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

Stastny, Petr; Lehnert, Michal; De Ste Croix, Mark B; Miroslav, Petr; Svoboda, Zdenek; Maixnerova, Eliska; Varekova, Renata; Botek, Michal; Petrek, Martin; Kocourkova, Lenka and Pawel, Cie

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-β 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 mount 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 that 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 musculotendinous 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± 0.2y) 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 (n = 146)	Handball (n=32)	Basketball (n=54)	Soccer (n=50)	Female (n=56)	Male (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 (Leg length and sitting height interaction)) - (0.001663 (age and leg length interaction)) + 0.007216 (age and sitting height interaction)) + (0.02292 (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 (Leg length and sitting height interaction)) + (0.0022 (age and leg length interaction)) + 0.005841 (age and sitting height interaction)) - 0.002658 (age and body mass interaction)) + 0.07693 (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)

Gene	**SNP ID**	**Chr: Position**	**Allele**	**Location**
COL5A1	rs12722	9:137734416	C/T	3´-UTR
	rs11103544	9:137735043	T/C	3´-UTR
PPARA	rs4253778	22:46630634	G/C	Intron
GDF5	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 ($kN \cdot m^{-1}$) 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 = Jump height (mm)/ground contact time (ms) and jump height (m) = (gravity · (Flight time)^2)/8, where gravity is 9.81 m·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 ORIGINE 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 χ^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 ω^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 χ^2.

Gene (N)	SNP	Genotype	Genotype Frequency	χ^2	EUR fr (n=503)	CEU fr (n=99)	Allele	Allele Frequency
COL5A1	rs11103544	TT	0.746	2.08	0.716	0.758	T	0.849
138	T>C	TC	0.217		0.264	0.232	C	0.161
		CC	0.036		0.020	0.010		
102	rs12722	TT	0.669	0.51	0.356	0.354	T	0.828
	C>T	CT	0.309		0.459	0.444	C	0.173
		CC	0.022		0.185	0.202		
GDF5	G>A	GG	0.236	3.55	0.161	0.091	G	0.405
127	rs143383	GA	0.374		0.421	0.414	A	0.595
		AA	0.390		0.417	0.495		
PPARA	C>G	CC	0.051	1.37	0.040	0.030	C	0.194
138	rs4253778	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, ω^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**		**χ^2 test**			**Pre PHV**		**Post PHV**		**χ^2 test**	
Gene (n)	**SNP**	**Geno type**	**n**	***f***	**n**	***f***	**χ^2**	**p**	**allele**	**n**	***f***	**n**	***f***	**χ^2**	**p**
COL5A1 54	rs11103544 T>C	TT	13	0.650	24	0.706	1.8	0.40	T	33	0.825	56	0.824	0.10	0.75
		TC	7	0.350	8	0.235			C	7	0.175	10	0.177		
		CC	0	---	2	0.059									
51	rs12722 C>T	TT	8	0.381	14	0.467	1.67	0.43	T	28	0.667	44	0.733	0.30	0.58
		CT	12	0.571	16	0.533			C	13	0.333	16	0.267		
		CC	1	0.048	0	---									
GDF5 46	G>A rs143383	AA	8	0.471	10	0.345	0.74	0.69	A	22	0.647	32	0.551	2.11	0.15
		AG	6	0.353	12	0.414			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	3.62	0.16	C	11	0.275	13	0.191	1.02	0.31
		CG	9	0.450	7	0.206			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	Genotype	Genotype						Allele						
		Genotype	Pre PHV		Post PHV		χ^2 test			Pre PHV		Post PHV		χ^2 test	
			n	*f*	n	*f*	χ^2	p	allele	n	*f*	n	*f*	χ^2	p
COL5A1 84	rs11103544 T>C	TT	11	0.846	53	0.753	1.14	0.56	T	24	0.923	119	0.842	1.31	0.25
		TC	2	0.154	13	0.178			C	2	0.077	23	0.157		
		CC	0	--	5	0.068									
49	rs12722 C>T	TT	10	0.769	32	0.556	1.11	0.29	T	23	0.884	68	0.944	1.03	0.31
		CT	3	0.231	4	0.444			C	3	0.115	4	0.056		
		CC	0	---	0	---									
GDF5 80	G>A rs143383	AA	5	0.385	28	0.418	0.32	0.85	A	16	0.615	80	0.597	0.03	0.86
		GA	6	0.462	24	0.358			G	10	0.385	54	0.402		
		GG	2	0.154	15	0.224									
PPARA 84	C>G rs4253778	CC	0	--	3	0.042	1.90	0.38	C	2	0.077	27	0.190	1.97	0.16
		CG	2	0.154	21	0.296			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 ± 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 ($mm \cdot ms^{-1}$)	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, ω^2= 0.14), where TT and CC homozygotes resulted in better FBT scores than TC heterozygotes (Figure 2).

