# Single nucleotide polymorphism A-511 G of IL-1 gene modifies anthropometric and physiological parameters of athletes

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## Abstract

Interleukin-1β is one of pro-inflammatory responses and plays an important role in cell proliferation, differentiation, and apoptosis. The gene of this cytokine is highly polymorphic, one of which is located on 5´flanking region at position -511 (rs16944, A-511 G). This allele encodes IL-1β protein with less activity in comparison with wild type. In this research, 40 number of professional athletes in futsal and volleyball fields have been studied in their anthropometric and physiological indexes and also IL-1$\beta$ genetic polymorphisms. Our results show that in subjects carrying IL-1$\beta$ A (-) SNP or wild type allele, the weight, body fat and some other physiological indexes have been increased. This might have relation with elevated plasma level of leptin which is conducted by IL-1$\beta$ A (-) allele. These findings suggest a remarkable relation between genetic polymorphisms of IL-1β gene and physical potential of athletes.

**Key words:** IL-1, Genotyping, anthropometric and physiological parameters

## Introduction

Athletic performance is controlled by many factors which is not well defined. Although the effect of inflammatory responses is one of researchers interests [1].

IL-1α and IL-1β are known as the first regulatory and inflammatory cytokine families. This cytokine is involved in inflammatory responses as an important mediator and also plays an important role in cell proliferation, differentiation, and apoptosis. Interleukin 1 family includes 9 genes forming a cluster on chromosome 2. Three genes of IL-*1α, IL-1β* and IL-1 receptor antagonist (IL-1 Ra) are located on chromosome 2q13-14 and encode three respective glycoproteins. IL-1α, IL-1β along with IL-1 Ra and interleukin 18 (IL-18) involve in up-and-down of acute inflammation [2]. Interleukin 1 beta (IL-1β) which is encoded by the IL-*1β* gene is a premature protein and consequently would form a mature type through cleavage process by cytosolic caspase 1 (interleukin 1 beta convertase) enzyme. IL-1β as a major regulator of adipose metabolism, is secreted by adipose tissue and reduces adipose tissue lipoprotein lipase activity and expression [3]. This cytokine like tumor necrosis factor-α (TNF-α) suppresses adipocyte differentiation, expression of fatty acid transport proteins and fatty acid translocase in adipose tissue. The concentration of IL-1β increases in the obese subjects [2][4]. The IL-1*β* gene demonstrates high polymorphic variations. A polymorphic allele located on the 5´down-stream region at position -511 (rs16944, A-511 G) has been considered to associate with some autoimmune diseases [5][6]. This polymorphism, A-511 G, influence the activity of IL-*1β* gene promoter and protects body against human leptospirosis [7][8]. During exercise, IL-6 production is increased by muscle fibers via a TNF-independent pathway. IL-6 elevates the ciculatory level of the other anti-inflammatory cytokines such as IL-1Ra and IL-10. It can also inhibit the production of the proinflammatory cytokine TNF-α [9][10]. Based on this research, we explore the possibility of having relation between single nucleotide polymorphism of IL-1β gene and athletes' body compositional properties. It is objected to propose nutritional and physical interventions which is specific for every athlete based on their genomic variations and in order to improve athletes' performance.

## Methods

## Subjects

This study subjected 40 professional male athletes including 21 (52.6%) members from futsal (FS) group and 19 (47.4%) volleyball (VL) members to enter for IL-1 genotyping. For the analysis of genotype 21 male athlete subjects entered to study of whom 10 (47.6%) members of FS group and 11 (52.4%) from VL group. As control 10 healthy subjects of non-athlete have been considered to juxtapose simultaneously. Before entering the study, an informed consent document was signed by all of the participants which was approved by University of Rome "Tor Vergata" Medical Ethics Committee.

