
|           |      | Control        | 0.31  | 0.56  | 0.13  | 0.25  | 0.13  |
|           |      |                |       |       |       |       |       |
| rs731236  | VDR  | G/A            |       |       |       |       |       |
|           |      | Case           | 0.64  | 0.34  | 0.02  | 0.948 | 0.930 |
|           |      | Control        | 0.63  | 0.31  | 0.06  | 0.64  | 0.04  |
| rs2239179 | VDR  | C/T            |       |       |       |       |       |
|           |      | Case           | 0.44  | 0.47  | 0.09  | 1.379 | 0.581 |
|           |      | Control        | 0.50  | 0.38  | 0.12  | 0.53  | 0.10  |
| rs1544410 | VDR  | G/A            |       |       |       |       |       |
|           |      | Case           | 0.59  | 0.36  | 0.05  | 1.151 | 0.815 |
|           |      | Control        | 0.63  | 0.31  | 0.06  | 0.64  | 0.04  |
| rs2228570 | VDR  | G/A            |       |       |       |       |       |
|           |      | Case           | 0.42  | 0.47  | 0.11  | 0.442 | 0.167 |
|           |      | Control        | 0.31  | 0.69  | 0.00  | 0.48  | 0.15  |
| rs12717991| VDR  | C/T            |       |       |       |       |       |
|           |      | Case           | 0.27  | 0.53  | 0.20  | 2.251 | 0.20  |
|           |      | Control        | 0.38  | 0.38  | 0.24  | 0.32  | 0.21  |
| rs11168275| VDR  | C/T            |       |       |       |       |       |
|           |      | Case           | 0.35  | 0.47  | 0.18  | 0.787 | 0.704 |
|           |      | Control        | 0.31  | 0.44  | 0.25  | 0.42  | 0.16  |
| rs4588    | GC   | G/T            |       |       |       |       |       |
|           |      | Case           | 0.55  | 0.37  | 0.08  | 1.60  | 0.465 |
|           |      | Control        | 0.75  | 0.25  | 0.00  | 0.66  | 0.06  |
| rs2282679 | GC   | G/T            |       |       |       |       |       |
|           |      | Case           | 0.55  | 0.36  | 0.09  | 1.590 | 0.469 |
|           |      | Control        | 0.75  | 0.25  | 0.00  | 0.66  | 0.06  |
| rs7041    | GC   | C/A            |       |       |       |       |       |
|           |      | Case           | 0.32  | 0.53  | 0.15  | 1.812 | 0.406 |
|           |      | Control        | 0.25  | 0.31  | 0.44  | 0.40  | 0.10  |
| rs10741657| CYP2R1| G/A           |       |       |       |       |       |
|           |      | Case           | 0.37  | 0.50  | 0.13  | 0.648 | 0.501 |
|           |      | Control        | 0.25  | 0.56  | 0.19  | 0.25  | 0.13  |
| rs10766197| CYP2R1| G/A           |       |       |       |       |       |
|           |      | Case           | 0.39  | 0.48  | 0.13  | 6.533 | 0.019 |
|           |      | Control        | 0.69  | 0.12  | 0.19  | 0.32  | 0.17  |
| rs10877012| CYP27B1| G/T          |       |       |       |       |       |
|           |      | Case           | 0.30  | 0.46  | 0.24  | 0.870 | 0.818 |
|           |      | Control        | 0.31  | 0.63  | 0.06  | 0.42  | 0.14  |
| rs12785878| DHCR7/| G/T           |       |       |       |       |       |
|           | NADSYN1|              |       |       |       |       |       |
|           |      | Case           | 0.38  | 0.44  | 0.18  | 0.903 | 0.859 |
|           |      | Control        | 0.38  | 0.56  | 0.06  | 0.23  | 0.22  |

**OR** – odds ratio, 95%CI – 95% confidence intervals. * The major allele was referred to as allele 1 and the minor allele as allele 2. ** The p-value of alleles was calculated using Fisher’s exact test to compare the participants with vitamin D deficiency and those without. # OR estimated by logistic regression analysis, adjusted for gender, age, BMI and study site. Bold numbers represent significant P-values. Vitamin D deficiency was defined as serum 25OHD <20 ng/ml. Case: participant with vitamin D deficiency, control: participant without vitamin D deficiency.
the incidence of vitamin D insufficiency is astonishingly high in Uygur and Kazak adults residing in Xinjiang, China. The median 25OHD concentration is as low as 10.4 ng/ml in Uygurs and 16.2 ng/ml in Kazaks. In Uygurs, the prevalence of vitamin D deficiency, in-sufficiency, and sufficiency is 91.2%, 5.8%, and 3.0%, respectively. Furthermore, it showed that MAFs in GC-rs7041 and CYP27B1-rs10877012 is significantly different between Uygurs and Kazaks. CYP2R1-rs10766197(A/G), DHC7/NADSYN1-rs12785878(T/G) is significantly associated with 25OHD deficiency in Uygur and Kazak ethnic populations.

