ORIGINAL ARTICLE

The contribution of selected variants in *ACE*, *MSTN* and *ADRB2* genes in the achievements of judo practitioners

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Manuscript Preparation
E Funds Collection

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Abstract

Background and Study Aim: I/D polymorphism of the *ACE* gene is one of the best known genetic variants which affects physical performance in humans. Another variant of genes which can play an important role in predisposition to sport engagement are Arg16Gly and Gln27Glu of the *ADRB2* gene and K153R of the *MSTN* gene. *ADRB2* is expressed on bronchial smooth muscle and is associated with lipid metabolism. These genotypes influence the RAS function, affecting the body's water and sodium balances. In turn, *MSTN* modulates myoblast proliferation and hence muscle mass and strength. This study aimed to answer the question of whether selected gene variants (I/D, Arg16Gly, Gln27Glu, and K153R polymorphisms in the *ACE*, *ADRB2*, and *MSTN* genes, respectively) have a relationship with achievements obtained by competitive judo athletes and their predisposition to injuries.

Material and Methods: In our study, 82 judo athletes from Poland and 26 from Croatia took part. We studied the contribution of selected polymorphisms of *ACE*, *ADRB2* and *MSTN* genes in judo practitioners. The study was conducted on DNA isolated from buccal cells. The genotypes of the *ACE*, *ADRB2* and *MSTN* genes were determined by ASA-PCR and RFLP-PCR.

Results: Genotypes ID and Arg16Gly were associated with better performances in competitions (most p*l*a*c*e*d* 2nd in competition, p=0.06). Furthermore, the I allele may predispose an individual to joint injuries (OR=1.69, p=0.03) while the Gln allele may protect an individual from spinal injuries (OR=0.58, p=0.054), respectively.

Conclusions: *ACE* and *ADRB2* genes may also influence leg muscle strength and body weight in judo participants.

Keywords: combat sports • endurance • genotype • SNPs • strength

Conflict of interest: Authors have declared that no competing interest exists

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Muscle strength – essential and basic physical capacity in combat sports by which the body moving status is modified [43].

Muscle tissue – noun the specialised type of tissue that forms the muscles and can contract and expand [44].

Endurance – noun the ability or power to bear prolonged exertion, pain or hardship endurance athlete [44].

Genetic – adjective relating to or contained in genes [44].

Injury – noun damage or a wound caused to a person's body [44].

Marker– noun 1. in games such as football and hockey, a player who stays close to an attacking player in the opposing team to prevent him or her from receiving the ball or scoring 2. a substance which reveals the use of a banned substance, found in drugs testing [44].

Metabolic pathway – noun a series of chemical reactions in the body, controlled by enzymes [44].

Performance – noun the level at which a player or athlete is carrying out their activity, either in relation to others or in relation to personal goals or standards [44].

INTRODUCTION

Individual genetic variability and favourable genetic endowment in conjunction with environmental factors seem to be necessary for attaining the highest level of athletic performance [1, 2]. The influence of genes involved in metabolic pathways and skeletal muscle structure has been analysed [3], but it is still unclear which genetic factors determine elite endurance performance [4]. However, there is evidence which suggests that genetic markers may explain, in part, individual variability in response to training [2]. In this study, we tested polymorphisms in selected genes which are candidates to the title genetic markers in sport achievements.

The most investigated polymorphism is the I/D (insertion/deletion) variant (rs4646994) in the *ACE* (Angiotensin-converting enzyme) gene which is also one of the first genes described in the subject of sports performance [5] and is located on chromosome 17 (17q13). The I/D polymorphism is involved with insertion and deletion of a 287 bp Alu repeat sequence located in 16 introns [6]. The I allele (insertion 287 bp) is associated with lower *ACE* concentration in serum [7] and with improved performance in endurance sports [8]. The D allele (deletion 287 bp) is associated with short aerobic performance [9]. The *ACE* gene influences the renin-angiotensin system (RAS) as it plays an important role in the regulation of blood pressure, water and sodium balance, and increased muscle tissue [8]. In addition, this gene is involved in left ventricular hypertrophy [10], myocardial infarction, cardiomyopathy and coronary artery disease [11].

The other gene that was studied in the context of sport performance is the *ADRB2* gene (beta-2 adrenergic receptor gene) located on chromosome 5, (5q31-q32) [12, 13]. The *ADRB2* gene is expressed on bronchial smooth muscle cells and is activated by catecholamine and epinephrine [14]. Moreover, the *ADRB2* (also B2AR) gene is associated with lipid metabolism. The most interesting single nucleotide polymorphisms (SNPs) in sport sciences are Arg16Gly (rs1042713) and Gln27Glu (rs1042714) which are in codon 16 and 27, respectively. In addition, the beta-adrenergic receptor is the main objective of illegal anabolic agents chosen by athletes of which one effect is bronchodilation. Corticosteroids together with anabolic agents also significantly increase anti-inflammatory activity. Individuals that carry 16Gly show a fast decrease in activity of the beta-2 receptor and loss of susceptibility to antagonists, while subjects with 27Glu are resistant to these effects [15, 16].