The SLR showed differences between *COLA1* rs12722 genotype groups in males ($F_{1, 39}$= 5.3, p= .027, ω^2= 0.11) and females ($F_{1, 37}$= 5.6, p= .027, ω^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, ω^2= 0.11), where GG homozygotes showed lower (better) test scores than AA and AG genotypes (Figure 3).

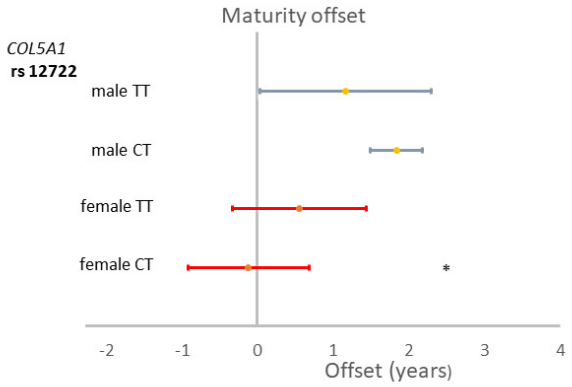

*Figure 1 Maturation offset differences in genotype groups. COL5A1= collagen alpha-1(V) chain. *significantly different than other genotype groups in the same sex.*

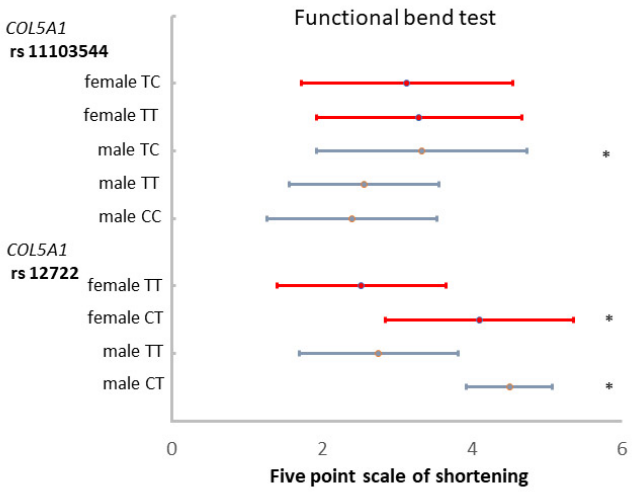

*Figure 2 Functional bend test in different genotype groups. The scale point 5 means lowest flexibility. COL5A1= Collagen alpha-1(V) chain. *significantly different than other genotype groups in the same sex.*

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

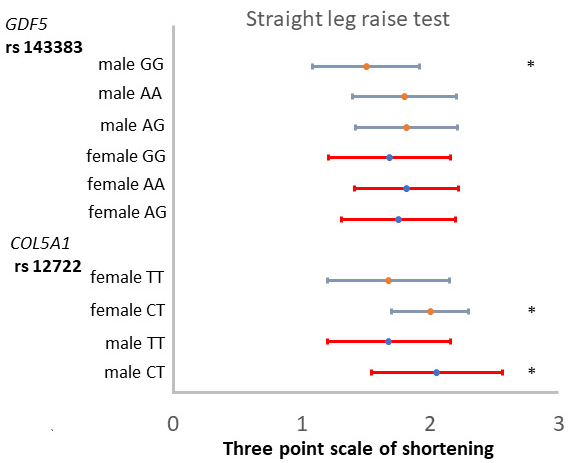

Figure 3 Straight leg raise test results in different genotype groups. The scale point 3 means lowest flexibility. COL5A1= *Collagen alpha-1(V) chain,* GDF5= *growth differentiation factor. *significantly different than other genotype groups in the same sex.*

