Associations of vitamin D binding protein variants with the vitamin D-induced increase in serum 25-hydroxyvitamin D

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Associations of vitamin D binding protein variants with the vitamin D-induced increase in serum 25-hydroxyvitamin D.

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Running title: Vitamin D, 25(OH) D, supplementation, DBP, gene-diet interaction.

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Abstract

Background: Vitamin D deficiency is a global problem that may be improved by vitamin D supplementation; however, the individual's response to the intervention varies. We aimed to investigate possible genetic factors that may modify the impact of environmental exposure on vitamin D status. The candidate gene variant we investigated was the Gc gene-rs4588 polymorphism at the vitamin D receptor (DBP) locus.

Methods: A total of 619 healthy adolescent Iranian girls received 50000 IU of vitamin D_3 weekly for 9 weeks. Serum 25(OH) D concentrations, metabolic profiles and dietary intake were measured at baseline and after 9 weeks of supplementation. The genotypes of the DBP variant (rs4588) were analyzed using the TaqMan genotyping assay.

Results: Our results revealed that the rs4588 polymorphism might be associated with serum 25-hydroxy vitamin D both at baseline (p value=0.03) and after intervention (p value=0.008). It seemed that the outcome of the intervention was gene-related so that the subjects with common AA genotype were a better responder to vitamin D supplementation (Changes (%) 469.5(427.1) in AA carriers vs. 335.8(530) in GG holders), and carriers of the less common GG genotype experienced a rise in blood glucose after 9 weeks (Changes (%) 0 (1.5)). Our findings also showed that the statistical interaction between this variant and supplementation was statistically significant (intervention effect p-value<0.001 and p-value SNP effect=0.03). The regression model also revealed that after adjusted for potential confounders, likelihood of affecting serum 25(OH)D in individuals who were homozygous for the uncommon allele G was less than those homozygous for the more common AA genotype (OR=4.407 (1.82-8.89); p=0.001).

Conclusion: Serum vitamin 25(OH) D following vitamin 25(OH) D_3 supplementation appears to be modified by genetic background. The Gc genetic variant, rs4588 encoding the vitamin D receptor seems to influence the response to vitamin D supplementation.

Key words: Total 25(OH) D, Supplementation, Gc gene, rs4588.

Introduction

In addition to its classical functions in bone and mineral metabolism, vitamin D has several roles in the human body including modulation cell proliferation, differentiation, apoptosis and immune function. Growing body of evidence has revealed associations between vitamin D deficiency and several health outcomes. The vitamin D receptor is expressed in numerous tissues [1], and there is a relationship between low serum 25(OH)D levels and risk of chronic diseases including cardiovascular disease, diabetes, and cancer [2]. The major sources of vitamin D in humans are dermal synthesis with U.V. exposure and dietary intakes such as oily fish and dairy products. Therefore, subjects with vitamin D deficiency need supplementation if the deficiency cannot be adequately corrected by changes in lifestyle such as more outdoor activities or a vitamin D rich diet. However, it has been shown that the serum 25(OH)D level also depends on the interaction between environmental and genetic factors [3, 4]. Several family and twin studies investigated the contribution of genetic background in association with variance in serum vitamin D, estimating heritability of serum vitamin D vary between 23% and 80 %[5].Various SNPs affect the serum 25(OH) D levels produced by the skin or received from the diet, it seems that they could also influence serum 25(OH) D level following supplementation. If so, it might be necessary to take genetic factors into account when recommending vitamin D supplementation.

The vitamin D binding protein (DBP), originally known as the group-specific component (Gc-globulin), is a multifunctional protein in an ascetic fluid, plasma, the cerebrospinal fluid

that also found on the surface of numerous cells. It binds to the different forms of vitamin D (ergocalciferol (D2) and cholecalciferol (D3), the 25-hydroxylated forms (calcifediol, and the active product, 1,25-dihydroxy vitamin D (calcitriol)). In the human body, the DBP is the major blood transporter of vitamin D [6] and is also a Macrophage Activating Factor (MAF) that has been a target for cancer treatments [7]. The main genetic variations (Gc1S, Gc1F and Gc2) are associated with differences in circulating 25(OH) D3 levels [3]. It appears that they may be determinants of vitamin D status in different genetic backgrounds [8]; moreover, several studies have shown a relationship between these variants and the response to vitamin D supplementation[9-11]. In addition, recently emerging evidence from gene-diet interaction analyses in large-scale observational studies and randomized intervention trials favour the idea that complex diseases may be due to interactions between lifestyle (e.g. diet) and genetic make-up [12]. However, this field is in its infancy and supporting data are still sparse; and little of the knowledge about gene-diet interaction has been applied in public health practice. The aim of the present study was to evaluate the influence of DBP variants on responding to vitamin D_3 supplementation