## Anthropometric measurements

Body composition of all participants were assessed by anthropometric measures includes; body weight, height, hip and waist circumferences also skinfolds and bioimpedence analysis with standard methods. Subjects were required to take off their clothes and shoes before measurements. Body weight (kg) and height (cm) were measured with approximation of 0.1 kg and 0.1cm respectively using a balance scale and stadiometry. The measurement of the circumstances of waist and hip were performed by a flexible steel metric tape with approximation of 0.5 cm. the abdominal circumstance was measured with horizontal distances around the area and also hip circumstance was assessed with measuring the distances between two superior iliac bones. Body mass index (BMI) is a measure of body fat based on height and weight and it is calculated through related formula:

$$BMI = \frac{body\ weight(kg)}{height(m2)}$$

## Dual X-ray absorptiometry (DXA)

Dual X-ray absorptiometry is a technique for scanning bone and measuring bone mineral density (BMD). We evaluated the body composition by means of DXA (Lunar model DPX-IQ, Lunar Corp., Madison) fan beam scanner. All subjects were given complete instructions on the method of assessment. They required to wear a standard cotton t-shirt, shorts and socks. DXA scan

recorded their results while they were laid down on the DXA without moving. Coefficient of variation (CV%=100×S.D./mean) intra and inter subject ranged from 2 to 5%. The radiation exposure was <8 SV.[11]

## Genetic analysis

The genomic DNA was extracted by the QIAamp DNAMini Kit (Quiagen, Valencia, CA) from whole blood and stored at −20 ◦C. The concentration of DNA extract was estimated by absorbance at 260 nm.

Our goal was identification of subject-specific SNPs in IL-1*β* (rs16944) gene and determining the proportion of each polymorphism in every participants. PCR primers for amplifying of the gene is designed. PCR reaction was performed using following primers: For amplification of IL-1*β* gene (rs16944), 3´- TGGCATTGATCTGGTTCATC -5´ as forward primer. The PCR was performed in a 25µl total volume containing 2.5mM $MgCl_2$, 9.9mM Tris–HCl (pH 8.8), 50mM KCl, 0.1% Triton-X 100, 0.20 M deoxyribonucleotide triphosphate (dNTPs), 1U of Taq DNA polymerase, and 0.2µM of each primer. The following cycling profile of PCR was set on 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 1 min, with a final extension of 72°C for 7 min. The final PCR products were analyzed on 2% agarose gel. The length of the attended amplicon was 114 and 237bp for IL-*1β*, representing mutated and wild alleles.

## Statistical analysis Data

The presented data are mean ± standard deviations (SD). T-student test is used to determine the significance of each value between carrier and non-carrier groups (P value≤0.05). The frequency of each allele in FS and VL groups was calculated, and compared with controls by one-sample chi-squre test. The statistical analysis was carried out by means of the Instat-GraphPad Software, SanDiego, CA, USA, version number 4.03, 2005.

# Results

Body composition and physiological parameters of FS and VL groups for IL-*1β* (Table 1).

## Allelic frequencies

### IL-1β

Among 21 attendant subjects of FS group, 4 (19.0%) participants with age media: 21.0 (SD=2.82) had wild allele (GG) and 16 (76.1%) of them with age media: 27.7 (SD=5.99) had mutated allele (GA-AA) both homozygotes and heterozygotes. In VL group from 19 attendants, there were 8 (42.1%) subjects with age media: 29.5 (SD=4.20) wild type allele (GG) and 10 (52.6%) with age media: 28.4 (SD=6.18) had mutated type (GA-AA), shown in Fig. 1.

## Physiological and Body composition parameters

### IL-*1β*

The differences among FS group were significant ($p<0.05$) and IL-*1β* affects Body mass index (BMI) (Δ%=12.40%) and reactance (Xc) (Δ%=-14.00%) in A (+) compared to A (-). Although there were no significant differences in age, height and waist/hip (W/H) ratio averages, abdomen, fat mass (FM), Percentage of Fat mass (FM%), Free fat mass (FFM), Percentage of Free fat mass (FFM%), Appendicular skeletal muscle mass (ASMM), muscle mass (MM), Body cell mass (BCM), Total body water (TBW) and Extra cellular Water (ECW) indexes which are shown in Fig. 2 and Fig. 3.