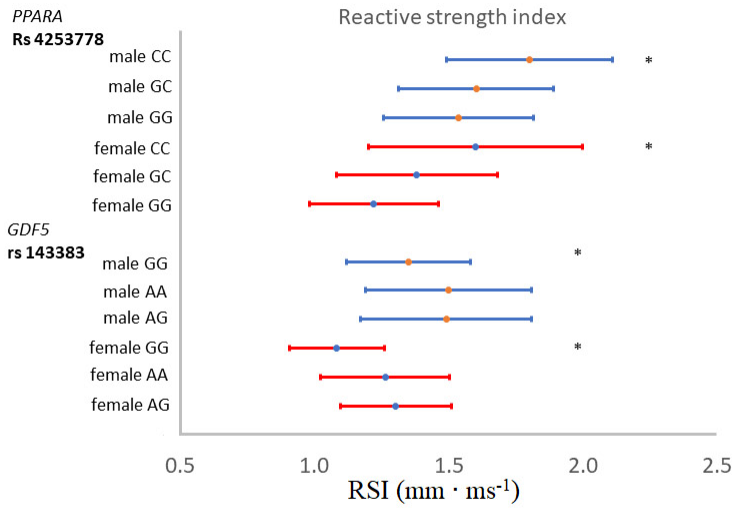

*Figure 4 Reactive strength index differences in genotype groups. PPARA= peroxisome proliferator-activated receptor, GDF5= growth differentiation factor. *significantly different than other genotype groups in the same sex.*

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 musculotendinous 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.

References

1. Aksenov, MO and Ilyin, AB. Training process design in weightlifting sports customized to genetic predispositions. *Teor Prakt Fiz Kult* 2017-January: 75-77, 2017.
2. Bell, RD, Shultz, SJ, Wideman, L, and Henrich, VC. Collagen gene variants previously associated with anterior cruciate ligament injury risk are also associated with joint laxity. *Sports Health* 4: 312-318, 2012.
3. Brown, J, Miller, CJ, Schwellnus, M, and Collins, M. Range of motion measurements diverge with increasing age for COL5A1 genotypes. *Scand J Med Sci Sports* 21, 2011.
4. Buxton, P, Edwards, C, Archer, CW, and Francis-West, P. Growth/differentiation factor-5 (GDF-5) and skeletal development. *J Bone Joint Surg Am* 83-A Suppl 1: S23-30, 2001.
5. Cejudo, A, de Baranda, PS, Ayala, F, and Santonja, F. Test-retest reliability of seven common clinical tests for assessing lower extremity muscle flexibility in futsal and handball players. *Phys Ther Sport* 16: 107-113, 2015.
6. Collins, M, Mokone, G, September, A, Van der Merwe, L, and Schwellnus, M. The COL5A1 genotype is associated with range of motion measurements. *Scand J Med Sci Sports* 19: 803-810, 2009.
7. Collins, M, O'Connell, K, and Posthumus, M. Genetics of musculoskeletal exercise-related phenotypes, in: *Genetics and Sports*. Karger Publishers, 2016, pp 92-104.
8. Collins, M and Posthumus, M. Type V collagen genotype and exercise-related phenotype relationships: a novel hypothesis. *Exerc Sport Sci Rev* 39: 191-198, 2011.
9. Cousminer, DL, Berry, DJ, Timpson, NJ, Ang, W, Thiering, E, et al. Genome-wide association and longitudinal analyses reveal genetic loci linking pubertal height growth, pubertal timing and childhood adiposity. *Hum Mol Genet* 22: 2735-2747, 2013.
10. Croisier, J-L. Factors associated with recurrent hamstring injuries. *Sports Med* 34: 681-695, 2004.
11. Dalleau, G, Belli, A, Viale, F, Lacour, J-R, and Bourdin, M. A simple method for field measurements of leg stiffness in hopping. *Int J Sports Med* 25: 170-176, 2004.
12. Engebretsen, AH, Myklebust, G, Holme, I, Engebretsen, L, and Bahr, R. Intrinsic risk factors for hamstring injuries among male soccer players: a prospective cohort study. *Am J Sports Med* 38: 1147-1153, 2010.
13. Flanagan, EP and Comyns, TM. The use of contact time and the reactive strength index to optimize fast stretch-shortening cycle training. *Strength Condit J* 30: 32-38, 2008.