Material and method

The 619 girls aged 12-17 years old were recruited between January and April 2015 in Mashhad city, by a randomized cluster sampling method. Informed consent was collected from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences. Participants with chronic diseases history, or who were taking any kinds of dietary supplements and anti-depressant or psychotropic drugs were excluded from the study. Subjects received 50,000 IU vitamin D_3/week over 9 weeks. The total 25(OH) D in serum and metabolic profiles were measured at the baseline and after the intervention (fig.1).

Anthropometric and biochemical measurements

Anthropometric parameters (e.g., height, body weight) and blood pressure were measured by trained technicians in both phases. With the metric system, the formula for BMI is weight in kilograms divided by height in meters squared. Weight and Height were measured by standardized procedures. A portable stadiometer was used so as to measure height (OTM, Tehran, Iran), being taken to the nearest 0.1 cm, no shoes, subjects stretching to the maximum height while the head positioned in the Frankfort line. For weight measuring, Rassa weight scale (Rassa, Tehran, Iran) was used to the nearest 100 gram while subjects had no shoes and wore light clothes.

Biochemical markers including serum high sensitivity C-reactive protein (Hs-CRP), fasting blood glucose (FBG) and lipid profile; total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were evaluated as described previously[13, 14]. The total serum 25(OH) vitamin D level (the sum of 25OHD2 and 25OHD3) was determined using an electrochemiluminescence method (ECL, Roche, Basel, Switzerland). Subjects were categorized based on serum 25(OH)D into 3 groups: Deficient group: Serum 25(OH)D level<50nmol/L; Sufficient group: 50nmol/L to <75nmol/L; Desirable one >75nmol/L[15].

Dietary and physical activity assessment

We used a validated food frequency questionnaire in order to assess dietary intakes [16]. To analysis energy and other nutrient intakes, we converted the reported portion size in FFQ to grams using household measures and then were introduced to the Nutritionist IV software. Moreover, the level of physical activity was studied by a validated questionnaire [16] and

reported as metabolic equivalents (METs) in hours per day. Demographic data, sun exposure and use of sunscreen were collected by an expert interviewer and by the use of a standard questionnaire[17].

DNA extraction and genotyping

Genomic DNA was extracted from EDTA blood samples using QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) according to the manufacturer's instructions. The purity and concentration of DNA samples were determined using the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of CYP2R1-rs10766197 polymorphism was carried out using Taq-man®-probes-based assay; PCR reactions were performed in 12.5 ml total volume, using 20 ng of DNA in TaqMan®n Universal MasterMix with specific primers and probes (Applied Biosystems Foster City, CA). To assess the allelic content, an ABIPRISM-7500 instrument equipped with the SDS version-2.0 software was used.

Statistical analysis

Data was analyzed using SPSS version 20, IBM (SPSS Inc., IL, USA). Variables are reported as the mean ± standard deviation (SD). Continuous variables were analyzed for normality using the Kolmogorov–Smirnov test. Analysis of variance (ANOVA) was performed to compare changes in biomarkers after intervention in different genotype groups. Post hoc analysis was done using Tukey's test. A Chi-square test with continuity correction was used to determine whether genotype frequencies followed the Hardy–Weinberg Equilibrium. Repeated measures analysis of covariance (ANCOVA) was performed to investigate the effect of the genotypes. Logistic regression also was performed to study the probability of change in serum 25(OH) D in different genetic models. For dietary analysis, all dietary variables were adjusted for the total energy intake using a residual model [32]. We

158 considered age, BMI, physical activity, sun exposure, and passive smoking, dietary intakes
159 (energy, vitamin D and polyunsaturated fatty) acid as potential confounders. Significance was
160 set at $p < 0.05$.

