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Official URL: <https://doi.org/10.1519/JSC.0000000000003142>

DOI: <http://dx.doi.org/10.1519/JSC.0000000000003142>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/6527>

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**The effect of *COL5A1*, *GDF5* and *PPARA* genes on a movement screen and neuromuscular performance in adolescent team sport athletes**

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Accepted for publication in the Journal of Strength and Conditioning Research. ISSN 1064-8011

## INTRODUCTION

Sports games are popular among adolescent at various performance levels, and are important for the development of physical health, mental health, and for social development (46, 64). Adolescence is characterized by maturation, which evokes muscle-tendon unit growth resulting in a steep increase in strength and power performance on the one hand (34) and an increased risk of injury on the other (45, 58). Both physical performance and non-contact injuries are multifactorial domains including many intrinsic and extrinsic factors, which should also include the genetic profile (38, 46). However, the effect of genetics on muscle injury predictors and neuromuscular performance in adolescents is scarce in current research.

It has been acknowledged that the risk of injury increases with chronological age and may be related to important periods during growth and maturation (45). According to a previous study, the 13 to 18-year-old age group is subject to the greatest risk and most injuries are likely to occur during this period (53, 58). Others have suggested that injury incidence is highest around the time of Peak Height Velocity (PHV) (62), and that females appear to have a greater relative risk of a non-contact injury compared with males when hours of athlete exposure are taken into account (63). The higher incidence of injury during PHV in women may be explained by anatomical, neuromuscular and hormonal differences (20), or by genetic factors influencing the soft tissue (2, 33, 56). Therefore, current efforts focus on injury prediction during the high risk maturation period, because a sustained injury itself is one of the strongest re-injury predictors (12, 23).

Physical performance and the risk of injury have been associated with ligament and tendon properties which are dependent on individual genotypes. The collagen alpha chain (*COL5A1*) gene has been associated with mechanical properties of tendon structure in the knee extensors in vivo (26), and tendon and ligament injuries (57). Specifically, the *COL5A1* rs12722 CT heterozygotes

has been linked to poorer flexibility than CC and TT homozygotes (6) during a sit and reach test. Peroxisome proliferator-activated receptor alpha (*PPARA*) and growth differentiation factor (*GDF5*) genes have been associated with power performance (48), stress fractures (66) and muscle regeneration (18). Specifically, it has been suggested that *PPARA* predicts anaerobic trainability (1), and aerobic trainability (43, 47, 49), while *GDF5* regulates the response of the proliferation satellite cell (18) (crucial after resistance training), meniscus injury incidents, and knee joint function recovery (14). Therefore, the combination of candidate genes determining the loading response, injury incident and recovery rate might be important during the period of maturation. Moreover, the above-mentioned genes polymorphisms might be related to stretch shortening cycle capability (determined from leg stiffness and reactive strength index) (30, 31), but the importance of these parameters still need to be identified within young sporting population. The collagen tissue quality is genetically determined, inter alia, by genes encoding collagens, where the *COL5A1* gene (rs12722, rs3196378, rs11103544) seems to play a key role in the probability of knee and achilles tendon injury (57) and muscle flexibility (6). Therefore, the relationship between mechanical properties such as leg stiffness and reactive strength index (RSI) might be correlated with the *COL5A1* gene in addition to genes related to performance (*PPARA*), injury and recovery predisposition (*GDF5*).

The genetic predisposition for collagen production (*COL5A1*), carbohydrate and protein metabolism (*PPARA*), cell differentiation and the transforming growth factor- $\beta$  superfamily (*GDF5*) has a potential to determine overuse injuries or complex phenotypes related to tissue properties such as leg stiffness (LS) or RSI (33). Specifically, the *GDF5* rs143383 A allele carriers have been shown to have lower *GDF5* transcriptional activity in chondrogenic cells than GG homozygotes, which might influence the amount of cartilage of the vertebrae, limb dimensions or

joint angles (42, 55). In addition to above mentioned genetic predisposition, the power and stretch shortening cycle performance is related to *PPARA* rs4253778, where C allele carriers have shown greater power related outcomes than TT homozygotes (48) in adult athletes, however this relationship has not yet been confirmed in adolescents.