The third gene studied in this paper is the *MSTN* gene (myostatin, also known as GDF-8) which is located in chromosome 2 (2q32.2) [17, 18]. *MSTN* encodes myostatin, a skeletal muscle-specific peptide which mainly functions to modulate myoblast proliferation and hence muscle mass and strength [19]. *MSTN* enters circulation as a latent precursor protein and then undergoes a proteolytic process. Mature peptide binds with the extr*ace*llular activing type II receptor (ActRIIB), which consequently causes the activation of the Smad pathway [20, 21]. One common SNP is the K153R polymorphism *(rs1805086)* which is associated with the thickness of muscle growth and with the ability to produce peak power during muscle contractions in young non-athletic men [21].

The aim of this study was the question of whether selected gene variants (I/D, Arg16Gly, Gln27Glu, and K153R polymorphisms in the *ACE*, *ADRB2*, and *MSTN* genes, respectively) have a relationship with achievements obtained by competitive judo athletes and their predisposition to injuries.

MATERIAL AND METHODS

Ethics statement

Studies were performed with the University of Rzeszow (Poland) ethical committee approval. Written informed consent was obtained from all subjects a priori.

Genetic material and DNA isolation

The study group consisted of 108 judo athletes: 62 men and 20 women from Poland (n=82, age: 16-35 years; weight 52-115 kg, years of practice: 6-27 years) and Croatia: 20 men and 6 woman (n=26, age: 16-35 years; weight 52-124 kg, years of practice: 5-26 years). Participants answered survey questions about their weight, height, years of practice, training frequency per week, injuries, strength and pl*ace*ment during championship tournaments. Additionally, Polish judo athletes are divided into non-elite and elite groups with the latter group currently on the national team. In the Croatian group, we did not find judo participants who were on the national team. In this described group of judo athletes, we included only participants who have a minimum

of 16 years of age that have been training for a minimum of 5 years, and we also included judo athletes, who are no longer competing but hold the black belt rank (e.g. trainers). Athletes who did not fall into the indicated ranges were excluded from this study. A number of 108 athletes included in the study is caused by relatively small numbers of individuals participating in this sport and meeting inclusion criteria, e.g. period of active training, consent to blood drawing. Additionally, we do not decide to mix judo athletes with other combat sport because of the specifics of judo discipline, which is completely different from wrestling, karate and even Jujitsu. On the other hand, we decided to mix Croatian and Polish groups because of its genetic similarity considering Y chromosome.

The material for the study was DNA isolated from buccal epithelial cells. DNA was isolated according to the protocol described by Bolla MK et al. [22], with minor modifications. First, the buccal epithelial cells were transferred to an Eppendorf tube. Subsequently, the samples were centrifuged for 2 min at 10,000 g and supernatant was removed. After centrifugation, 400 µl of 10 mMNaCl-EDTA was added, and the samples were centrifuged again for 2 min at 10,000 g. After centrifugation, the supernatant was removed, and 100 µl of 20 mM NaOH was added, and the samples were then heated for 10-20 min at 95°C. DNA was precipitated using 95% ethanol and washed two times with 70% ethanol. The DNA was dissolved in 1X TE (Tris-EDTA) buffer and stored for further analysis at −20°C.

Genotyping

In order to determine the genotypes of the *ACE* gene, the following primers were used: h*ace*5a: 5'-TGGGACCACAGCGCCCGCCACTAC-3'; h*ace*5c 5'-TCGCCAGCCCTCCCATGCCCATAA-3' [23], and the specific primer flanking Alu sequences 5`-CTGGAGACCACTCCCATCCTTTCT-3` [7]. PCR reactions were carried out in a total volume of 20 µl, containing 2.5, 10, and 15 pmol of the h*ace*5a primer flanking Alu sequences and the h*ace*5c primer, respectively 0.5 U of Taq polymerase (Kapa Biosystems), 0.2 mM dNTP Mix (Thermo Scientific) and 1X reaction buffer with 1.5 mM $MgCl_2$. PCR assays were performed using the following conditions: the initial denaturation at 95°C for 5 min, denaturation at 95°C for 35 s, annealing at 62.6°C for 60 s and elongation at 72°C for 35 s, with a cycle number of 35.

Table 1. Types of PCR and name of the restriction enzyme which was used to diagnose alleles in the genes studied.