Among VL group the significant differences ($p<0.05$) were highlighted and IL-*1β* A (+) remarkably influenced on weight (Δ%=-22.6%), BMI (Δ%=-11.2%), resistance (R) (Δ%=+2.40%), BCM (Δ%=-17.70%), TBW (Δ%=-18.20%), ECW (Δ%=-18.00%), BCMI (Δ%=-7.37%), Waist, abdomen, hip, FM, ASMM, MM and MB. Although in Age, height, PA and FMM no significant differences were analyzed which are shown in Fig. 4 and Fig. 5. According to the

50th percentile, all IL-*1β* A (+) present a BMI<23.1 and a R≥460 Ω ($p<0.05$). Otherwise, 83.3% of IL-*1β* A (+) had a BCMI<12 ($p<0.05$).

## Discussion

Professional exercises may cause physical micro-injuries to skeletal muscles. This causes inflammation and results in increasing pro-inflammatory cytokines like IL-*1β* in blood which is one of the primary cytokines with an important role in the regulation of inflammatory responses. It could protect body from secondary tissue damages and myopathy. IL-*1β* released due to prolonged strenuous exercise results in elevation of plasma levels of leptin [12]. The hormone Leptin is released by adipocytes and regulates appetite in demand and body weight [13]. The main concern of the research is to examine whether the polymorphisms of IL-*1β* gene could be associated with the athlete's physiological and body composition parameters. Furthermore, various significant differences between FS and VL subjects have been assessed. The allelic differences between three groups of FS, VL and control subjects are illustrated In Fig. 1 and Fig. 2. There were not any significant differences in IL-*1β* polymorphisms among FS, VL and Control subjects. The control group are random subjects whom we analyzed their IL-*1β* polymorphisms. Incompatibly as indicated by Fig. 2, It may determine that genetic constitution is associated with athletic performance in addition to epigenetic and environmental factors [1]. The hormone leptin has been known to be regulated by immune responses [8] and it is constantly correlated with increasing of body fat. As our results showed in Fig. 4, VL group with IL-*1β* A(-) has higher weigh, waist, abdomen and Hip measures than the carrier subjects. It is because of the effect of A(+) allele on the activity of promoter which can reduce leptin gene expression and therefore causes low level of fat accumulation [1][9][10][14][15]. Within this study we have calculated BMI in FS and VL subjects based on the collected data and as shown in Fig. 3 there is significant difference between IL-*1β* Polymorphism group holders. It has been considered that BMI is correlated with IL-*1β* polymorphisms and subjects with a G/A transition in nucleotide number 511 of IL-1β promoter among FS group have higher BMI than subjects carried wild type of this polymorphism. In the Fig. 3 and Fig. 4, it has been documented that some of body compositions and physical parameters have correlation with IL-*1β* polymorphisms which assures relation to athlete's physical performance. The relationship between single nucleotide polymorphisms and physical

performance also has been shown in other studies [11][16][17][18]. The more differences aren't significant in this project and it may be because of low number of subjects. Although some studies show The relation between physical activity of athletes and IL-*1β* polymorphisms, there are some others suggest that there is no correlation between athlete's physical activity and circulating mRNA levels of IL-*1β* [1].