Stiffness of the whole limb is affected by muscle and tendon mechanical properties as well as elastic properties of the joint structures and stiffness arising from muscle actions (21). Lower limb stiffness points to an ability to generate strength and to be able to resist deformation resulting from movement, including a direct transition from eccentric to concentric muscle contraction (44) in a stretch shortening cycle. Therefore, leg stiffness should be closely related to the RSI (30), which has been reported as a predictor of injury in adolescent athletes (51). Leg stiffness and RSI are typically based on a squat jump movement pattern, where the hip extensor (posterior) muscle chain is crucial for successful technical execution of the test. This posterior muscle chain includes several critical muscle groups such as the hamstrings and low back extensors, whose shortening or other imbalances have been identified as injury predictors (10, 16, 24, 40).

The current research has identified muscle flexibility, functional movements screens, leg stiffness, RSI and muscle strength as injury predictors, all of which depend on the collagen tissue condition (21). As the predictability of musculoskeletal conditions by genetic factors is not sufficiently documented, the purpose of this study was to determine whether the *COL5A1*, *PPARA* and *GDF5* genes are associated with muscle functions and stretch shortening cycle performance in adolescent athletes.

## **MATERIALS AND METHODS**

### **Experimental approach to the problem**

At the beginning of the competitive season, a cross-sectional measurement of anthropometry and muscle function were performed by each participant. An injury record for the past 12 months was obtained by a physician specialized in neurophysiology and muscle function. The participants were screened for anthropometry, DNA, muscle function, and neuromuscular performance (RSI and LS during vertical jumps). The participants were also requested not to exercise in excess of their normal training habits 2 days before the test in order to exclude the effects of delayed muscle soreness on muscle function (37).

### **Subjects**

The participants were 146 youth players (age 13-15y,  $14.4 \pm 0.2$ y) of various team sports (basketball n= 54, soccer n= 50, handball n= 32), both sex (90 male, 56 female) with a high potential of lower limb soft tissue injury (Table 1). All participants were players in the highest league in their sport with at least 6 years of organized training experience and their current habitual training cycle met the following criteria as minimum: 6 training sessions per week, 160min of conditioning work, 120min of technical-tactical training, 190min of game time and 130min of warm ups . The research and the informed consent form were approved by the institutional ethics committee of the Palacky University Olomouc, Faculty of Physical Culture in accordance with the ethical standards of the Helsinki Declaration of 2013, and a signed written informed consent form was obtained from the parents of all adolescents participating in this study before measurements.

Table 1. Participant characteristics according to game and sex.

	All participants	Handball	Basketball	Soccer	Female	Male
	(n = 146)	(n=32)	(n=54)	(n=50)	(n=56)	(n=90)
Body mass (kg)	60.30 ± 14	56.77 ± 8	62.95 ± 13	59.97 ± 18	57.32 ± 8.7	62.72 ± 12
Stature (cm)	169.25 ± 12	164.43 ± 7	173.36 ± 11	168.45 ± 15	164.53 ± 10	172.49 ± 9
Age (years)	14.36 ± 1.18	14.10 ± 1.21	14.54 ± 1.15	14.37 ± 1.05	14.14 ± 1.32	14.57 ± 1.09
Leg Length (cm)	81.27 ± 7.98	78.91 ± 3.8	84.86 ± 6	78.81 ± 10	79.30 ± 9.1	82.73 ± 8.1

## Procedures

### *Biological maturity*

Biological maturity was determined using the gender-specific equation determined by Mirwald (41) based on measurement of leg length, body mass, standing and sitting heights. The equation for maturity offset in males (years) was  $-9.236 + (0.0002708 \text{ (Leg length and sitting height interaction)}) - (0.001663 \text{ (age and leg length interaction)}) + 0.007216 \text{ (age and sitting height interaction)} + (0.02292 \text{ (body mass by height ratio)})$  with reported coefficient of determination  $R^2 = 0.915$ , and standard error of estimate  $SEE = 0.490$  (41). The equation for maturity offset in female (years) was  $-9.376 + (0.0001882 \text{ (Leg length and sitting height interaction)}) + (0.0022 \text{ (age and leg length interaction)}) + 0.005841 \text{ (age and sitting height interaction)} - 0.002658 \text{ (age and body mass interaction)} + 0.07693 \text{ (body mass by height ratio)}$  with reported coefficient of determination  $R^2 = 0.910$ , and  $SEE = 0.499$  (41). The maturity offset was used as categorical value to identify the group of participants before PHV (pre-PHV) and after PHV (post-PHV), where any negative maturity offset prediction was classified as pre-PHV and any positive prediction as post-PHV. The numerical value of maturity offset was used as a value representing biological maturity in other analyses.