Gene alleles	Methods	Alleles	Primers	Primer annealing	Enzyme	Patterns
ACE	ASA-PCR	I	sense: h*ace*5a: 5'-TGGGACCACAGCGCCCGCCACTAC-3'; antisense: h*ace*5c 5'-TCGCCAGCCCTCCCATGCCCATAA-3'; specific primer flanking Alu sequences: 5`-CTGGAGACCACTCCCATCCTTTCT-3`	62.6°C		522+335 bp
		D				234 bp
ADRB2 (B2AR)	RFLP-PCR	Arg16	sense: 5'-GAACGGCAGCGCCTTCTTGCTGGCACCCCAT-3` antisense: 5'- CTGCCAGGCCCATGACCAGATCAG-3`	64°C	Eco130I	242 bp
		Gly16				214+28 bp
		Gln27			Fnu4HI	181+55+6 bp
		Glu27				236+6 bp
MSTN (GDF-8)	RFLP-PCR	K153	sense: 3`-TGGATGGAAAACCCAAATGT-5' antisense: 3`- GCCTGGGTTCATGTCAAGTT-5`	55°C	ApaI	200 bp
		R153				138+62bp

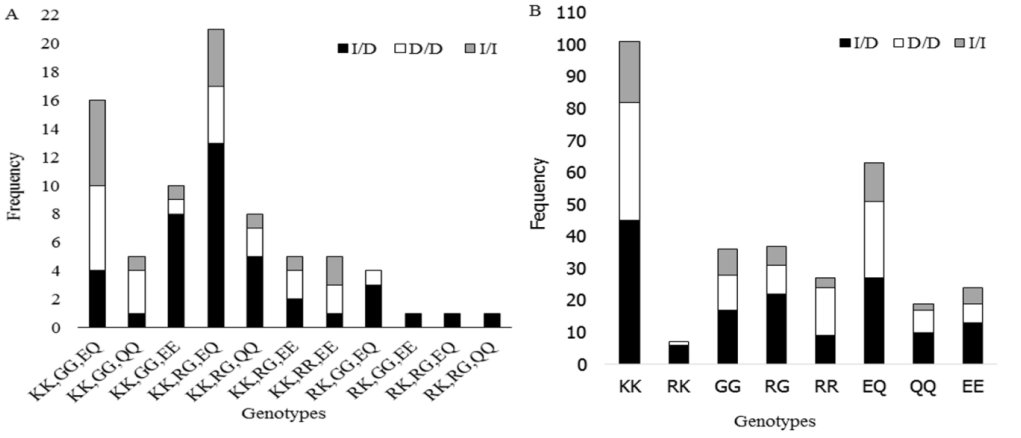

Figure 1. The distribution of genotype combinations in all judo athletes.

To determine the genotypes of the *ADRB2* gene by the RFLP-PCR technique, the following primers were used: sense (AB3) 5′-GAACGGCAGCGCCTTCTTGCTGGCACCCCAT-3` and antisense (AB2) 5′- CTGCCAGGCCCATGACCAGATCAG-3`[24]. PCR assays were carried out in a total volume of 20 µl containing 10 pmol of each primer, 0.5 Taq polymerase (Kapa Biosystems), 0.2 mM dNTP Mix (Thermo Scientific) and 1X reaction buffer with 1.5 mM $MgCl_2$. PCR conditions were as follows: 2 min of initial denaturation at 94°C for 2 min, denaturation at 94°C for 30 s, primer annealing at 64°C for 45 s, elongation at 72°C for 60 s, with a cycle number of 35. PCR products were digested for 16 hours at 37°C by restriction enzymes 4U Eco130I (Thermo Scientific) and 3U Fnu4HI (Thermo Scientific), in a separate tube. The genotypes of the *MSTN* (GDF-8) gene were determined by the RFLP-PCR technique. For this gene, the following primers were used: 3`-TGGATGGAAAACCCAAATGT-5' and 3`- GCCTGGGTTCATGTCAAGTT-5`. The primers were designed using Primer3 Software. PCR assays were carried out in a total volume of 10 µl containing 10 pmol of each primer, 0.5 U Taq polymerase (Kapa Biosystems), 0.2 mM dNTP Mix (Thermo Scientific) and 1X reaction buffer with $MgCl_2$. PCR conditions were as follows: 2 min of initial denaturation at 95°C for 2 min, denaturation at 95°C for 30 s, primer annealing 55°C for 45 s, elongation 72°C for 60 s, with a cycle number of 35. PCR products were digested for 16 hours at 37°C by ApaI enzyme (Thermo Scientific). All PCR products were separated on 2.5% agarose gels stained with Midori Green (Nippon Genetics). The patterns for all genotypes are included in Table 1.

Statistical analysis

An analysis of the distribution of compliance with the Hardy-Weinberg disequilibrium of genotypes was performed using a chi-square test. The correlation between the results obtained during competitions, body weight, skeletal muscle strength and studied polymorphisms were assessed using a chi-square test. The medians between groups were analysed by the Wald Wolfowitz test. Moreover, a statistical analysis between genotypes and selected traits was tested using two-way ANOVA. The level of significance was set at 0.05. The groups of athletes were selected based on the combinations of genotypes presented in Figure 1. Statistical analyses were performed using the Statistica package (Statsoft, Poland).