An important implication of these findings is to provide fit nutritional and physical interventions for every athlete which is based on their genetic variations and in order to improve appropriately athlete's performance. This study has not been yet performed for Italian athletes' population. Certainly, a wide investigation should be performed to show the significant differences of main physiological parameters between carrier and non-carrier groups in order to determine the role of IL-*1β* polymorphisms. Gene expression analysis in transcription and translation levels should be performed for IL-*1β* gene and also other epigenetic variation of these two genes like methylation within deferent athlete population could be investigated in complementary studies. Some environmental factors like intense and frequent exercises were shown to have effects on the number of satellite cells of skeletal muscles [12][19][20], therefore the association between this physiological changes and epigenetics is one of future studies' objective. In conclusion, our findings suggest that there are correlations between IL-*1β* polymorphisms at one side and anthropometric and physiological characters of the body under the exercise on the other side. This idea can potentially conduct the genetic based imprint of nutrition and physical performance of athletes.

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| **Groups** | **IL-1β/ FS** | | **IL-1β /VL** | |
|---|---|---|---|---|
| | GG | GA-AA | GG | GA-AA |

| | | | | |
|---|---|---|---|---|
| Age (years) | 21.00±2.828 | 27.75±5.994 | 29.50±4.203 | 28.40±6.188 |
| Height (cm) | 186.00 ±8.485 | 178.00±5.451 | 201.75±6.020 | 192.80±6.685 |
| Body weight (kg) | 72.15±4.879 | 74.86±5.057 | 101.55±9.500 | 83.06±7.667 |
| BMI | 20.85±.494 | 23.68±1.164 | 24.92±1.685 | 22.30±.538 |
| Waist (cm) | 76.00 | 80.18±3.305 | 88.12±2.174 | 79.40±4.350 |
| Abdomen (cm) | 82.75±5.303 | 85.00±5.700 | 94.62±3.350 | 82.64±4.070 |
| Hip (cm) | 97.00±3.535 | 97.93±4.739 | 108.25±2.629 | 98.70±2.334 |
| R | 522.50±4.949 | 439.87±36.431 | 447.75±6.344 | 485.40±19.957 |
| Xc | 68.50±3.535 | 59.62±4.779 | 60.50±1.914 | 66.00±6.442 |
| PA | 7.45±.353 | 7.73±.373 | 7.70±.316 | 7.70±.452 |
| FM (kg) | 10.60±.141 | 11.80±4.630 | 19.25±4.863 | 12.68±2.719 |
| FM% | 14.70±.848 | 14.73±4.335 | 18.77±2.896 | 15.14±2.138 |
| FFM (kg) | 61.55±4.737 | 57.97±22.088 | 82.25±5.076 | 58.60±26.349 |
| FFM% | 85.30±.848 | 85.26±4.335 | 81.22±2.89 | 71.06±31.13 |
| ASMM | 26.00±1.979 | 27.80±2.654 | 34.92±2.19 | 29.54±2.57 |
| MM (kg) | 34.05±2.474 | 36.25±3.115 | 43.55±2.19 | 37.74±3.102 |
| BCM | 37.10±2.121 | 37.17±12.419 | 50.37±3.58 | 42.80±3.28 |
| BCM% | 60.30±1.272 | 61.30±1.492 | 55.15±7.58 | 49.82±11.16 |
| TBW | 44.85±3.606 | 48.77±4.787 | 60.37±3.83 | 51.06±4.539 |
| TBW% | 62.10±.848 | 62.30±3.226 | 59.57±2.182 | 61.98±1.63 |
| ECW | 17.85±1.909 | 18.97±1.915 | 23.52±1.52 | 19.92±2.219 |
| ECW% | 39.80±1.131 | 38.93±1.256 | 39.02±1.14 | 38.94±1.50 |
| MB | 1825.50±61.518 | 1936.62±121.130 | 2211.00±104.361 | 1992.40±95.80 |
| BCMI | 10.75±.353 | 12.80±1.195 | 12.37±.660 | 11.52±.277 |