161 **Results**

162 *Influences of supplementation on circulation 25(OH)D in relation to the DBP gene*
163 *variant*

164 To examine the effect of DBP variant on the serum levels of the total 25-hydroxy vitamin D
165 after the intervention, subjects were categorized across rs4588 genotypes. The results
166 demonstrated a significant trend in the distribution of vitamin D status (desirable, sufficiency
167 and deficiency) among different genotypes at baseline and after supplementation P-trend =
168 0.03 and 0.008 respectively (Table 1). As shown the serum 25(OH) D response depended on
169 the SNP in DBP (Table. 1 and 2). Our data revealed that after adjusting for potential
170 confounders including age, BMI and physical activity, passive smoking, energy intake,
171 dietary intake of vitamin D and poly-unsaturated fatty acid, sun exposure the SNP rs4588
172 could modulate response to vitamin D supplementation (p-value of intervention effect <0.001
173 and p-value SNP=0.03) (Fig. 1). Accordingly, serum 25(OH) D increased in all genotype
174 groups, but carriers who had the common AA genotype had higher 25-hydroxy vitamin D
175 concentrations after 9 weeks of intervention. The regression model also indicated that the
176 probability of rise in serum 25(OH) D in individuals who had homozygous rare genotype GG
177 was 4.407-fold less than carriers of the common AA genotype (after adjusted for
178 confounders) (OR=4.407(1.82-8.89), 0.001) (table 3). The regression analysis was also
179 significant in unadjusted model (OR=3.72(1.94-7.139),<0.001). Potential confounders were
180 age, BMI, physical activity and passive smoking, energy intake, dietary intake of vitamin D
181 and poly-unsaturated fatty acid, sun exposure.

Influence of supplementation on metabolic profile in DBP variant

Further analysis showed that although fasting blood glucose fell in carriers of common A allele, it rose in individuals with GG genotype (before intervention 88.7±9.6 mg/dl and after intervention= 91.04±14.3 mg/dl) (Table 2). Furthermore, serum 25-hydroxy vitamin D increased in all genotypes after supplementation, and as data showed elevation was more significant in AA common genotype %469.5(427.1).

Discussion

Influence of supplementation on circulation 25(OH) D in DBP variant

The aim of the current trial study was to examine the response to supplementation with respect to a genetic variant of the DBP locus in a healthy group of Iranian girl adolescences. The results demonstrated that this polymorphism was statistically related to serum 25-hydroxy vitamin D at both baseline and follow-up. We found that intake of 50000 IU/D vitamin D_3 per week had beneficial effects on the total 25(OH)D circulation in all genotype groups. However, the carriers of the common AA genotype were better responders to vitamin D supplementation on the basis of elevation serum 25(OH)D.

Genome-wide association studies (GWAS) have demonstrated associations between various single-nucleotide polymorphisms (SNPs) and the vitamin D metabolism pathway with serum 25(OH)D level [18]. Several of these SNPs have been associated with the elevating serum 25(OH)D_3 in response to the vitamin D supplementation [10, 19]. In a cohort of Chinese individuals, it was demonstrated that DBP variants including rs1155563, rs2282679, T436K and D432E were statistically related to a lower level of serum 25(OH) D_3[20]. Similarly, results of a cross-sectional study on Danish Caucasian population suggested DBP phenotype as an independent predictor of serum 25(OH)D_3 [21]. In two independent European studies, it was shown that there was a significant association between SNP rs2282679 with 25(OH)

D3 concentrations [18]. Moreover, Fu et.al examined 436KK in a healthy population, they also suggested 436KK homozygosity influenced the level of serum 25-hydroxy vitamin D at baseline and also modified the response to vitamin D3 supplementation [22].

Influence of supplementation on fasting blood glucose in DBP variant

We also showed that fasting blood sugar increased in the carriers of uncommon GG genotype after intervention by vitamin D supplementation while this parameter was dropped in individuals who had a common A allele. However, although the variable of "percentage of change after intervention" was not statistically significant. It appears that clinical outcome in response to vitamin D supplementation was gene-dependent.