The proportion of combined genotypes compared with I/D polymorphisms of the *ACE* gene: 1- KK, Gly16Gly, Glu27Gln; 2- KK, Gly16Gly, Gln27Gln; 3- KK, Gly16Gly, Glu27Glu, 4- KK, Arg16Gly, Glu27Gln; 5- KK, Arg16Gly, Gln27Gln; 6- KK, Arg16Gly, Glu27Glu; 7- KK, Arg16Arg, Glu27Glu; 8- RK, Gly16Gly, Glu27Gln; 10- RK, Gly16Gly, Glu27Glu; 11- RK, Arg16Gly, Glu27Gln; 12- RK, Arg16Gly, Gln27Gln. Numbers/ Combination of genotypes 9- RK, Gly16Gly, Gln27Gln, 13- RK Arg16Gly, Glu27Glu, 14- RK,Arg16Arg, Glu27Gln; 15- RK Arg16Arg, Glu27Gln ;16- RK, Arg16Arg, Glu27Glu are not included in this figure because there were not present such a case (Figure 1A). The proportion of RK, Arg16Gly and Gly27Gln polymorphisms compared with I/D polymorphisms. The following notation is used in both figures: Glu → E, Gln → Q, Arg → R, Gly → G (Figure 1B).

Table 2. A contribution of the genotypes and alleles in judo athletes from Poland and Croatia.

Gen	Genotype/allele	Polish judo		All Polish judo athletes	Croatian judo athletes	Total judo athletes
		non-elite	elite			
		participants: N (%)				
ACE	I/D	30 (39.47)	4 (66.66)	34 (40.74)	17 (65.38)	51 (46.78)
	D/D	31 (40.79)	2 (33.33)	33 (40.74)	5 (19.23)	38 (34.86)
	I/I	15 (19.74)	-	15 (18.52)	4 (15.39)	19 (17.43)
	D	92 (60,53)	8 (66,66)	100 (61.11)	27 (51.92)	127 (58.8)
	I	60 (39.47)	4 (33.34)	64 (38.89)	25 (48.08)	89 (41.2)
ADRB2	Gly16Gly	25 (33.33)	2 (33.33)	27 (33.33)	11 (42.31)	38 (35.18)
	Arg16Gly	28 (37.33)	2 (33.33)	30 (35.33)	10 (38.46)	40 (37.03)
	Arg16Arg	23 (29.34)	2 (33.33)	25 (31.34)	5 (19.23)	30 (27.79)
	16Gly	78 (51.31)	6 (50)	84 (51.22)	32 (61.54)	116 (53.7)
	16Arg	74 (48.69)	6 (50)	80 (48.78)	20 (38.46)	100 (46.3)
	Glu27Gln	48 (63.15)	5 (83.33)	53 (64.63)	13 (50)	66 (61.11)
	Gln27Gln	13 (17.12)	-	13 (15.86)	6 (23.07)	19 (17.7)
	Glu27Glu	15 (19.73)	1 (16.66)	16 (19.51)	7 (26.93)	23 (21.29)
	27Glu	78 (51.32)	7(58.33)	85 (51.83)	27 (51.92)	112 (52.31)
	27Gln	74 (48.68)	5 (54.67)	79 (48.17)	25 (48.08)	104 (47.69)
MSTN	KK	73 (96.05)	4 (66.66)	77 (93.9)	24 (92.31)	101(93.58)
	RK	3 (3.95)	2 (33.33)	5 (6.1)	2 (7.69)	7 (6.42)
	RR	-	-	-	-	-
	K	149 (98.02)	10 (83.33)	159 (96.95)	50 (96.15)	209 (96.78)
	R	3 (1.98)	2 (16.67)	5 (3.05)	2 (3.85)	7 (3.21)

RESULTS

In all judo participant groups, the most common genotypes were: ID (46.8%), Arg16Gly (37%), Glu27Gln (61.1%) and KK (93.6%). In elite Polish judo athletes (n = 6), (the small number of competitors is due to the small number of weight classes in this discipline (−60, −66, −73, −81, −90, +100), the most common genotypes were: ID (66.7%), Glu27Gln (83.3%), and KK (66.7%). In Arg16Gly polymorphisms, there was no difference between the frequencies of genotypes (Table 2). The median between the frequency of I/D polymorphism between judoka groups differed significantly between Polish judo participants (including Polish elite) and Croatian (p=0.007). The distribution of genotypes and alleles of *ACE* and *MSTN* was consistent with Hardy-Weinberg equilibrium while genotypes and alleles of the *ADRB2* gene were not consistent with Hardy-Weinberg equilibrium.

Judokas with ID and Gly27Gly genotypes may have better results during the tournament than judokas with ID and Arg16Gly genotypes – these genotypes are associated with athletes who got mostly p*lace* different than 1st (Figure 2). Additionally, judokas with other genotypes had worse results in tournaments (data not shown) compared to athletes with genotypes combination ID and Gly27Gly or ID and Arg16Gly.