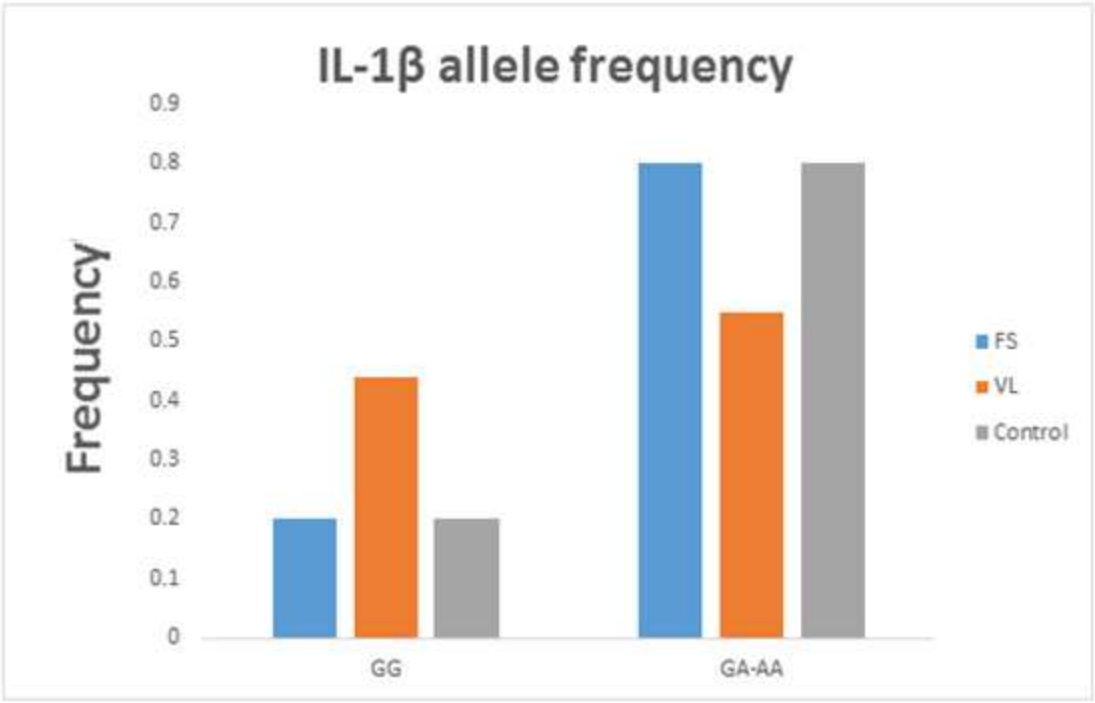


Fig. 1. IL-1β allele frequencies in Italian athletes. FS and VL are Futsal and Volleyball groups respectively. Allele frequencies were evaluated in 21 and 19 subjects of FS and VL groups, respectively. The frequencies are given in percent.