Emerging evidence has reported a relationship between vitamin D supplementation and clinical diabetes. The functions of vitamin D on the treatment of pre-diabetes and diabetes through anti-inflammatory mechanisms and improving insulin sensitivity have been proposed in emerging evidence. Therefore, genetic variants in the DBP gene locus appear to have an impact on FBG and diabetes. Proteomic investigations have reported DBP as a hepatic acute phase reactant that in diabetes it would be upregulated [23]. However, the results from various ethnicities were inconsistent. Studies on Japanese and Indian population supported our finding. They revealed that DBP variants influenced fasting plasma insulin levels in normal glucose tolerance[24, 25]. In Japanese with Gc1S-2 and Gc1S-1S, fasting plasma insulin was higher than in those who were homozygotes for Gc1F. Furthermore, in a cohort study by Baier on non-diabetics, results revealed that exon 11 polymorphisms in this locus were correlated with blood glucose responses to oral glucose; however, they found no association with fasting glucose plasma and insulin levels [26]. Another study on Hispanic and Caucasian individuals revealed no associations between DBP variants and levels of

insulin [27]. Various cohorts and cross sectionals have failed to show a strong association between DBP and diabetics outcome.

Conclusion

We have found that Gc-rs4588 was could modify responses to high dose vitamin D supplementation. We also revealed that clinical outcome of supplementation may be gene-related so that in the carriers of the rare homozygous genotype of rs4588, fasting blood glucose increased after the intervention.

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Table 1. Vitamin D status before and after vitamin D supplementation according to DBP genotypes.

Vitamin D status (N=619)	AA		AG		GG	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Desirable	25 (6.9)	200 (65.8)	13 (6)	101 (58.4)	1 (2.5)	8 (33.3)
Sufficiency	27 (7.5)	56 (18.4)	14 (6.5)	33 (19.1)	0 (0)	6 (25)
deficiency	310 (85.6)	48 (15.8)	189 (87.5)	39 (22.5)	39 (97.5)	10 (41)

Note: Σ2 test showed a P_{trend} of 0.03 at baseline; P_{trend} at follow-up is **0.008**. Data is presented as frequencies (%). Deficiency: Serum 25(OH)D level < 50 nmol/l. Sufficiency: Serum 25(OH) D level between 50 to 75 nmol/l. Desirable: Serum 25(OH)D level > 75 nmol/l.

Table 2. Comparisons of the variables before and after 25-hydroxyvitamin D3 supplementation in different genetic modes.

Variable		AA (N=362)	AG (N=216)	GG (41)	*P-value*
Anthropometric					
BMI (kg/m^2)	Baseline	21.7±3.9	21.9±4.4	21.6±4.0	0.1
	Follow-up	21.4±4.4	21.7±4.4	21.5±4.2	0.12
	Change (%)	0(-4.2)	0(-3.8)	0(-2.7)	0.7
Blood pressure					
SBP (mmHg)	Baseline	101.0±12.5	101.3±13.3	100.8±11.9	0.38
	Follow-up	100.4±13.0	100.3±13.3	101.5±10.4	0.57
	Change (%)	0(-11.3)	0(-18.3)	0(-19)	0.8
DBP(mmHg)	Baseline	67.9±9.6	67.1±9.7	68.1±11.4	0.1
	Follow-up	64.4±10.8	64.8±10.3	65±10.0	0.6
	Change (%)	0(-20)	0(-22)	0(-23)	0.18
Lipid profile					
Cholesterol (mg/dl)	Baseline	165.1±28.7	162.5±28	156.1±23.1	0.2
	Follow-up	155.6±27.0	152.1±29.7	148.3±16.6	0.4
	Change (%)	-6.3(-16)	-6.5(-16.2)	-6(-17.4)	0.2
TG (mg/dl)	Baseline	83.7±36	81.4±34.3	76.3±26.8	0.8
	Follow-up	81.5±35	77.1±32.3	81.7±35	0.1
HDL(mg/dl)	Baseline	47.91±8.8	46.8±9	46.5±7.9	0.5
	Follow-up	45.8±8.7	44.5.3±7.8	46.8±8.7	0.4
	Change (%)	-4.2(-14.2)	-3.2(-18.3)	-3.4(-19.7)	0.6
LDL(mg/dl)	Baseline	101.1±23.6	101.5±24	96.6±19	0.8
	Follow-up				0.3
	Change (%)	-10(-28.7)	-14(-22.1)	-10.7(-22.4)	0.1
Serum glucose					
FBG (mg/dl)*	Baseline	88.3±11.1	86.9±9.8	88.7±9.6	0.1
	Follow-up	86.7±11.3	85.2±11.8	91.04±14.3	0.03
	Change (%)	-2.5(-6)	-2.1(-4)	0(1.5)	0.2
Serum metabolite					
Vitamin D** (nmol/L)	Baseline	29.2±26.6	24.1±20.8	15.6±18.7	0.01
	Follow-up	95.6±41.8	83.3±41	63.06±37.5	<0.001
	Change (%)	469.5(427.1)	320.9(433.8)	335.8(530)	0.001