In this study we did not find correlation between polymorphisms of *MSTN*, *ADRB2* and *ACE* gene (Tables 3, 4, 5) with:

1) the results obtained during competitions: KR polymorphism (χ^2=8.5; p=0.3); Gln27Glu polymorphism (χ^2=13.05, p=0.91); Arg16Gly polymorphism (χ^2 =16.07, p=0.31); I/D polymorphism (χ^2=18.5, p=0.19). However, individuals with KK and Glu27Gln genotypes mostly occupied 2nd and 3rd p*lace* in competitions: 25% (2nd p*lace*) and 32%

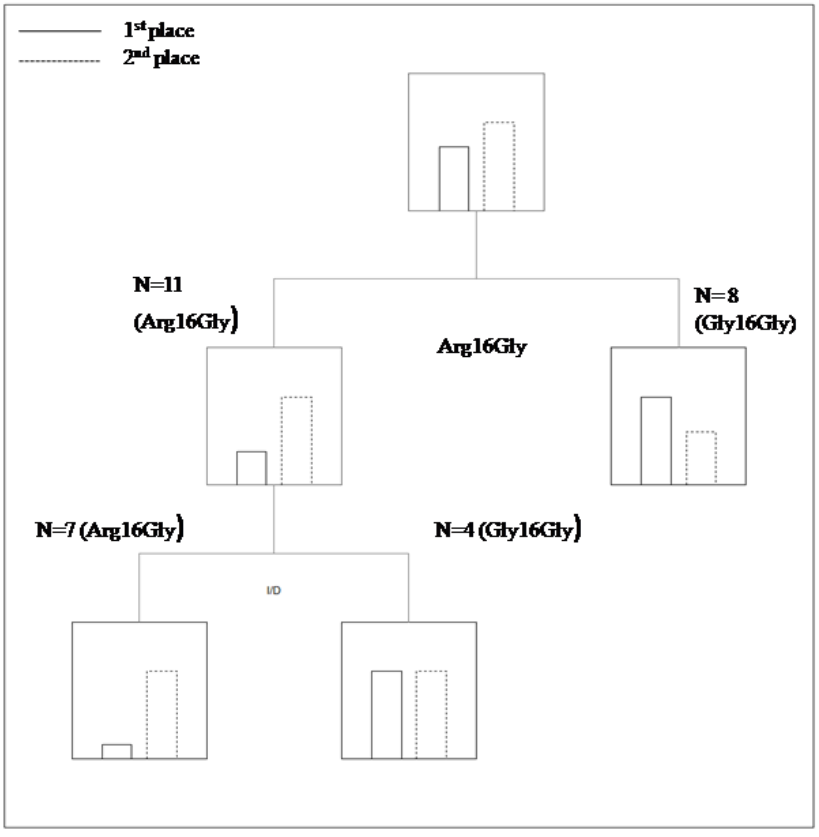

Figure 2. The contribution of genotypes Arg16Gly, GlyGly, ID, DD and p*lace*ment that judokas achieved in a competitive match (solid line- 1st p*lace*, dotted line- 2nd p*lace*). In this analysis, we present the combinations of alleles and p*lace*ment with statistically significant differences.

(3rd p*lace*) of participants with Glu27Gln genotype; 27% (2nd p*lace*) and 33.33% (3rd p*lace*) for participants with KK genotype, respectively. We also did not find any correlation between the frequency of training per week and the result during competitions (χ^2=94.62, p=0.03).

2) body weight: KR polymorphism: (χ^2=3.61, p=0.31); Gln27Glu polymorphism (χ^2=4.63, p=0.59); Arg16Gly polymorphism (χ^2=4.5, p=0.34); I/D polymorphism (χ^2=4.8, p=0.9) and 23% of individuals with ID genotype mainly take part in competitions at 73 kg.

3) skeletal muscle strength: leg muscle strength: KR polymorphism (χ^2=3.5, p=0.63), but individuals with the KK genotype (49.6%) trained by weight in classes between 70 and 100 kg; Gln27Glu polymorphism (χ^2=27.75, p=0.024) and Arg16Gly polymorphisms (χ^2=6.21, p=0.80); I/D polymorphism: (χ^2=0.77, p=0.37); bicep strength: KR polymorphism: (χ^2=0.16, p=0.92); Gln27Glu polymorphism (χ^2=7.24, p=0.30), these heterozygous individuals were divided into groups that trained biceps with 10-30 kg (50%) and 31-60 kg (50%); Arg16Gly polymorphisms (χ^2=1.99, p=0.74); I/D polymorphism: (χ^2=6.51, p=0.16) but athletes with ID genotype (72%) mainly trained with weights between 31-60 kg.