14. Ge, W, Mu, J, and Huang, C. The GDF5 SNP is associated with meniscus injury and function recovery in male Chinese soldiers. *Int J Sports Med* 35: 625-628, 2014.
15. Göeken, LN. Instrumental straight-leg raising: results in healthy subjects. *Arch Phys Med Rehabil* 74: 194-203, 1993.
16. Greene, HS, Cholewicki, J, Galloway, MT, Nguyen ,CV, and Radebold, A. A history of low back injury is a risk factor for recurrent back injuries in varsity athletes. *Am J Sports Med* 29: 795-800, 2001.
17. Hammami, R, Chaouachi, A, Makhlouf, I, Granacher, U, and Behm, DG. Associations between balance and muscle strength, power performance in male youth athletes of different maturity status. *Pediatr Exerc Sci* 28: 521-534, 2016.
18. Hatazawa, Y, Ono, Y, Hirose, Y, Kanai, S, Fujii, NL, Machida, S, et al. Reduced Dnmt3a increases Gdf5 expression with suppressed satellite cell differentiation and impaired skeletal muscle regeneration. *FASEB J* 32: 1452-1467, 2018.
19. Hays, WL. Statistics Fort Worth. *TX: Harcourt Brace College Publications*, 1994.
20. Hewett, TE, Myer, GD, and Zazulak, BT. Hamstrings to quadriceps peak torque ratios diverge between sexes with increasing isokinetic angular velocity. *J Sci Med Sport* 11: 452-459, 2008.
21. Hughes, G and Watkins, J. A risk-factor model for anterior cruciate ligament injury. *Sports Med* 36: 411-428, 2006.
22. Janda, V. *Muscle function testing.* Cornwalt: Elsevier, 2013.
23. Jonhagen, S, Nemeth, G, and Eriksson, E. Hamstring injuries in sprinters: the role of concentric and eccentric hamstring muscle strength and flexibility. *Am J Sports Med* 22: 262-266, 1994.
24. Kiesel, K, Plisky, PJ, and Voight, ML. Can serious injury in professional football be predicted by a preseason functional movement screen? *North American journal of sports physical therapy: NAJSPT* 2: 147, 2007.
25. Kishore, A, Žižková, V, Kocourková, L, and Petřek, M. A dataset of 26 candidate gene and pro-inflammatory cytokine variants for association studies in idiopathic pulmonary fibrosis: frequency distribution in normal Czech population. *Front Immunol* 6, 2015.
26. Kubo, K, Yata, H, and Tsunoda, N. Effect of gene polymorphisms on the mechanical properties of human tendon structures. *Springer Plus* 2: 343, 2013.
27. Kuszewski, M, Saulicz, E, Gnat, R, Knapik, A, and Wandzel, P. Body Proportions and the Results of the Toe-Touch Test. *J Hum Kinet* 16: 83, 2006.
28. Laguette, M-J, Abrahams, Y, Prince, S, and Collins, M. Sequence variants within the 3′-UTR of the COL5A1 gene alters mRNA stability: implications for musculoskeletal soft tissue injuries. *Matrix Biol* 30: 338-345, 2011.
29. Leońska-Duniec, A, Ahmetov, II, and Zmijewski, P. Genetic variants influencing effectiveness of exercise training programmes in obesity - An overview of human studies. *Biol Sport* 33: 207-214, 2016.
30. Lloyd, RS, Oliver, JL, Hughes, MG, and Williams, CA. Reliability and validity of field-based measures of leg stiffness and reactive strength index in youths. *J Sports Sci* 27: 1565-1573, 2009.
31. Lloyd, RS, Oliver, JL, Hughes, MG, and Williams, CA. The influence of chronological age on periods of accelerated adaptation of stretch-shortening cycle performance in pre and postpubescent boys. *J Strength Condit Res* 25: 1889-1897, 2011.