Calcium (mg/dl)	Baseline	9.4±0.5	9.4±0.56	9.0±0.97	0.001
	Follow-up	9.7±0.5	9.7±0.5	9.7±0.5	0.1
	Change (%)	4.4(-0.5)	2.1(8.6)	2.1(9.6)	0.07
Phosphate (mg/dl)	Baseline	3.9±0.40	3.8±0.40	3.8±0.40	0.1
	Follow-up	4.1±0.40	4.0±0.40	4.0±0.38	0.5
	Change (%)	5(12.4)	5(13.4)	4.8(13.8)	0.6
Creatinine (mg/dl)	Baseline	0.65±0.09	0.63±0.09	0.6±0.1	0.5
	Follow-up	0.69±0.08	0.7±0.07	0.68±0.08	0.4
	Change (%)	14.3(16.7)	14.3(16.7)	0 (16.7)	0.6

Abbreviation: BMI, body mass index; SBP, systolic blood pressure, DBP, diastolic blood pressure; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; FBG, fasting blood glucose; WBC, white blood cell; Hs-CRP, high sensitivity reactive protein. Note: Change = ((Follow up – Baseline)/Baseline)/100;

Fig.2.Serum 25(OH) D stratified by a polymorphism in Gc gene-rs4588. Values are means ± std. Repeated measures adjusted for multiple comparisons by Bonferroni test for serum 25(OH) D_3 levels. Covariates used: age, BMI and physical activity, passive smoking, energy intake, dietary intake of vitamin D and poly-unsaturated fatty acid, sun exposure.

Table 3. Association of GC variant- rs4588 with circulation levels of 25(OH) D (N=619).

Genotype	AA	AG	GG
		OR (95% CI), p-value	
Model.1	Reference	1.44(0.975-2.14), 0.07	3.72(1.94-7.139),<0.001
Model.2	Reference	1.48(0.9-2.2), 0.06	3.80(1.95-7.39),<0.001
Mdel.3	Reference	1.40(0.19-2.3), 0.10	4.40(1.82-8.89), 0.001

Model. 1: adjusted for no confounder.
Model.2: adjusted for age, BMI, physical activity, sun protection, passive smoking.
Model.3: adjusted for both confounders in model 2 and vitamin D intake and dietary poly unsaturated fatty acid.