Individuals with combinations of genotype ID and Arg16Gly (p=0.06) (Figure 2) mostly p*lace*d 2nd during competitions. Moreover, judokas with the combination of genotype Glu27Gln and ID competed at bodyweight up to 90 kg (p=0.007) for the most part and athletes with the combination of genotype Gly16Gly and DD may have the most leg muscle strength (p=0.03).

Moreover, statistical analyses showed that the combination of the I allele and II genotype might lead to increased risk of joint injuries (OR=1.69, p=0.03; OR=6.13, p=0.05, respectively). In addition, the Gln27Gln genotype may have an influence on decreasing risk of spine injuries (OR=0.24, p=0.02) (Table 4).

Table 3. Joint injuries in judo participant groups.

Gene	Genotype/ allele	N (%) in the group without injuries	N (%) in the group with injuries	Odds ratio (OR)	Confidence interval (CI) 95%	p value
ACE	DD	27 (38.57)	11 (32.4)	Ref.	-	-
	ID	40 (57.14)	12 (33.8)	1.135	0.553-2,.330	0.73
	II	3 (4.29)	12 (33.8)	6.136	1.573-23.942	0.005
	Total					
	D-allele	94 (67.14)	34 (48.6)	Ref.	-	-
	I-allele	46 (32.86)	36 (51.4)	1.690	1.047-2.728	0.03
ADRB2	Gly16Gly	22 (29.73)	14 (40)	Ref.	-	-
	Arg16Gly	34 (45.95)	11 (31.4)	0.508	0.5196-1.321	0.16
	Arg16Arg	18 (24.32)	10 (28.6)	0.873	0.314-2.428	0.79
	Total					
	Gly-allele	78 (52.7)	39 (55.7)	Ref.	-	-
	Arg-allele	70 (47.3)	31 (44.3)	0.886	0.500-1.568	0.67
	Glu27Glu	18 (24.32)	6 (17.1)	Ref.	-	-
	Gln27Glu	43 (58.1)	23 (65.8)	1.159	0.389-3.453	0.79
	Gln27Gln	13 (17.58)	6 (17.1)	0.722	0.190-2.752	0.63
	Glu-allele	79 (53.4)	35 (50)	Ref.	-	-
	Gln-allele	69 (46.6)	35 (50)	0.873	0.494-1.543	0.64
MSTN	KK	70 (94.6)	32 (91.4)	Ref.	-	-
	RK	4 (5.4)	3 (8.6)	1.641	0.347-7.763	0.52
	Total					
	K-allele	144 (97.3)	67 (95.7)	Ref.	-	-
	R-allele	4 (2.7)	3 (4.3)	1.612	0.351-7.405	0.7

DISCUSSION

This is the first molecular study on such a large group of judo athletes, and we decided to focus on this type of discipline because it is poorly investigated and there are few papers published on this topic. Moreover, there is no data in the literature concerning the impact of Arg16Gly, Gln27Glu, and K153R polymorphisms in the described group and the combination of polymorphisms presented in this study have not yet been addressed in any judo participant group.

In this paper, we present distribution of the I/D polymorphism in the *ACE* gene, Glu27Gln and Arg16Gly polymorphisms in the *ADRB2* gene and K153R polymorphism in the *MSTN* gene among judo athletes from Poland and Croatia. The contribution of haplotypes on the Y chromosome are similar [25], and the frequency of genotypes in I/D polymorphism was significantly different. We tested which variants are desirable in this sport and which variants can protect from or predispose to injury, and affect achievement.

The I/D polymorphism in the *ACE* gene is the most widely studied genetic variant in the context of sport achievements. Researchers have shown a linear relationship between the distribution of I allele (which is associated with higher cardiovascular fitness and ability to endure longer exercises) and the length exercise performance by athletes [26]. In contrast, the D allele is associated with a short aerobic capacity [27], and in our study, the D allele was more frequent. In a thesis presented by Myerson S et al. [28], a group of judo athletes was also analysed but at a much smaller group number (20 judo and taekwondo athletes formed one group). Myerson S et al. demonstrated that the less frequent genotype was DD (20%). The other genotypes, ID and II, occurred with the same frequency (40%). The differences

Table 4. Spine injuries in judo participant groups.