32. Lloyd, RS, Radnor, JM, Croix, MBDS, Cronin, JB, and Oliver, JL. Changes in sprint and jump performances after traditional, plyometric, and combined resistance training in male youth pre-and post-peak height velocity. *J Strength Condit Res* 30: 1239-1247, 2016.
33. Maffulli, N, Margiotti, K, Longo, UG, Loppini, M, Fazio, VM, and Denaro, V. The genetics of sports injuries and athletic performance. *MLTJ Muscles, Ligaments and Tendons Journal* 3: 173-189, 2013.
34. Malina, RM, Eisenmann, JC, Cumming, SP, Ribeiro, B, and Aroso, J. Maturity-associated variation in the growth and functional capacities of youth football (soccer) players 13–15 years. *Eur J Appl Physiol* 91: 555-562, 2004.
35. Malina, RM and Kozieł, SM. Validation of maturity offset in a longitudinal sample of Polish boys. *J Sports Sci* 32: 424-437, 2014.
36. Marfell-Jones, MJ, Stewart, A, and De, Ridder J. *International standards for anthropometric assessment.* New Zealand: International Society for the Advancement of Kinanthropometry, 2012.
37. Marginson, V, Rowlands, AV, Gleeson, NP, and Eston, RG. Comparison of the symptoms of exercise-induced muscle damage after an initial and repeated bout of plyometric exercise in men and boys. *J Appl Physiol* 99: 1174-1181, 2005.
38. McCabe, K and Collins, C. Can Genetics Predict Sports Injury? The Association of the Genes GDF5, AMPD1, COL5A1 and IGF2 on Soccer Player Injury Occurrence. *Sports* 6: 21, 2018.
39. McMahon, TA and Cheng, GC. The mechanics of running: how does stiffness couple with speed? *J Biomech* 23: 65-78, 1990.
40. Mikkelsson, LO, Nupponen, H, Kaprio, J, Kautiainen, H, Mikkelsson, M, and Kujala, UM. Adolescent flexibility, endurance strength, and physical activity as predictors of adult tension neck, low back pain, and knee injury: a 25 year follow up study. *Br J Sports Med* 40: 107-113, 2006.
41. Mirwald, RL, Baxter-Jones, AD, Bailey, DA, and Beunen, GP. An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc* 34: 689-694, 2002.
42. Miyamoto, Y, Mabuchi, A, Shi, D, Kubo, T, Takatori, Y, Saito, S, et al. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. *Nat Genet* 39: 529-533, 2007.
43. Nishida, Y, Iyadomi, M, Tominaga, H, Taniguchi, H, Higaki, Y, Tanaka, H, et al. Influence of Single-Nucleotide Polymorphisms in PPAR-δ, PPAR-γ, and PRKAA2 on the Changes in Anthropometric Indices and Blood Measurements through Exercise-Centered Lifestyle Intervention in Japanese Middle-Aged Men. *Int J Mol Sci* 19: 703, 2018.
44. Padua, DA, Arnold, BL, Perrin, DH, Gansneder, BM, Carcia, CR, and Granata, KP. Fatigue, vertical leg stiffness, and stiffness control strategies in males and females. *J Athletic Training* 41: 294, 2006.
45. Parkkari, J, Pasanen, K, Mattila, VM, Kannus, P, and Rimpelä, A. The risk for a cruciate ligament injury of the knee in adolescents and young adults: a population-based cohort study of 46 500 people with a 9 year follow-up. *Br J Sports Med* 42: 422-426, 2008.
46. Pearson, D, Naughton, GA, and Torode, M. Predictability of physiological testing and the role of maturation in talent identification for adolescent team sports. *J Sci Med Sport* 9: 277-287, 2006.