Fig.1. Cohort flowchart

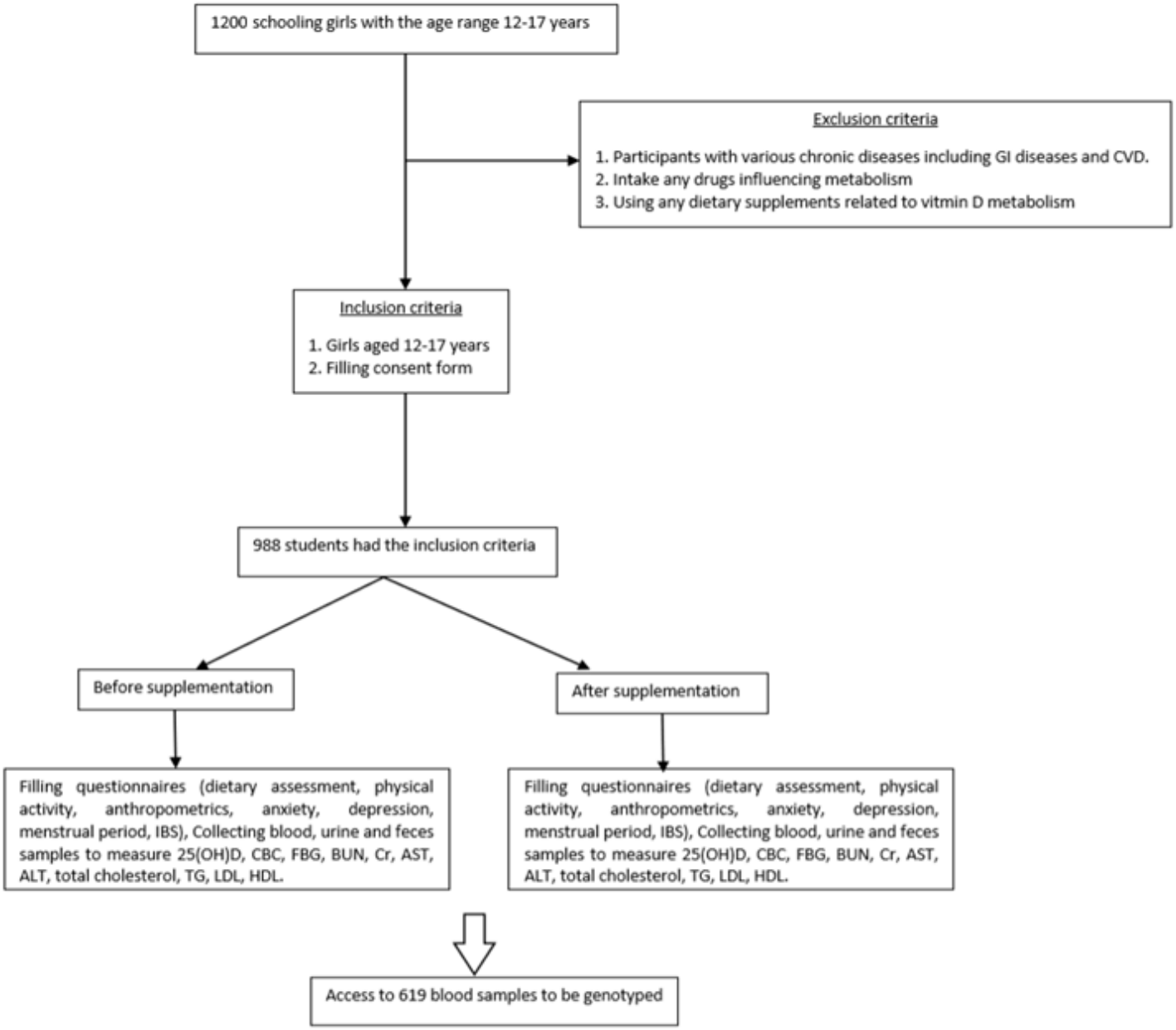
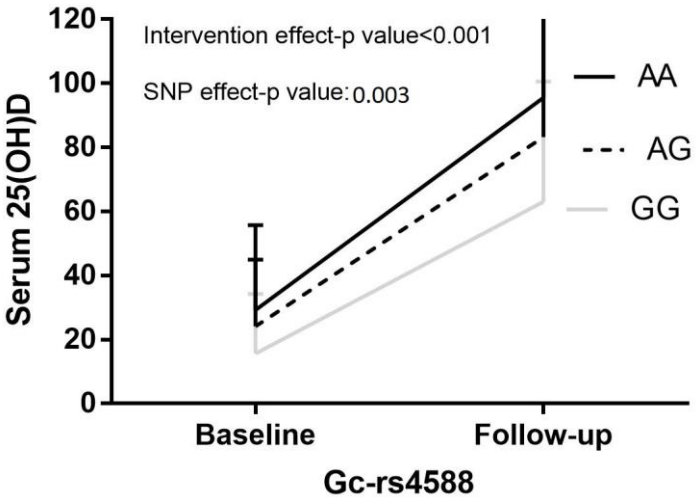


Fig.2.Serum 25(OH) D stratified by a polymorphism in Gc gene-rs4588. Values are means ± std. Repeated measures adjusted for multiple comparisons by Bonferroni test for serum 25(OH) D levels. Covariates used: age, BMI and physical activity, passive smoking sun exposure, vitamin D intake and dietary poly unsaturated fatty acid.


Intervention effect-p value<0.001
SNP effect-p value: 0.003
Serum 25(OH)D
120
100
80
60
40
20
0
Baseline
Follow-up
Gc-rs4588
AA
AG
GG

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