### *Anthropometry*

Anthropometry measurements were used to describe the participants, biological maturity estimation and normalization of leg stiffness. All measurements were undertaken by an experienced anthropometric technician according to the procedures of the International Society for the Advancement of Kinanthropometry (ISAK) (36). Leg length, tibia length, standing and sitting heights were measured using the A-226 Anthropometer (Trystom, Olomouc, CZ) with sliding telescopic sleeves. Body mass has been measured using the 2-axis force platform PS-2142 (Pasco, Roseville, USA).

### *DNA analyses*

DNA was extracted from Flinders Technology Associates Classic cards (Cat. no. WB120305; Whatman International Ltd, Piscataway, NJ) according to the Whatman FTA® Elute protocol. A panel of SNPs in the genes associated with genes previously related to tendon structure, ligament structure and muscle function were selected as candidate variants for the present study (Table 2); the selected SNPs were examined using MALDI-TOF MS based MassARRAY (Agena Bioscience, San Diego, CA, USA) genotyping assay (25).

Table 2. Analysed gene variants by MassARRAY (ADS v.20)

<b>Gene</b>	<b>SNP ID</b>	<b>Chr: Position</b>	<b>Allele</b>	<b>Location</b>
<i>COL5A1</i>	rs12722	9:137734416	C/T	3'-UTR
	rs11103544	9:137735043	T/C	3'-UTR
<i>PPARA</i>	rs4253778	22:46630634	G/C	Intron
<i>GDF5</i>	rs143383	20:34025983	A/G	5'-UTR



### *Functional movement screen tests*

The test of muscle functions included a functional bend test (FBT), passive straight leg raise test (SLR), and individual muscle tests of the hip adductors, rectus femoris, tensor fascia lata, and iliopsoas. All manual muscle tests had acceptable reliability: FBT test (CV = 9.86%; ICC = 0.89), SLR (CV = 5.46%; ICC = 0.85), lower limb muscle tests (SEM below 10%, ICC above 0.88) (5). The tests were selected according to the fact that altered musculotendinous functions such as flexibility may be associated with musculotendinous injuries (10, 16, 24, 40, 51). The functional screening measurements were conducted by the same experienced researcher. The SLR was performed according to the procedures of Göeken (15) using a three point scale (1 flexible, 3 moderate, 3 stiff); the FBT, known also as the Thomayer or toe touch test was performed according to the procedures of Janda (22, 27) using a five point scale (1 hyperflexible, 2 flexible, 3 medium, 4 stiff, 5 extremely stiff); and individual muscle tests were performed according to the procedures of Janda (22) using a three point scale (1 flexible, 3 moderate, 3 stiff). All functional tests were assessed twice and the average score was used for further analyses.

### *Leg stiffness*

Absolute LS was measured during the sub-maximal bilateral hopping test performed using a mobile 2-axis force platform PS-2142 (Pasco, Roseville, USA) at a hopping frequency of 2.5 Hz. This frequency was chosen to ensure that the movement patterns are reflective of typical spring-mass model behaviour (30). Relative LS was normalized to leg length and body mass (39). The participants were asked to hop two-legged on the force plates for 20 consecutive hops. Leg stiffness ( $\text{kN}\cdot\text{m}^{-1}$ ) was calculated using the measures of body mass, contact times and flight times, which is known to be a valid and reliable method (11) with a reported ICC = 0.93 and CV = 9.48% in children (30).

### *Reactive strength index*

The RSI was determined during a 5 maximum hop test which was performed on a mobile contact mat (FITRO Jumper, Fitronic, Slovakia). The RSI been shown to have high test-retest reliability (13) with reported ICC = 0.90 and CV = 14.24% in children (30). The participants were instructed to maximize jump height and minimize ground contact time (11) and performed 3 trials. The RSI variable was calculated using the equation by Flanagan and Comyns (13), where  $RSI = \text{Jump height (mm)} / \text{ground contact time (ms)}$  and  $\text{jump height (m)} = (\text{gravity} \cdot (\text{Flight time})^2) / 8$ , where gravity is  $9.81 \text{ m} \cdot \text{s}^{-1}$  and flight time in seconds. The first hop served as a countermovement jump and was consequently excluded from analysis, with the 4 remaining hops averaged for analysis of RSI. Players performed three trials with 2 min rest between trials. The greatest value recorded from the three attempts was used in further analysis.

