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A variant in CYP2R1 predicts circulating vitamin D levels after supplementation with high-dose of vitamin D in healthy adolescent girls.

Sayyed Saeid Khayyatzadeh[1,2,3,*], Mehrane Mehramiz[1,4,*], Habibollah Esmaeily[5,*], Seyed Jamal Mirmousavi[6], Leila Khajavi[1], Fatemeh Nejati Salehkhani[1], Parichehr Hanachi[7], Hamidreza Bahrami-Taghanaki[9], Saeed Eslami[10], Hasan Vatanparast[8], Gordon A. Ferns[11], Amir Avan[1,4,#] , Majid Ghayour-Mobarhan[1,#]

Affiliations:

1)Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2) Nutrition and food security research centre, Shahid sadoughi university of medical sciences, Yazd, Iran
3)Department of nutrition, faculty of health, Shahid sadoughi university of medical sciences, Yazd, Iran
4) Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5) Department of Biostatistics, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.
6) Community Medicine, Community Medicine Department, Medical School, Sabzevar University of Medical Sciences, Sabzevar, Iran.
7) Department of Biology, Biochemistry Unit, Al Zahra University, Tehran, IR Iran
8) Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
9) Chinese and Complementary Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
10) College of Pharmacy and Nutrition, University of Saskatchewan, Health Sciences E-Wing, Saskatoon, Saskatchewan, Canada.
11) Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.

#**Corresponding authors:**

Majid Ghayour-Mobarhan, MD, PhD, Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Tel: +985118002288, Fax: +985118002287; Email: ghayourm@mums.ac.ir

Amir Avan, PhD, Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +985118002298, Fax: +985118002298; Email: avana@mums.ac.ir & amir_avan@yahoo.com

* Equally contributed as the first author

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Abstract*:*

Aim: The determinants of serum vitamin D seems to be environmental factors (dietary and supplementary intake and exposure to ultraviolet light) and genetic factors. We aimed to study the relationship between a vitamin D-associated genetic polymorphism and serum 25(OH)D concentrations in healthy adolescent girls in Iran, and its effects on a high dose supplement of vitamin D.

Material and method: A total of 616 healthy adolescent girls with mean age 15 received 50000 IU of vitamin D3 weekly over 9 weeks. Serum vitamin D levels and other metabolic factors were measured at baseline and after the intervention. The genotyping of the CYP2R1 variant (rs10741657) was performed by TaqMan genotyping assays.

Results: Regardless of genetic background, at baseline, 87% of adolescent girls were vitamin D deficient (serum 25(OH)D level<50nmol/l). High-dose supplementation with VitD reduced the proportion of girls who were deficient substantially to about 24%. Genetic analysis revealed that although at baseline there was not a gene-vitamin D association (P trend=0.1), the response to supplementation appeared to be modulated by this variant (P trend<0.001). However, other anthropometric and biochemical measures were not affected by this intervention, over this short period. Serum 25(OH)D was increased in all participants although the carriers of the minor A allele seemed to be better responders so that the percentages of change serum vitamin D in the holder of AA and AG genotypes were 539.4±443.1and 443.7±384.6 respectively, compared to those with common GG genotype (363.3±354.0). Our regression analysis revealed that the probability of an increase in serum 25(OH)D in a participant with AA genotype was 2.5 fold greater than those with a GG genotype (OR=2.5 (1.4-4.4); p value=0.002).

Conclusion: Based on our findings, it appears that the rs10741657 variant of the CYP2R1 gene modulates the response to high-dose of vitamin D supplementation.