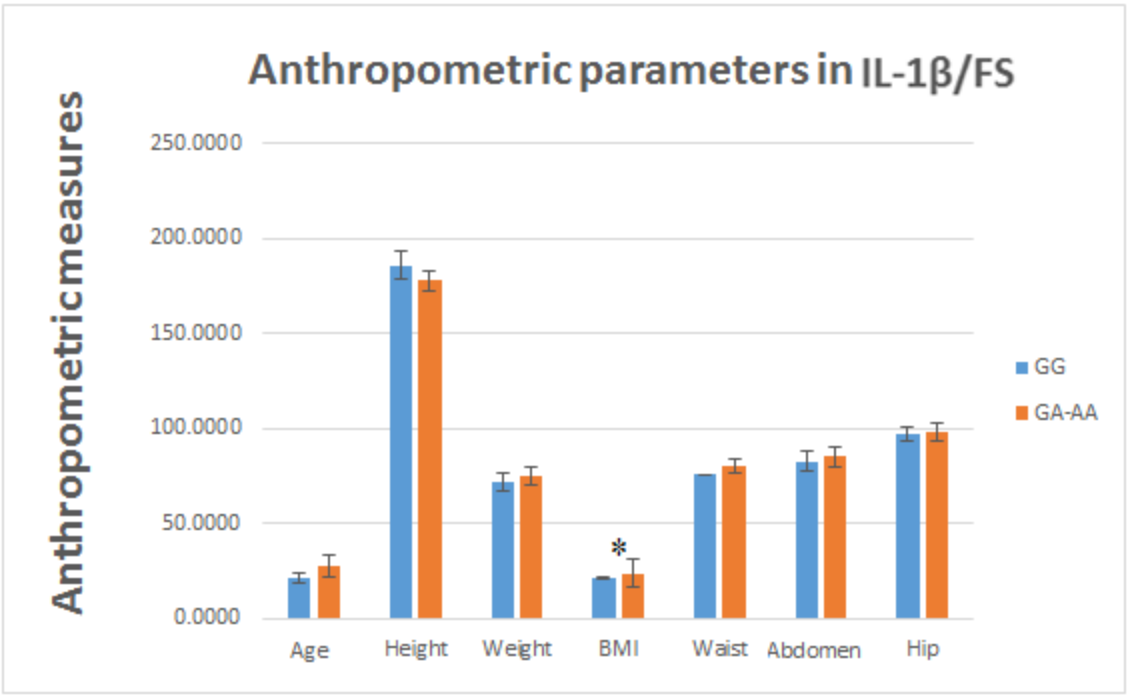


Fig. 2. Anthropometric parameters in FS group in polymorphisms of IL-1β. The significant difference among carrier and non-carriers subjects of IL-1β polymorphisms is just in BMI index which is shown with star. BMI (body mass index).

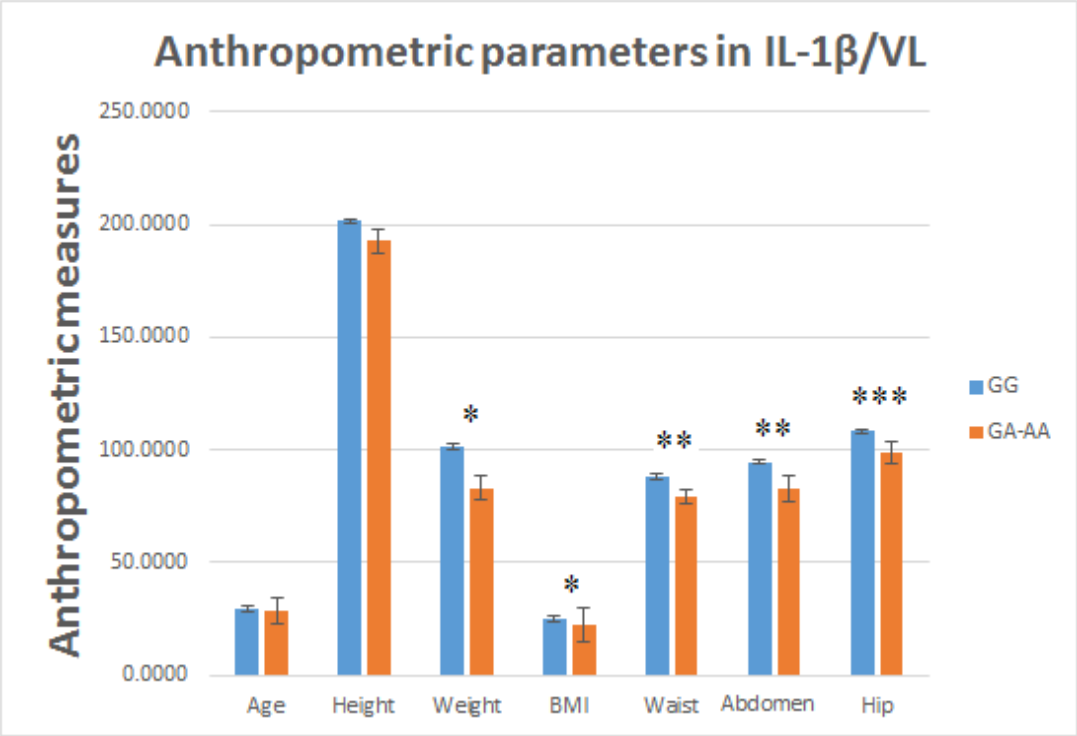


Fig. 3. Anthropometric parameters in VL group in polymorphisms of IL-1β. The significant differences among carrier and non-carriers subjects of IL-1β polymorphisms are in Weight, BMI, Waist, Abdomen, Hip indexes which are shown with star.