### **Statistical analyses**

The phenotype and genotype data are presented in the supporting information file (Supplementary file 1). The data was processed using the ORIGIN software (version 2018b SR0, OriginLab, Wellesley Hills, MA, USA,) where statistical significance was set up at  $\alpha < .05$ . All analyses were performed separately for each sex. Genotype and allele frequencies between pre-PHV and post-PHV groups were compared using  $\chi^2$  test to identify potential differences in maturity status. In addition to frequency analyses, the phenotypes according to maturity status were compared using a Wilcoxon-Mann-Whitney U test. All variables in the genotype groups were tested for normality using the Kolmogorov-Smirnov test. As all variables were normally distributed, the data are expressed as mean and standard deviations. A MANOVA for unequal sample sizes (phenotype outcome x gender x polymorphism) was used to evaluate the differences between genotype groups, where  $p < 0.05$ , post-hoc Tukey tests, with Hays  $\omega^2 > 0.09$  were considered significant. The  $\omega^2$

0.10-0.29, 0.30-0.49 and >0.50 were considered as weak, moderate and strong associations, respectively (19).

## RESULTS

The genotype frequency did not disrupt the Hardy Weinberg equilibrium (HWE) and did not show any differences in genotype frequency in comparison with an EU population and the population of Utah with Northern and Western European Ancestry (Table 3). Some genotype groups contained a low sample of carriers: men rs12722 CC genotypes (n= 1), women rs12722 CC genotypes (n= 1), women rs11103544 CC genotypes (n= 2). The low sample groups were eliminated from statistical analyses if appropriate. The Kolmogorov-Smirnov test revealed no grounds for rejecting the hypothesis of normality in any genotype group included in MANOVA.

Table 3. Genotype frequencies and comparison with EU population (EU) and population of Utah with Northern and Western European Ancestry (CEU), allele frequency and Hardy-Weinberg equilibrium (HWE) expressed by  $\chi^2$ .

Gene (N)	SNP	Genotype	Genotype Frequency	$\chi^2$	EUR fr (n=503)	CEU fr (n=99)	Allele	Allele Frequency
<b>COL5A1</b> 138	rs11103544 T>C	TT	0.746	2.08	0.716	0.758	T	0.849
		TC	0.217		0.264	0.232	C	0.161
		CC	0.036		0.020	0.010		
102	rs12722 C>T	TT	0.669	0.51	0.356	0.354	T	0.828
		CT	0.309		0.459	0.444	C	0.173
		CC	0.022		0.185	0.202		
<b>GDF5</b> 127	G>A rs143383	GG	0.236	3.55	0.161	0.091	G	0.405
		GA	0.374		0.421	0.414	A	0.595
		AA	0.390		0.417	0.495		
<b>PPARA</b> 138	C>G rs4253778	CC	0.051	1.37	0.040	0.030	C	0.194
		CG	0.275		0.304	0.333	G	0.806
		GG	0.673		0.656	0.636		

Biological maturity status did not show any differences between pre and post PHV groups (Table 4) in genotype frequencies, allele frequencies (Table 5 and 6) and in phenotype values (Table 7). The MANOVA showed differences in maturity offset between female *COL5A1* rs12722 genotype

groups ( $F_{1,78} = 12.1$ ,  $p = .029$ ,  $\omega^2 = 0.22$ ), where CT heterozygotes showed a lower maturity offset than TT homozygotes (Figure 1).

**Figure Table 4.** Basic anthropometrics characteristic of sex and maturation groups and differences between pre and post peak height velocity groups by Wilcoxon-Mann-Whitney U test.

Test	Male (n= 90)			Female (n= 56)		
	Pre PHV (n= 19)	Post PHV (n= 71)	T test p	Pre PHV (n= 35)	Post PHV (n= 21)	U test p
Age	13.26 ± 0.48	14.78 ± 1.0	<0.001	13.0 ± 0.75	14.84 ± 0.94	<0.001
Body mass (kg)	54 ± 8.7	65 ± 11.4	<0.18	51 ± 7.3	60 ± 7.7	<0.001
Height (cm)	156 ± 0.10	175 ± 9.19	<0.001	160 ± 5.1	167 ± 5.9	<0.001
Sitting Height (cm)	80 ± 8.8	84.1 ± 8.0	<0.001	82 ± 3.5	87 ± 4.46	0.18
Leg length (cm)	75 ± 9.1	83.9 ± 8.1	<0.001	77.9 ± 4.2	80.1 ± 9.0	<0.001
Tibia length	45.5 ± 4.7	49.56 ± 5.5	0.003	43.48 ± 4.1	46.06 ± 3.4	0.001