Keywords: CYP2R1, rs10741657, vitamin D, supplementation

Introduction

In human, ~~the main sources of vitamin D are cutaneous synthesis and diet although it is influenced by other environmental factors including environmental factors and genetic background~~ vitamin D can be synthetize by either the skin, or through dietary intake, such as fatty fish, egg yolk, some mushrooms. Meanwhile, the ultraviolet irradiance at northern latitudes is too low to produce enough vitamin D over the winter season; therefore, the fortified foods with vitamin D and supplements have been the effective ways to receive adequate vitamin D[1]. ~~Since the diet source of vitamin D is rare, fortified foods with vitamin D and supplements have been the effective ways to receive adequate vitamin D~~ [2]. However, Vitamin D deficiency is a widespread public health problem globally. This issue is related to clinical complications such as autoimmune diseases, various cancers, obesity, cardiovascular disorders, and metabolic syndrome and even pregnancy outcome. ~~Currently, serum 25-hydroxyvitamin D concentrations have been used to determine vitamin D status, but due to lack of the accuracy in the diagnostic essay and the lack of reference standard, this bio-factor is under questioned. However, the scientists cannot yet reach a consensus on the healthy range of serum 25-hydroxyvitamin D concentrations in various population groups.~~ Growing bodies of evidence suggested the influences of environmental and genetic background on vitamin D variation in people. Some studies have reported an inverse association between body mass index (BMI) and variation in serum 25(OH)D level 3, 4, suggesting volumetric dilution, storage of vitD and up-regulation of the vitamin D receptor (VDR) in the adipose tissue might lead to lower response to vitamin D intake in obese people4, 5 ; however, the results have been controversial6, 7. Moreover, An age-related reduction in renal faction and also calcium absorption leads to declining in 1,25(OH)2D8, 9.On the other hand, studies on twins and their families have revealed heritability of the serum vitamin D levels. Additionally, emerging evidence has studied the genetic locus related to this hormone. Recently, several genetic determinants of circulating

vitamin D have been suggested, including Gc, CYP2R1 and CYP24A1, VDR, DHCR1 [10]. CYP2R1 accounts for the hydroxylation of vitamin D in the first stage of vitamin D activation[11] and researchers have attached importance to gene variants regarding vitamin D status [10, 12, 13]. The current study was carried out to determine the potential effect of the rs10741657 polymorphism located on chromosome 11p15.2, in terms of responding to high-dose vitamin D supplementation in 616 healthy Iranian girls suffering from vitamin D deficiency.

Material and method

Study population

A cohort of 616 adolescent girls, with average age 15 years old, were recruited by a randomized cluster sampling method[14]. The study ran between January and April 2015 in Mashhad city, and consent forms were filled by all participants according to protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences. The exclusion criteria were a history of the various chronic disease, receiving any kind of dietary supplementation, anti-depressant or psychotropic drugs. Subjects received 50,000 IU vitamin D/week for 9 weeks.

Anthropometric and biochemical measurements

Various anthropometric parameters including height (cm), body weight (kg) as described before. Moreover, biochemical factors; 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), Serum calcium (Ca), phosphate (P) were evaluated [15-17]. Serum 25(OH) vitamin D level was measured using an electrochemi-luminescence method (ECL, Roche, Basel, Switzerland). (%). We categorized serum 25 (OH) D status as deficient for serum 25(OH)D level <50 nmol/l, sufficient for a serum 25(OH)

D level between 50 to 75 nmol/l, and proposed optimal group with serum 25(OH)D level > 75 nmol/l. All measurements were done at baseline and following 9 weeks of intervention[17].

DNA extraction and genotyping

Genomic DNA was extracted from blood samples using a QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) following the manufacturer's instructions. The purity and concentration of DNA samples were determined using the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotyping analysis of CYP2R1-rs10741657 polymorphism was carried out using a 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). We re-genotyped 10 per cent of samples, resulting in 100% reproducibility. The allelic content was evaluated using the ABIPRISM-7500 instrument with the SDS version-2.0 software.

Statistics analysis

Normally distributed variables were reported as the mean ± standard deviation (SD), and non-parametric data was shown as median (Q3-Q1). The Kolmogorov–Smirnov test was performed for the analysis of the normality of continuous variables. We also did an analysis of variance (ANOVA) to compare changes in biomarkers after intervention in different genotypic 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. Moreover, to investigate the effect of the genotypes, repeated measures analysis of covariance (ANCOVA) was used, together with a logistic regression model, we examined the probability of changes in serum 25(OH) D in various genetic models. Data were analyzed using SPSS version 20, IBM (SPSS Inc., IL, USA), and significance was set at $p < 0.05$.