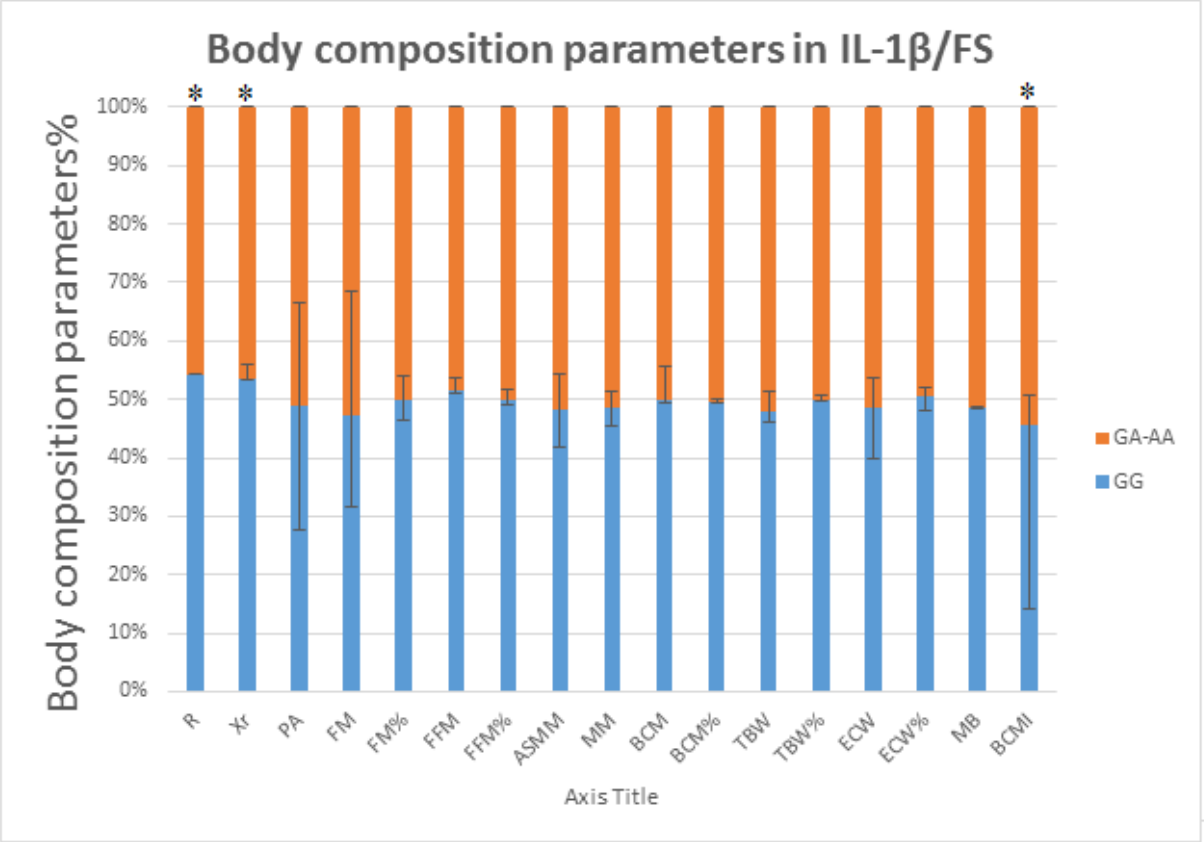


Fig. 4. Body composition indexes in FS group in polymorphisms of IL-1β. Significant differences are shown with stars. R (resistance), Xr, PA (Phase angle), FM (fat mass), FFM (free fat mass), ASMM (appendicular skeletal muscle mass), MM (muscle mass), BCM (body cell mass), TBW (total body water), ECW (energy cost of walking), MB, BCMI (Body cell mass index).