PHV = peak height velocity. Wilcoxon-Mann-Whitney U test

**Table 5.** Gene and allele frequency comparison in pre and post peak height velocity groups in females.

Female			Genotype								Allele					
			Pre PHV		Post PHV		$\chi^2$ test				Pre PHV		Post PHV		$\chi^2$ test	
Gene (n)	SNP	Geno type	n	f	n	f	$\chi^2$	p	allele	n	f	n	f	$\chi^2$	p	
COL5A1 54	rs11103544 T>C	TT	13	0.650	24	0.706			T	33	0.825	56	0.824	0.10	0.75	
		TC	7	0.350	8	0.235	1.8	0.40	C	7	0.175	10	0.177			
		CC	0	---	2	0.059										
51	rs12722 C>T	TT	8	0.381	14	0.467			T	28	0.667	44	0.733	0.30	0.58	
		CT	12	0.571	16	0.533	1.67	0.43	C	13	0.333	16	0.267			
		CC	1	0.048	0	---										
GDF5 46	G>A rs143383	AA	8	0.471	10	0.345			A	22	0.647	32	0.551	2.11	0.15	
		AG	6	0.353	12	0.414	0.74	0.69	G	9	0.353	26	0.448			
		GG	3	0.176	7	0.241										
PPARA 54	C>G rs4253778	CC	1	0.050	3	0.088			C	11	0.275	13	0.191	1.02	0.31	
		CG	9	0.450	7	0.206	3.62	0.16	G	29	0.725	55	0.808			
		GG	10	0.500	24	0.706										

$f$  = frequency, PHV= peak height velocity

**Table 6.** Gene and allele frequency comparison in pre and post peak height velocity groups in males.

Gene (n)	SNP	Geno type	Genotype						Allele				$\chi^2$ test		
			Pre PHV		Post PHV		$\chi^2$ test		Pre PHV		Post PHV				
			n	f	n	f	$\chi^2$	p	allele	n	f	n	f	$\chi^2$	p
COL5A1 84	rs11103544 T>C	TT	11	0.846	53	0.753			T	24	0.923	119	0.842	1.31	0.25
		TC	2	0.154	13	0.178	1.14	0.56	C	2	0.077	23	0.157		
		CC	0	--	5	0.068									
49	rs12722 C>T	TT	10	0.769	32	0.556			T	23	0.884	68	0.944	1.03	0.31
		CT	3	0.231	4	0.444	1.11	0.29	C	3	0.115	4	0.056		
		CC	0	---	0	---									
GDF5 80	G>A rs143383	AA	5	0.385	28	0.418			A	16	0.615	80	0.597	0.03	0.86
		GA	6	0.462	24	0.358	0.32	0.85	G	10	0.385	54	0.402		
		GG	2	0.154	15	0.224									
PPARA 84	C>G rs4253778	CC	0	--	3	0.042			C	2	0.077	27	0.190	1.97	0.16
		CG	2	0.154	21	0.296	1.90	0.38	G	24	0.923	115	0.810		
		GG	11	0.846	47	0.662									

*f* = frequency, PHV= peak height velocity

**Table 7.** The summary of functional test results and performance tests by sex and maturation and differences between pre and post peak height velocity groups by Wilcoxon-Mann-Whitney U test.

Test	Male (n= 90)			Female (n= 56)		
	Pre PHV (n= 19)	Post PHV (n= 71)	U test p	Pre PHV (n= 35)	Post PHV (n= 21)	U test p
Functional bend test	2.69 ± 1.37	2.68 ± 1.10	0.98	2.9 ± 1.48	3.38 ± 1.3	0.70
Straight leg raise test	1.62 ± 0.51	1.75 ± 0.43	0.30	1.6 ± 0.50	1.82 ± 0.38	0.21
Iliopsoas shortening	1.08 ± 0.29	1.11 ± 0.31	0.78	1.05 ± 0.23	1.00 ± 0.09	0.36
Rectus femoris	1.91 ± 0.29	1.90 ± 0.30	0.89	1.37 ± 0.50	1.50 ± 0.51	0.18
Tensor fascia latae	1.42 ± 0.51	1.28 ± 0.45	0.32	1.21 ± 0.42	1.08 ± 0.29	0.21
Hip abductors	1.08 ± 0.29	1.18 ± 0.39	0.42	1.05 ± 0.23	1.02 ± 0.17	0.67
Reactive strength index (mm·ms <sup>-1</sup> )	1.35 ± 0.33	1.41 ± 0.37	0.19	1.24 ± 0.34	1.29 ± 0.39	0.75
Relative leg stiffness	35 ± 6.7	41 ± 9.1	0.15	31 ± 7.2	37 ± 8.3	0.71

U = Wilcoxon-Mann-Whitney U test. The values of functional test are point on three or five point scale.