Results

Influences of supplementation on circulation 25(OH) D in the total population, regardless of the genetic make-up

A shown in Figure 1, at baseline, the serum vitamin D in about 87% of the studied population was <50nmol/L (vitamin D deficient), with approximately 19% and 6% in the vitamin D sufficient and proposed optimal categories, respectively. The proportion of individuals categorized as deficient fell sharply after supplementation with high-dose of vitamin D, to approximately 20%. On the other hand, the share of subjects having vitamin D at sufficient levels increased by about 13%. On supplementation, the percentage of girls with a proposed optimal level of vitamin D increased to 60.5%. It is noteworthy that in total population mean±SD of serum 25(OH)D before supplementation was 26.2±23.7 mg/dl and after supplementation became 90.0±42.2 mg/dl

Influences of supplementation on circulation 25(OH) D in CYP2R1 variant

To examine the influence of CYP2R1 variant on the circulation levels of vitamin D after the intervention, subjects were categorized by rs10741657 genotype. There was no significant trend in the distribution of vitamin D status (proposed optimal, sufficiency and deficiency) among different genotypes at baseline (P-trend = 0.1). However, supplementation for 9 weeks led to a significant trend (P-trend =0.001) (Table 1), with a reduction in the percentage of subjects with a low serum vitamin D. It appeared that responding to the serum 25 (OH) D was dependent on the genotype at the CYP1 locus (Fig. 2); during the supplementation, serum (OH) D increased in all groups, but carriers who had the common A allele, had higher vitamin D concentrations. Perhaps the SNP rs10741657 modulated response to vitamin D supplementation (*p*-value of intervention effect=0.001 and *p*-value of SNP effect=0.006) (Fig. 2). The results of the regression analysis also showed that in the additive model, the probability of increasing serum 25(OH)D, in individuals

who had the homozygous genotype AA was two and a half fold higher than those who were homozygous for the common GG genotype (OR=2.5 (1.4-4.4); p value=0.002). The regression model was also significant using a recessive model (OR=1.65 (1.1-2.4); p value=0.008) and dominant model (OR=2.05 (1.2-3.4); p value=0.007) (Table 3). Data was adjusted for potential confounders such as age, BMI, and season.

Influence of supplementation on metabolic profile in CYP2R1 variant

Further analysis showed that changes in various clinical and anthropometrics measures after intervention were not variant-dependent which meant that neither at baseline nor after the intervention, we could not see any difference among carriers of different genotypes (table 2). However, individuals possessing an uncommon "A" allele were better responder to supplementation than those with GG genotype in terms of serum 25(OH) D; the percentage of changes in serum 25(OH) D for participants with GG, AG, and AA genotypes were 363.3±354, 443.7±384.6 and 539.4±443.1 respectively (P value (GG vs AA/AG)=0.003).

Discussion

The purpose of the current study was to investigate whether a specific variant at the CYP2R1 locus on chromosome 11p15.2 was associated with an altered response to high-dose of vitamin D supplementation. Although at baseline, the distribution of individuals with different genotypes was not statistically significant in different vitamin D groups, after the intervention, the changes in serum 25 (OH)D did appear to be influenced by this variant. Our data showed that holders of less common variant might be a better responder to vitamin D supplementation. The logistic model also demonstrated that the likelihood of increase in serum 25(OH)D in the homozygotes of minor AA genotype might be 2.5 fold more than those with common GG genotypes. However, our data revealed no changes in other biochemical parameters after the intervention.

It is becoming evident that the individual response to a dietary program varies dependent on genetic factors [18]. There is growing evidence that genetic factors are determinants of vitamin D status in different ethnic groups [19-22]. Looking at potential determinants of 25(OH)D, our group previously reported a significant difference among different genotypes of CYP1 SNP rs10766197 in terms of responding to high-dose of vitamin D supplementation; the changes in serum 25 (OH) D were much more in individuals with common GG genotype; however, this intervention deteriorate inflammation status in the holder of this genotype [17]. Similarly, a German study demonstrated an association between serum vitamin D and the rs10741657 SNP [12]. Another study conducted on individuals with gestational diabetes mellitus suggested that both genetic susceptibility and uterine environment appeared to be involved in GDM[13]. Arabi et.al examined influences of 2 different doses of VitD supplementation in 218 overweight individuals in the elderly population (>60 years) in terms of skeletal measures. Accordingly, it seemed that in their study, the serum 25(OH)D at baseline was related to CYP2R variants; however, these variants did not affect response to vitamin D supplementation[23]. Bu et al. studied 49 SNPs in genes related to metabolism of Vitamin D in 156 healthy Caucasian subjects, after adjusting for potential confounders, they found that variants in the CYP2R1 and Gc genes appeared to modulate serum 25(OH)D [24]. Nissen et.al demonstrated that variants in CYP2R1 and Gc genes might be associated with circulating VitD and those haplotypes might lead to lower serum vitamin D in 201 healthy Danish families [25].