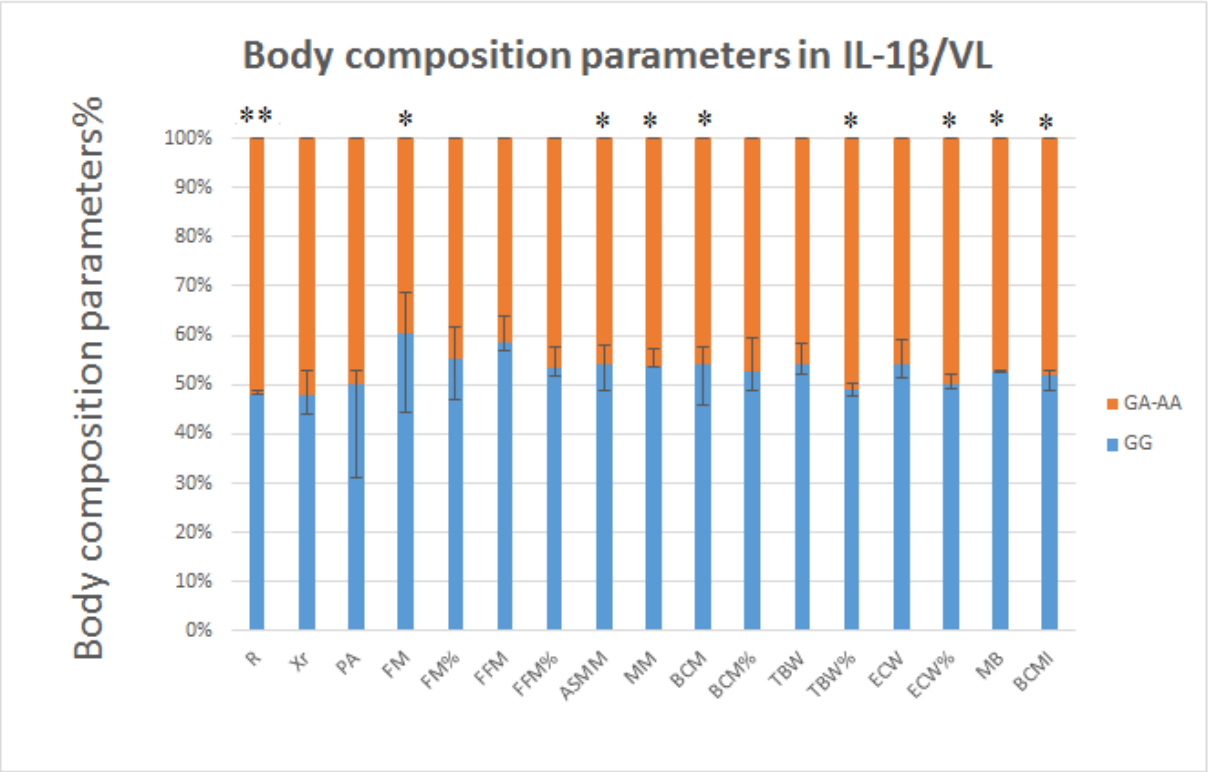


Fig. 5. Body composition indexes in VL group in polymorphisms of IL-1β. Significant differences are shown with stars. Two stars mean 0.01≤P value<0.05 which are more significant. The measures are given in percent. R (resistance), Xr, PA (Phase angle), FM (fat mass), FFM (free fat mass), ASMM (appendicular skeletal muscle mass), MM (muscle mass), BCM (body cell mass), TBW (total body water), ECW (energy cost of walking), MB, BCMI (Body cell mass index).