Gene	Genotype/ allele	N (%) in the group without injuries	N (%) in the group with injuries	Odds ratio (OR)	Confidence interval (CI) 95%	p value
ACE	DD	22 (31.8)	16 (45)	Ref.	-	-
	ID	34 (49.3)	18 (40)	0.728	0.308-1.722	0.47
	II	13 (18.9)	6 (15)	0.635	0.199-2.029	0.44
	D-allele	78 (56.5)	50 (62.5)	Ref.	-	-
	I-allele	60 (43.5)	30 (37.5)	0.780	0.444-1.371	0.38
ADRB2	Gly16Gly	21 (30.4)	15 (37.5)	Ref.	-	-
	Arg16Gly	27 (39.2)	18 (45)	0.933	0.383-2.276	0.88
	Arg16Arg	21 (30.4)	7 (17.5)	0.467	0.158-1.377	0.16
	Gly-allele	69 (50)	48 (60)	Ref.	-	-
	Arg-allele	69 (50)	32 (40)	0.677	0.382-1,165	0.15
	Glu27Glu	19 (27.5)	5 (12.5)	Ref.	-	-
	Gln27Glu	41 (59.4)	25 (62.5)	0.549	0.196-1.535	0.25
	Gln27Gln	9 (13.1)	10 (25)	0.237	0.062-0.900	0.03
	Glu-allele	79 (57.2)	35 (56.2)	Ref.	-	-
	Gln-allele	59 (42.8)	45 (43.8)	0.581	0.333-1.013	0.054
MSTN	KK	65 (94.2)	37 (92.5)	Ref.	-	-
	RK	4 (5.8)	3 (7.5)	1.318	0.280-6.210	0.73
	Total					
	K-allele	134 (97.1)	77 (96.2)	Ref.	-	-
	R-allele	4 (2.9)	3 (3.8)	1.305	0.285-5.985	0.72

in genotype frequency between our study (ID-46.78%; DD- 34.86%; II- 17.43%) and that described in Myerson S et al. may result from the number of individuals and population or criteria differences between groups which took part in this study. The aforementioned thesis did not take into consideration the results obtained by judokas. A high frequency of the ID and II genotype may be the most desirable due to a limited period of effort, but also by increased glucose uptake in skeletal muscle. Cieszczyk P et al. [29] compare polymorphisms in the angiotensin-converting enzyme I gene between individuals not practising sports (n=115) were ID-50.4%; DD-30.4%; II-19.2% while in Judokas group (n=28) ID-64.3%; DD-28.6%; II-7.1%. Comparing our result to control group from Cieszczyk P et al. [29] study we not observed any differences, higher than 5%, in the distribution of genotypes. These differences in the distribution of genotypes show that more studies comparing athletes and sedentary controls are needed.

On the other hand, distribution of ID, DD and II genotypes in athletes which are involved in strength power training (examined in the context of the speed of muscle contraction) indicates that individuals with the D allele perform the same exercise more efficiently, and this allele is a major contributor to increases in muscle strength (+/-S.E.M: II 9.0 +/-1.7 %; ID, 17.6 +/-2.2 %; DD, 14.9 +/-1.3 %) [30]. These results correspond with our study; in our judoka group, we found a correlation between the combination of Gly16Gly and DD genotypes with leg strength (p=0.03). Additionally, individuals with Gln27Glu mainly performed bicep curls with a weight between 10-60 kg but ID heterozygous individuals mainly exercised with loads between 31-60 kg. This observation suggests that the D allele can influence strength. However, the I/D

Table 5. Injuries other than spine or joint in judo participant groups.

Gene	Genotype/ allele	N (%) in the group without injuries	N (%) in the group with injuries	Odds ratio (OR)	Confidence interval (CI) 95%	p value
ACE	DD	22 (29.7)	16 (45.7)	Ref.	-	-
	ID	37 (50)	15 (42.8)	0.557	0.231-1.344	0.19
	II	15 (20.3)	4 (11.5)	0.367	0.102-1.315	0.12
	D-allele	81 (54.7)	47 (67.1)	Ref.	-	-
	I-allele	67 (45.3)	23 (32.9)	0.592	0.326-1.072	0.08
ADRB2	Gly16Gly	24 (32.4)	12 (34.3)	Ref.	-	-
	Arg16Gly	32 (43.2)	13 (37.1)	0.812	0.315-2.093	0.66
	Arg16Arg	18 (24.4)	10 (28.6)	1.111	0.393-3.138	0.84
	Gly-allele	80 (54)	37 (52.8)	Ref.	-	-
	Arg-allele	68 (46)	33 (47.2)	1.049	0.593-1.855	0.87
	Glu27Glu	18 (24.3)	6 (17.8)	Ref.	-	-
	Gln27Glu	44 (59.4)	22 (62.8)	1.500	0.522-4.313	0.45
	Gln27Gln	12 (16.3)	7 (20)	1.750	0.471-6.502	0.4
	Glu-allele	80 (54)	34 (48.6)	Ref.	-	-
	Gln-allele	68 (46)	36 (51.4)	1.246	0.705-2.201	0.45
MSTN	KK	68 (91.9)	34 (97.1)	Ref.	-	-
	RK	6 (8.1)	1 (2.9)	0.333	0.038-2.881	0.29
	RR	0 (0)	0 (0)	-	-	-
	K-allele	142 (95.9)	59 (98.3)	Ref.	-	-
	R-allele	6 (4.1)	1 (1.7)	0.343	0.040-2.905	0.45

polymorphism of the *ACE* gene is not the main determinant of skeletal muscle power [31].