Conclusion:

We found that although the rs10741657 on CYP2R1 gene was not associated with baseline serum 25 (OH) D in healthy adolescent Iranian girls, it may modulate the response to high-dose vitamin D supplementation so that participants with a minor AA genotype showed a higher level of vitamin D concentration after supplementation.

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Figure 1. Comparison of the vitamin D status categories before and after 9 weeks of vitamin D supplementation in the total population. Deficiency: Serum 25(OH)D level<50nmol/L. Sufficiency: 50nmol/L<Serum 25(OH)D level<75nmol/L. Proposed optimal>75nmol/L.

Table 1. Vitamin D status groups before and after 9 weeks of vitamin D supplementation according to CYP2R1-rs10741657 genotypes.

Table 2. Comparisons of the variables before and after 9 weeks of vitamin D supplementation

Fig.2.Serum 25(OH)D stratified by a polymorphism in the CYP1 gene. Values are means ± SD. Two-way ANCOVA repeated measures adjusted for multiple comparisons by Bonferroni test for serum 25(OH)D levels. Covariates used: age, gender, physical activity, smoking status.

Table 3. Association of CYP2R1 gene rs10741657 variant with changes in serum vitamin D After 9 weeks supplementation (under different genetic models).

Table 1. Vitamin D status groups before and after 9 weeks of vitamin D supplementation according to CYP2R1-rs10741657 genotypes.

Vitamin D status (N=616)	GG (N=269)		AG (N=261)		AA (N=86)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Proposed optimal	13 (4.8)	111 (53.9)	15 (5.7)	140 (63.3)	10 (11.6)	71 (82.7)
Sufficiency	18 (6.7)	34 (16.5)	15 (5.7)	46 (20.8)	8 (9.3)	13(14.7)
deficiency	238 (88.5)	61 (26.9)	231 (88.5)	35 (15.8)	68 (79.1)	2 (2.7)

Note: Σ2 test showed a $P_{trend}$of 0.1 at baseline; P_{trend} at 9- week follow-up is **<0.001** 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. Proposed optimal: Serum 25(OH)D level > 75 nmol/l.

Table 2. Comparisons of the variables before and after 9 weeks of vitamin D supplementation