The FBT showed differences between *COL5A1* rs12722 genotype groups in males ( $F_{1, 39} = 10$ ,  $p = .003$ ,  $\omega^2 = 0.18$ ) and females ( $F_{1, 37} = 8.5$ ,  $p < .001$ ,  $\omega^2 = 0.16$ ), where CT heterozygotes had lower functional test scores than TT homozygotes (Figure 2). The FBT showed differences between male

*COL5A1* rs11103544 genotype groups ( $F_{2, 83} = 8.1$ ,  $p = .049$ ,  $\omega^2 = 0.14$ ), where TT and CC homozygotes resulted in better FBT scores than TC heterozygotes (Figure 2).

The SLR showed differences between *COL1A1* rs12722 genotype groups in males ( $F_{1, 39} = 5.3$ ,  $p = .027$ ,  $\omega^2 = 0.11$ ) and females ( $F_{1, 37} = 5.6$ ,  $p = .027$ ,  $\omega^2 = 0.10$ ), where CT heterozygotes showed lower test scores than TT homozygotes (Figure 3). The SLR showed differences in males between *GDF5* rs143383 genotype groups ( $F_{2, 82} = 5.9$ ,  $p = .030$ ,  $\omega^2 = 0.11$ ), where GG homozygotes showed lower (better) test scores than AA and AG genotypes (Figure 3).

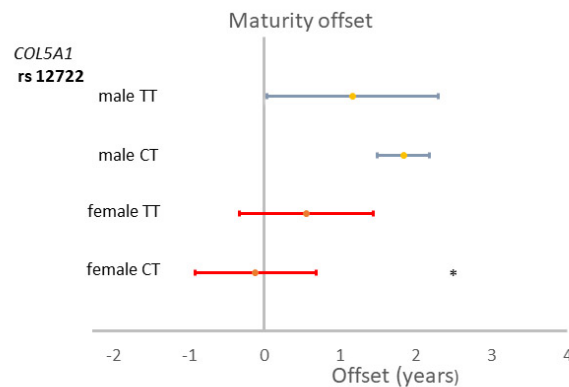


Figure 1 Maturation offset differences in genotype groups. *COL5A1* = collagen alpha-1(V) chain. \*significantly different than other genotype groups in the same sex.

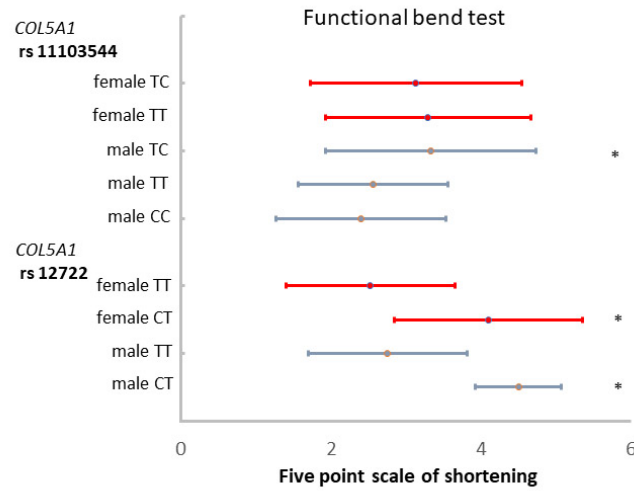


Figure 2 Functional bend test in different genotype groups. The scale point 5 means lowest flexibility. COL5A1= Collagen alpha-1(V) chain. \*significantly different than other genotype groups in the same sex.

The RSI differed between *GDF5* rs143383 genotype groups in males ( $F_{2, 73} = 5.8$ ,  $p = .050$ ,  $\omega^2 = 0.10$ ) and females ( $F_{2, 48} = 3.9$ ,  $p = .033$ ,  $\omega^2 = 0.11$ ), where AA homozygotes and AG heterozygotes had greater RSI than GG homozygotes (Figure 4). The best RSI differed between *PPARA* rs4253778 in males ( $F_{2, 74} = 5.9$ ,  $p = .049$ ,  $\omega^2 = 0.11$ ) and females ( $F_{2, 49} = 4.6$ ,  $p = .034$ ,  $\omega^2 = 0.12$ ), where CC homozygotes had a greater RSI than GG homozygotes and GC heterozygotes (Figure 4).