Furthermore, in our study, we found an association between ID and Glu27Glu genotypes and joint and spine injuries, respectively (Table 3, Table 4). To our knowledge, no such association has been described in the literature, and there are no described physiological or pathological pathways which can influence with injuries. Perhaps, lower frequency of injuries is not directly connected with polymorphisms of examined genes but with traits, which are regulated by these genes. In consequence, athletes with specific combinations of genotypes could be more susceptible to injuries than other athletes. In addition, more studies and more athletes should take part in studies are needed to determine the effect of described genotypes on injuries.

We did not find a correlation between KR polymorphism of the *MSTN* gene and leg muscle strength and biceps strength, but we observed that individuals with the KK genotype (49.6%) mainly trained leg muscles with loads between 70-100 kg. However, results presented by Santiago C et al. suggest that this polymorphism does influence peak power muscle strength [32]. Meyrson S et al. [28] suggests that this polymorphism affects muscle growth thickness. Differences in results between our investigation and the studies mentioned may be caused by differences in athletic discipline and group sample size.

The last analysed SNP`s are located in the androgen receptor gene. *ADRB2* is activated by adrenaline or noradrenaline [33], and *ADRB2* receptors are also involved in catecholamine function, thermogenesis and energy balance [14, 19, 34]. Distribution of polymorphisms in our studies (n=108) were Gly16Gly-35.18%; Gly16Arg-37.03%; Arg16Arg-27.79% and Gln27Gln-17.7%; Glu27Gln-61.11%; Glu27Glu-21.29%. Distributions

of mentioned genotypes in Sawczuk M et al. study in control group which were sedentary volunteers (n=354) were Gly16Gly-35.9%; Gly16Arg-47.7%; Arg16Arg-16.4% and Gln27Gln-33.1%; Glu27Gln-50.8%; Glu27Glu-16.1% [45]. The Arg-16Gly and Gln27Glu polymorphisms are associated with hypertension in Malaysian subjects ($p<0.05$) [13]. Homozygous Gly16Gly individuals demonstrate larger cardiac output in rest and during exercise ($p=0.048$) when compared with Arg16Arg homozygous ($p=0.035$) [35]. In a Caucasian male individuals, high diastolic blood pressure was associated with *ADRB2* haplotypes (OR=1.5) containing the pro-down regulatory Glu27 variant [36]. Earlier results presented can answer to the presence of a high frequency of Glu27Gln genotype and Gly16 allele in our carriers. Moreover, the Arg16 Gln27 haplotype might have some predictive value for poor outcomes in heart failure [37]. Arg16Gly and Gln27Glu polymorphisms are associated with response to bronchodilation [38]. Moreover, Janikowska G et al. [39] observed in road cyclists, correlations between *ADRB2* and *ROCK1* gene expression effects ($p=0.038$) during the effort. While Prior SJ et al. [34] found an association of Arg16Gly and Glu27Gln haplotypes with VO_{2max} and body composition ($\chi^2=22.1$, $p=0.015$). Our results also suggest that the combination of ID genotype of the *ACE* gene with Arg16Gly of the *ADRB2* gene has an influence on results obtained during competitions. The two genes: *Adrb2* and *ACE* have an influence on blood pressure and cardiac output, and this may be a reason for the correlation of these two genes with results obtained during competitions. Moreover, these genes can also affect the delivery of oxygen and nutrients to muscles.

The ADR genotype influences the accumulation of intermuscular fat in response to strength training. In addition, other studies have shown that intermuscular fat is significantly reduced (-2.3 ± 1.0 cm^3, $p=0.028$) with strength training in individuals carrying the Glu27 allele [40]. Homozygote Glu27Glu individuals were found to be more obese and had higher concentrations of triglyceride, leptin, and insulin compared with Gln27 homozygous in Saudi populations [41]. In our study, we demonstrated that the combination of Glu27Gln genotype with ID genotype of the *ACE* gene has an influence on relatively high body mass individuals (90 kg), but we did not observe obese participants in our group. On the other hand, Tringali C et al. [42] showed that the ADRB and FTO alleles are linked to a low body mass index and low-fat mass.

Analysis of gene markers which are associated with strength and physical performance in individuals that practice judo can provide new knowledge concerning the influence of genetic variants on success in this discipline.

CONCLUSIONS

Our investigation revealed that the combination of polymorphisms of the *ADRB2* gene (Arg16Gly genotype) and *ACE* gene (ID genotype) may have an influence on body mass weight and the results obtained during competitions between judo practitioners. It may suggest that the *ADRB2* gene plays a significant role during competition in the discipline of judo and hence may be a component of high achievement. In judo participants, I/D and Arg16Gly polymorphisms may influence leg muscle strength, and interestingly, I/D and Glu27Gln might influence body mass in judo practitioners. To confirm these observations, it will be necessary to expand our study on a larger group.

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