Variable		GG(n=269)	AG(n=261)	AA (n=87)
Anthropometric				
BMI (kg/m^2)	Baseline	21.9±4.2	21.6±4.4	21.7±4
	After 9 weeks	21.7±4.2	21.6±4.4	21.5±4
	Change (%)	-0.1±6.6	-0.2±5.1	-0.8±3.9
WHR(cm)	Baseline	0.8±0.07	0.8±0.07	0.8±0.07
	After 9 weeks	0.8±0.3	0.8±0.1	0.8±0.1
	Change (%)	1.9±4.1	-0.6±9.3	0.6±6.9
SBP(mmHg)	Baseline	101.2±12.1	101.3±13.1	99.1±12.6
	After 9 weeks	100.9±12.3	100.1±13.6	99.6±12.6
	Change (%)	0.2±13.1	-0.5±14	1.2±15
DBP(mmHg)	Baseline	68.4±9.7	66.9±11.4	66.5±9.8
	After 9 weeks	66.0±10.1	64.9±9.9	62.4±11.2
Lipid profile				
Cholesterol(mg/dl)	Baseline	165.3±28.2	162.6±28.4	162.1±26.8
	After 9 weeks	154.7±27.9	153.9±27	151.2±28.2
	Change (%)	-5.2±19.6	-4.4±13.9	-3.9±14.1
TG(mg/dl)	Baseline	81.7±33.4	82.6±35.1	80.9±35.3
	After 9 weeks	77.7±33	81.1±32.0	73.5±28
	Change (%)	-0.3±33	4.8±32.5	-1.05±31.4
HDL(mg/dl)	Baseline	48.2±9.1	45.4±8.4	45.5±7.3
	After 9 weeks	47.2±8.7	45.3±8.4	45.04±7
	Change (%)	-3±14.3	-2.3±15	0.8±15.7
LDL(mg/dl)	Baseline	102.1±22.7	100.2±23.8	99.7±20.7
	After 9 weeks	92.6±20	91.2±32	90.0±24.4
	Change (%)	-8.5±19	-7.2±20	-7±20.9
FBS(mg/dl)	Baseline	88.6±11.7	87.1±12	85.9±9.4
	After 9 weeks	87.1±12	86.8±11.6	83.9±10
	Change (%)	-1.4±13	-1.4±12	-3.1±11.3
Inflammatory measures				
WBC(10^9/L)	Baseline	6.35±3.3	6.3±1.7	6.1±1.7
	After 9 weeks	5.7±1.5	6.07±1.4	5.9±1.5
	Change (%)	-0.2±3.4	3.3±3	5.5±4.4
Hs-CRP(mg/L)	Baseline	1.4±1.7	1.47±1.9	1.6±2.1
	After 9 weeks	1.5±1.44	1.52±1.5	1.4±1.2
	Change (%)	0.9±2.6	1±2.9	0.6±1.9
Serum electrolytes				
VitD*(nmol/L)	Baseline	26.0±23.0	26.0±24.1	30.6±28.7
	After 9 weeks*	81.1±42.9	91.1±40.4	111.6±37.3

	Change (%)**	363.3±354.0	443.7±384.6	539.4±443.1
Ca(mg/dL)	Baseline	9.34±0.6	9.5±0.5	9.4±0.5
	After 9 weeks	9.6±0.5	9.7±0.5	9.7±o.4
	Change (%)	3.3±7.7	2.3±8	2.1±7
Phosphorus(mg/dL)	Baseline	3.91±0.4	3.9±0.4	3.8±0.4
	After 9 weeks	4.09±0.36	4.1±0.4	4.05±0.4
	Change (%)	5.3±11.3	5.3±9.8	6.2±11
Creatinine(mg/dL)	Baseline	0.6±0.1	0.6±0.09	0.65±0.09
	After 9 weeks	0.7±0.08	0.7±0.09	0.7±0.08
	Change (%)	13.3±38	9.6±14.2	8.9±14
BUN(mg/dL)	Baseline	12.09±3.04	12.6±3.02	12.1±2.7
	After 9 weeks	13.9±3.3	14.03±3.3	12.9±3.4
	Change (%)	29.8±143	22.1±12	11.1±32

Note: Change = ((Follow up – Baseline)/Baseline)/100; Co-dominant genetic model (GG genotype vs. AG+AA genotypes); Dominant genetic model (GG+AG genotypes vs. AA genotype). *P value $_{(GG\ vs\ AA/AG)}$<0.001, **P value $_{(GG\ vs\ AA/AG)}$=0.003

Table 3. Association of CYP2R1 gene rs10741657 variant with changes in serum vitamin D After 9 weeks supplementation in Iranian population (under different genetic models).

Additive model	GG	AG	AA
	Reference (Common genotype)	OR (CI95%), *p* value	OR (CI95%), *p* value
	1	1.7 (0.9-3), 0.05	2.5 (1.4-4.4), **0.002**
Recessive	GG/AG	AA	
	Reference	OR (CI95%), *p* value	
	1	1.65 (1.1-2,4), 0.008	
Dominant	GG	AA/AG	
	Reference	OR (CI95%), *p* value	
	1	2.05 (1.2-3.4), 0.007	

Data was adjusted for age, BMI percentile, physical activity, passive smoking.