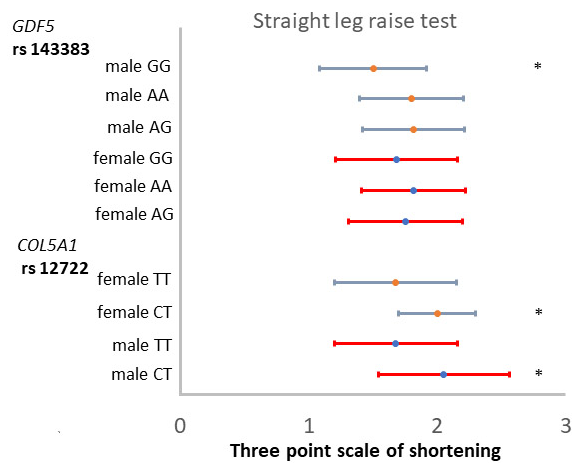


Figure 3 Straight leg raise test results in different genotype groups. The scale point 3 means lowest flexibility. COL5A1= Collagen alpha-1(V) chain, GDF5= growth differentiation factor. \*significantly different than other genotype groups in the same sex.

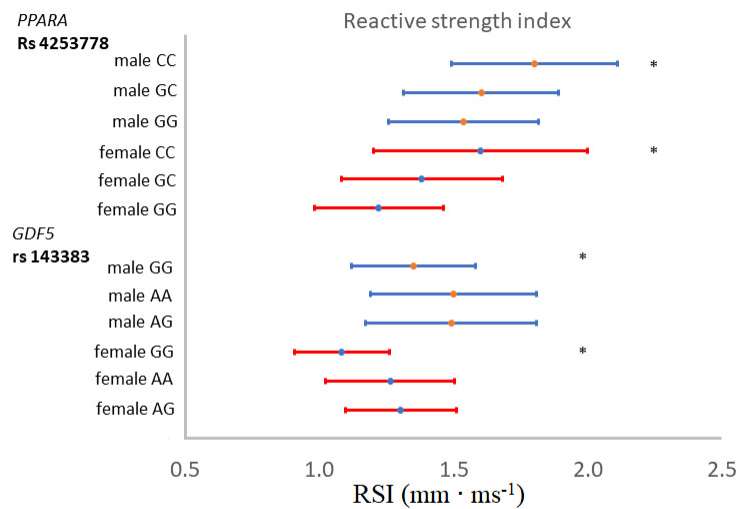


Figure 4 Reactive strength index differences in genotype groups. PPARA= peroxisome proliferator-activated receptor, GDF5= growth differentiation factor. \*significantly different than other genotype groups in the same sex.



## DISCUSSION

The main finding of this study is that *COL5A1* and *GDF5* gene variants are associated with injury risk predictors represented by functional movement tests scores in adolescents. *PPARA* and *GDF5* gene variants are also associated with RSI, and *COL5A1* genes variants might determine the maturation status in females. Specifically, *COL5A1* is a good predictor of muscle functional screening for males and females. Previous studies have shown that CT heterozygotes in *COL5A1* rs12722 are associated with decreased flexibility in the posterior fascial chain (hamstrings, erector spinae) during a sit and reach and straight leg raise test, where heterozygotes were less flexible than homozygous individuals (TT and CC genotypes) (6). The results of the present study support this previous findings (6) for a team sport population in relation to posterior muscle chain function, which is the muscle group tested in the FBT. Moreover, TC heterozygotes in *COL5A1* rs11103544 were associated with a lower FBT score than TT homozygotes in males and GG male heterozygotes in *GDF5* rs143383 with a better SLR score than AA and AG. Thus, a new possible relationship with functional muscle test in *COL5A1* and *GDF5* has been identified in the current study. However, the sample size did not allow *COL5A1* and *GDF5* gene interactions; therefore, this type of analysis should be performed in a future study with larger samples.

It has previously been reported that *COL5A1* rs12722 variation has an effect on ROM during aging and with respect to physical activity (3), however the present study is the first to include a group of young athletes at a high risk of musculoskeletal injury. Although there is no experimental evidence, the authors believe that increased type V collagen production is influenced by *COL5A1* rs12722 T allele variant (8), which might be especially important in terms of changes to bone and soft tissue mechanical properties during the period of maturation. Seven polymorphisms in 3'-UTR of *COL5A1* forming T allele of rs12722 has been associated with

increased mRNA stability (28). This suggests that T allele of rs12722 could be responsible for different connective tissue phenotypes, where increased stiffness can be beneficial for increased performance but simultaneously also for increased injury risk (7). Moreover, maturity offset was delayed in female rs12722 CT heterozygotes, which might mean that these individuals might be under a higher risk of injury due to muscle function tests and hormonal factors in general (20). An opposite trend of faster maturation in rs12722 TT female homozygotes might mean that those girls might be preferred for elite teams due to a biological age bias. To our best knowledge, our finding that *COL5A1* rs11103544 was related to FBT in males seems to be novel since this polymorphism relationship to the range of movement has been suggested, but not confirmed by previous research (6).