47. Pérusse, L, Ruchat, SM, Rankinen, T, Weisnagel, SJ, Rice, T, Rao, DC, et al. Improvements in glucose homeostasis in response to regular exercise are influenced by the PPARG Pro12Ala variant: Results from the HERITAGE family study. *Diabetologia* 53: 679-689, 2010.
48. Petr, M, Št'astný, P, Pecha, O, Šteffl, M, Šeda, O, and Kohlíková, E. PPARA intron polymorphism associated with power performance in 30-s anaerobic Wingate Test. *PLoS One* 9: e107171, 2014.
49. Petr, M, Stastny, P, Zajac, A, Tufano, JJ, and Maciejewska-Skrendo, A. The Role of Peroxisome Proliferator-Activated Receptors and Their Transcriptional Coactivators Gene Variations in Human Trainability: A Systematic Review. *Int J Mol Sci* 19: 1472, 2018.
50. Portas, MD, Parkin, G, Roberts, J, and Batterham, AM. Maturational effect on Functional Movement Screen™ score in adolescent soccer players. *J Sci Med Sport* 19: 854-858, 2016.
51. Raschner, C, Platzer, H-P, Patterson, C, Werner, I, Huber, R, and Hildebrandt, C. The relationship between ACL injuries and physical fitness in young competitive ski racers: a 10-year longitudinal study. *Br J Sports Med*: bjsports-2012-091050, 2012.
52. Ring-Dimitriou, S, Kedenko, L, Kedenko, I, Feichtinger, RG, Steinbacher, P, Stoiber, W, et al. Does genetic variation in PPARGC1A affect exercise-induced changes in ventilatory thresholds and metabolic syndrome? *J Exerc Physiol Online* 17: 1-18, 2014.
53. Rumpf, MC and Cronin, J. Injury incidence, body site, and severity in soccer players aged 6–18 years: implications for injury prevention. *Strength Condit J* 34: 20-31, 2012.
54. Rumpf, MC, Cronin, JB, Oliver, JL, and Hughes, MG. Vertical and leg stiffness and stretch-shortening cycle changes across maturation during maximal sprint running. *Human movement science* 32: 668-676, 2013.
55. Sanna, S, Jackson, AU, Nagaraja, R, Willer, CJ, Chen, WM, Bonnycastle, LL, et al. Common variants in the GDF5-UQCC region are associated with variation in human height. *Nat Genet* 40: 198-203, 2008.
56. September, A, Posthumus, M, and Collins, M. Application of genomics in the prevention, treatment and management of Achilles tendinopathy and anterior cruciate ligament ruptures. *Recent patents on DNA & gene sequences* 6: 216-223, 2012.
57. September, AV, Cook, J, Handley, CJ, van der Merwe, L, Schwellnus, MP, and Collins, M. Variants within the COL5A1 gene are associated with Achilles tendinopathy in two populations. *Br J Sports Med* 43: 357-365, 2009.
58. Shea, KG, Pfeiffer, R, Wang, JH, Curtin, M, and Apel, PJ. Anterior cruciate ligament injury in pediatric and adolescent soccer players: an analysis of insurance data. *Journal of Pediatric Orthopaedics* 24: 623-628, 2004.
59. Sovio, U, Bennett, AJ, Millwood, IY, Molitor, J, O'Reilly, PF, Timpson, NJ, et al. Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. *PLoS Genet* 5: e1000409, 2009.
60. Stefan, N, Thamer, C, Staiger, H, Machicao, F, Machann, J, Schick, F, et al. Genetic variations in PPARD and PPARGC1A determine mitochondrial function and change in aerobic physical fitness and insulin sensitivity during lifestyle intervention. *J Clin Endocrinol Metab* 92: 1827-1833, 2007.
61. Steinbacher, P, Feichtinger, RG, Kedenko, L, Kedenko, I, Reinhardt, S, Schönauer, AL, et al. The single nucleotide polymorphism Gly482Ser in the PGC-1α gene impairs

exercise-induced slow-twitch muscle fibre transformation in humans. *PLoS ONE* 10, 2015.
62. Van der Sluis, A, Elferink-Gemser, M, Coelho-e-Silva, M, Nijboer, J, Brink, M, and Visscher, C. Sport injuries aligned to peak height velocity in talented pubertal soccer players. *Int J Sports Med* 35: 351-355, 2014.
63. Waldén, M, Hägglund, M, Werner, J, and Ekstrand, J. The epidemiology of anterior cruciate ligament injury in football (soccer): a review of the literature from a gender-related perspective. *Knee Surg Sports Traumatol Arthrosc* 19: 3-10, 2011.
64. Washington, R, Bernhardt, D, Gomez, J, Johnson, M, Martin, T, Rowland, T, Set al. Organized sports for children and preadolescents. *Pediatrics* 107: 1459-1462, 2001.
65. Wu, DD, Li, GM, Jin, W, Li, Y, and Zhang, YP. Positive selection on the osteoarthritis-risk and decreased-height associated variants at the GDF5 gene in East Asians. *PLoS One* 7: e42553, 2012.
66. Zhao, L, Chang, Q, Huang, T, and Huang, C. Prospective cohort study of the risk factors for stress fractures in Chinese male infantry recruits. *J Int Med Res* 44: 787-795, 2016.