Xinjiang province in north-western China is located at 34° to 48° N latitude and has a long winter. There are 10.53 million Uygurs and 1.48 million Kazaks in Xinjiang. Although Uygur and Kazak ethnic populations have much lower vitamin D levels than other ethnic populations [22–25], vitamin D levels in Uygurs are even lower than in Kazaks [11]. In ethnic Chinese Han, the average concentration of 25OHD is 26.9 ng/ml, much higher than that in Uygurs and Kazaks [26]. The phenomena may be due to the following reasons. First, Uygurs and Kazaks geographically reside in areas with less sunlight exposure time. Kashgar, where most Uygurs reside, is at near 39° N latitude and has approximately 73 sunny days per year. Second, the Uygur people prefer to wear long trousers and long sleeves, as required by their culture. This habit may further reduce their sunlight exposure. Third, low vitamin D content in food and little vitamin D medical supplementation might exacerbate the situation.

VDR directly mediates the hormonal effects of 1, 25(OH)₂D₃ and there is some evidence showing that VDR-rs2228570 is associated with higher risk of multiple sclerosis [13,14] and female breast and reproductive system cancer [27–29]. Therefore, the genetic variation in VDR may influence the vitamin D levels. The association between VDR and plasma vitamin D levels has not been investigated in Uygur and Kazak ethnic populations. Our study showed that there is no significant association between common genetic variants (rs7975232, rs731236, rs2239179, rs1544410, rs2228570, rs12717991, rs11168275) and vitamin D deficiency, which is consistent with other previous studies [30].

GC, encoding vitamin D-binding protein (DBP), is a key transporter for vitamin D and its metabolites (including 25OHD and 1,25(OH)₂D₃) in circulation [31]. Recent studies have reported an association between SNPs in this gene and 25OHD concentrations [25,32]. GC-rs7041 and rs4588 variants (in exon 11) leads to a Glu/Asp amino acid change at codon 416 and a Thr/Lys amino acid change at codon 420 [33]. They are reported to be consistently associated with lower levels of 25OHD. However, we did not find any significant association between GC-rs2282679, rs4588 and rs7041 with vitamin D deficiency. The possible explanations for these discrepancies include: (1) The significant association between vitamin D levels and the allele frequency of GC SNPs may only exist in some specific ethnic population [10,11]. For example, GC-rs7041 polymorphism was associated with 25OHD levels in Arab and South Asian populations, but not in South East Asians [21]. A recent study, including 506 Northeastern Han Chinese children, did not find any significant association between GC-rs2282679, rs4588 and rs7041 and 25OHD levels as well [34]. (2) Total 25OHD concentration, measured in our clinical assays is composed of GC-bound fraction and free fraction. It is possible that the SNPs in GC may influence GC-bound fraction, not total 25OHD levels.

CYP2R1 is a microsomal vitamin D hydroxylase that hydroxylates vitamin D at the 25-C position for 25OHD synthesis (calcidiol) in the liver. We found that CYP2R1-rs10766197 is significantly associated with Vitamin D deficiency in the Uygur population, but not in the Kazak population. CYP2R1-rs10741657 is not related with vitamin D deficiency in Uygurs or Kazaks. A genome-wide association study, including 30 000 individuals of European descent, found that CYP2R1-rs10741657 was significantly associated with 25OHD levels [20]. This association was also confirmed by another study, which recruited 745 healthy white subjects [35]. The difference between these studies may reflect the ethnic variation.

Gene DHC7/NADSYN1 encodes 7-dehydrocholesterol (7DHC) reductase, which catalyzes 7DHC into cholesterol, providing substrate for vitamin D synthesis [20]. Our study found that DHC7/NADSYN1-rs12785878 was significantly associated with vitamin D deficiency in the Kazak ethnic population. Our findings are in line with several [20,36], but not all, studies. Recently, Zhang et al. linked DHC7/NADSYN1 SNPs (rs3829251, rs12785878) to decreased serum 25OHD levels in Han Chinese children [34], and Cooper et al. linked rs12785878 to vitamin D deficiency in whites [37]. However, a study of 1549 individuals (Arabs, South Asians, and Southeast Asians living in Kuwait) did not find any association between genotypes of DHC7/NADSYN1 (rs7944926, rs12785878, rs4944957, rs12800438, rs3794060, and rs3829251) and serum 25OHD levels in any of the 3 population groups [21].

Our study has some limitations. First, we selected only 5 candidate genes containing 14 SNPs that have been shown in previous GWAS reports to have an association with vitamin D level and are known to have a biological impact in vitamin D metabolism. Second, our study had a smaller sample size compared to the other studies. Despite of these limitations, our study has several advantages. The study uncovered a severe vitamin D deficiency in the Uygur and Kazak ethnic populations, who live in places without sufficient sunlight exposure. Furthermore, we revealed, for the first time, that polymorphisms in CYP2R1-rs10766197, DHC7/NADSYN1-rs12785878 were significantly associated with vitamin D deficiency in Uygurs and Kazaks.
Conclusions

Vitamin D deficiency is highly prevalent in Uygur and Kazak ethnic populations living in Xinjiang, China, indicating that, dietary or medically, vitamin D supplementation is necessary. Furthermore, CYP2R1 -rs10766197, DHCR7/NADSYN1 -rs12785878 SNPs is coupled with 25OHD deficiency in Uygur and Kazak populations, reflecting ethnic gene variation in vitamin D metabolism.

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Competing interests

The authors declare that they have no competing interests.
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