Although GDF5 protein is involved in bone and tissue growth in youth and adults (4), this did not identify a direct link with *GDF5* and players' maturity offset. The *GDF5* rs143383 A allele carriers has been previously associated with decreased stature and sitting height (55, 65) in Euro-American population and British population (55). Our study did not find an association between stature, sitting height or maturation offset (derived from stature and sitting height) and rs143383, which might be explained by our relatively low sample or the ongoing maturation process itself. Moreover, *GDF5* rs143383 has not been associated with pubertal height grow in a previous genome wide association (GWAS) study (9). Therefore, it is possible that *GDF5* gene expression does not differ at different stages of maturity estimated by anthropometrics, such it was showed for other polymorphisms like Disruptor of telomeric silencing 1-like (DOT1-like) or Mitogen-activated protein kinase 3 (MAPK3) (9, 59).

The results of the present study suggest that *GDF5* rs143383 polymorphism might play a role in male SLR score, where GG homozygotes do not have a increased test score; on the other

hand, GG homozygotes had lower RSI in both sexes. Thus, it might be speculated that rs143383 GG homozygotes showed equal development of performance and mechanical properties of the lower limbs, which might protect these individuals from a potential injury. The situation in which performance is ahead of mechanical property development might be understood as a potential injury risk factor, especially in the period of accelerated growth, during which most anthropometric changes take place (34, 35).

CC homozygotes in *PPARA* gene in the present study showed better jump performance represented by RSI, which had been suggested by previous studies (1, 48). However, the finding that this predisposition is identical in adults and adolescents should be considered when training methods are selected. Especially as *PPARs* and their coactivators are associated with improvements in training programs for weight reduction (29), aerobic performance (49, 52, 60, 61) and resistance training load capabilities (1). In this manner, the ketogenesis and other metabolic factors determined by *PPARA* indicate an individual response to strength and power training (1), and satellite cell proliferation determined by *GDF5* can indicate a potential to regenerate from a long term physical load (18). Regarding the fact that the interaction of these genes in terms of performance, injury prevention and fatigue factors was not analysed, the authors suggest that this analysis should be performed in future studies.

Limitations of the present study include the relatively small sample size and potential effects of other environmental (e.g. dietary) or genetic factors, therefore the results of the present study cannot be generalized to other populations. Validation in other cohorts and further studies are necessary to address the detailed role of the chosen polymorphisms of *COL5A1*, *GDF5* and *PPARA* genes within the complex phenotype of strength and power performance. Our polymorphisms selection has been performed in relation to muscle function and performance

phenotypes, but not to specific polymorphisms previously related to growth and maturation, which we suggest for future studies performed on adolescents. The maturation status has been found to have effect on functional movement including SL (50) and the muscle strength and power performance (17). Our phenotype results (without considering anthropometry in Table 4) showed no difference between pre and post PHV young male (Table 7), which is in accordance with previous studies where e.g. leg stiffness and RSI did not significantly differ between pre and post PHV in males (31, 54). This might be explained by low range of our PHV groups (13-15y) or by complex training effects, where plyometric training might be more effective in pre PHV than post PHV participants and where other training responses might be similar in both maturity groups (32). Although numbers of training sessions slightly differ between sports and the sexes, all participants were in a structured training program (with a minimum of 6 training sessions per week) designed to promote progressive musculoskeletal adaptation. Moreover, all participants had been in systematic training for a number of years (minimum 6 years) which might mean that any potential confounding variables did not influence our genotype results.

### **Practical application**

The present study showed that CT genotype in *COL5A1* rs12722 is a possible predictor of decreased muscle function in the posterior hip muscle chain, causing shortening in FBT and SLR test. Therefore, *COL5A1* rs12722 CT heterozygotes should be involved in specific programs targeting hamstring and posterior hip muscle chain flexibility, muscle functions and any other muscle imbalances. Woman with *COL5A1* rs12722 TT homozygosity might be used as a predictor of faster maturation, therefore their carriers might have a biological advantage in adolescent categories, and their performance should not be overestimated in practice. *PPARA* rs4253778 CC homozygotes and *GDF5* rs143383 AG and AA genotypes might have greater stretch shortening

cycle performance represented by RSI, therefore those athletes have a good potential to develop strength, power and speed in training.

**Acknowledgement:** This study has been supported by the GACR grant NO. 16-13750S and by Charles University grant UNCE/HUM/032.

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