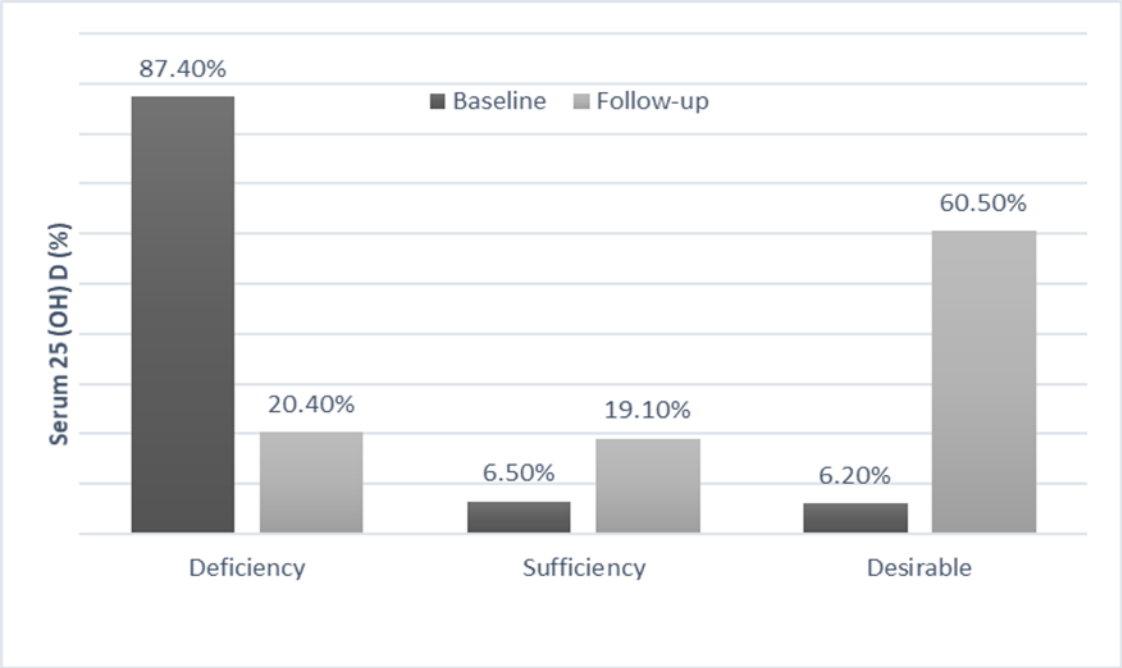

Figure. 1

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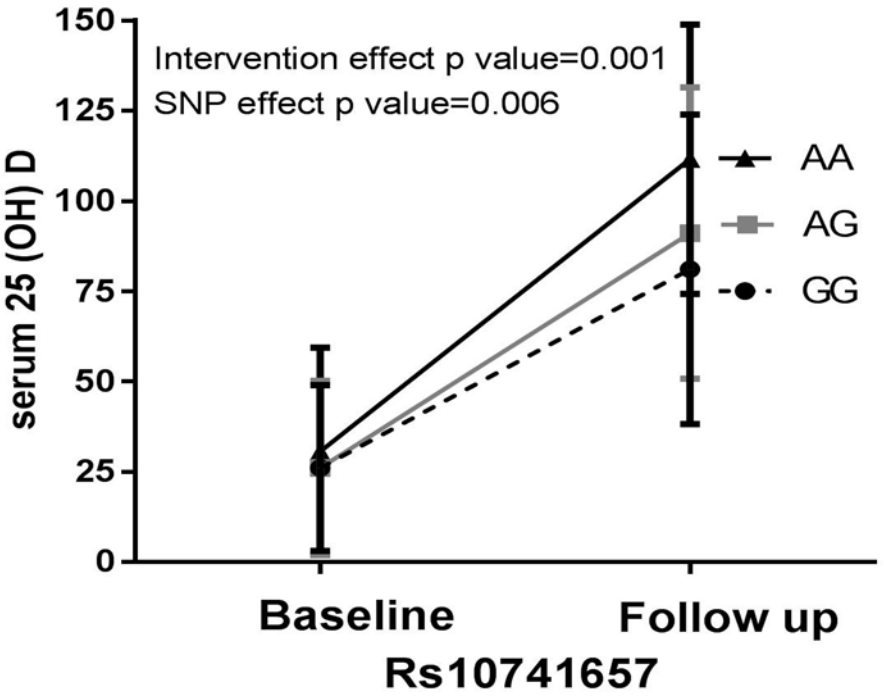

Figure. 2

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