

The association of *ACE*, *ACTN3* and *PPARA* gene variants with strength phenotypes in middle school-age children

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Abstract The aim of the study was to determine the association between *ACE* I/D, *ACTN3* R577X and *PPARA* intron 7 G/C gene polymorphisms and strength-related traits in 457 middle school-age children (219 boys and 238 girls; aged 11 ± 0.4 years). The assessment of different phenotypes was conducted with a number of performance tests. Gene polymorphisms were determined by PCR. The *ACE* D allele was associated with high results of standing long-jump test in boys [II 148.3 (16.3) cm, ID 152.6 (19.6) cm, DD 158.2 (19.1) cm; $P = 0.037$]. The *ACTN3* R allele was associated with high results of performance tests in males only in combination with other genes (standing long-jump test: $P = 0.021$; handgrip strength test: $P < 0.0001$). Furthermore, the male carriers of the *PPARA* gene C allele demonstrated the best results of handgrip strength testing than GG homozygotes [GG 14.6 (4.0) kg, GC/CC 15.7 (4.3) kg; $P = 0.048$]. Thus, the *ACE*, *ACTN3* and *PPARA* gene variants are associated with strength-related traits in physically active middle school-age boys.

Keywords Polymorphism · Gene · Genetics · Physiological development · Strength

Introduction

It has long been recognized that the inter-individual variability of physical performance traits and the ability to become an elite athlete have a strong genetic basis. The genetic factors that influence these phenotypes are now being sought [1, 2]. Several family, twin, case–control and cross-sectional studies suggest an important role of genetics along with epigenetic (i.e. stable and heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence such as DNA methylation and histone deacetylation) and environmental factors in the determination of individual differences in physical performance and training responses.

It is now very well established that a genetic component of the variance in any phenotype (i.e. height, muscle mass, strength, athlete status, etc.) is determined by small changes in the structure of DNA, which are called polymorphisms. There are no less than 50 million polymorphic variants in the human genome, which make all individuals different. The most common types of DNA sequence variants are single-nucleotide and insertion/deletion (I/D) polymorphisms. Genetic variations can affect the amount and structure of mRNA/protein, and therefore may account for the main share of genetic factors in human phenotypic variability.

The heritability of muscle strength has been shown to range from approximately 30 to 80 % [3, 4]. Muscle strength/power phenotypes are accepted to be polygenic in nature—that is, multiple genetic factors influence the observed phenotype. To date, over 20 genetic variants have been associated with strength and power-related phenotypes [1, 5], of which the *ACE*, *ACTN3* and *PPARA* gene polymorphisms are three of the most studied.

The I/D polymorphism of the angiotensin I-converting enzyme (*ACE*) gene denotes a substantial individual

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variation in renin–angiotensin system activity with the D allele being associated with higher ACE activity. Circulating ACE activity is significantly correlated with isometric and isokinetic quadriceps muscle strength [6]. Such an effect may depend upon increased ACE-mediated activation of the growth factor angiotensin II, and increased degradation of growth-inhibitory bradykinin. Accordingly, greater training-related increases in quadriceps muscle strength [7, 8], peak elbow flexor muscle strength and biceps muscle cross-sectional area [9] have been associated with the D allele. Similarly, several studies have shown the D allele to be associated with greater strength and muscle volumes at baseline [10–12]. In addition, the D allele was associated with elite power athlete status [13]. On the other hand, the ACE I allele was reported to be associated with endurance athlete status and endurance-related phenotypes such as proportion of slow-twitch type I muscle fibres, VO_{2max} , fatigue resistance and cardiac output (reviewed in [1]). However, it should be noted that few studies have reported conflicting results (i.e. no relationship between the ACE I/D polymorphism and strength/power phenotypes or the presence of association of the ACE I allele with strength/power phenotypes) [13].

The α -actinins constitute the predominant protein component of the sarcomeric Z line in skeletal muscle fibres, where they form a lattice structure that anchors together actin containing thin filaments and stabilizes the muscle contractile apparatus [14]. Expression of the α -actinin-3 (ACTN3) is limited to fast muscle fibres responsible for generating force at high velocity. A common genetic variation in the ACTN3 gene that results in the replacement of an arginine with a stop codon at amino acid 577 (rs1815739 C/T polymorphism in exon 16; R577X) has been identified. The X allele contains a sequence change that completely prevents the production of functional α -actinin-3 protein. Several case–control studies reported that ACTN3 RR genotype is over-represented or ACTN3 XX genotype is under-represented in strength/sprint athletes in comparison with controls [14–16]. Although some contradictory results exist [14], the meta-analysis of nine studies confirmed this kind of association [15]. The hypothesis that ACTN3 R allele may confer some advantage in power performance was also supported by several cross-sectional studies [17–22].

Peroxisome proliferator-activated receptor α (PPAR α) is a ligand-activated transcription factor that regulates the expression of genes involved in fatty acid uptake and oxidation, glucose and lipid metabolism, left ventricular growth and control of body weight. Jamshidi et al. [23] have shown that British army recruits homozygous for the rare PPARA C allele of the intron 7 G/C (rs4253778) polymorphism had a 3-fold greater increase in LV mass in

response to training than G allele homozygotes. The hypothesis that intron 7 C allele is associated with the hypertrophic effect due to influences on cardiac and skeletal muscle substrate utilization was supported by the findings that the PPARA C allele is over-represented in Russian power-oriented athletes and associated with an increased proportion of fast-twitch muscle fibres in *m. vastus lateralis* of physically active healthy men [24].

Twin studies have shown that genes play a larger role in younger age groups, as the environment may be more homogeneous and has had less time to take effect [25–27]. Consequently, in these age groups, there is expected to be a proportionately higher genetic contribution to phenotypic variation and more readily recognisable interactions between genetic and environmental components. At present, there are two studies related to the search of the associations of gene polymorphisms with physical, physiological and skill parameters in children. In these studies, Moran and colleagues showed an association between the ACE gene I/D polymorphism and both handgrip strength and vertical jump in female Greek adolescents [28], as well as a relationship between the ACTN3 gene R577X polymorphism and 40-m sprint time in male Greek adolescents [29].

The aim of the present study was to investigate individually, and in combination, the associations of ACE (I/D), ACTN3 (R577X) and PPARA (intron 7 G/C) gene polymorphisms with several anthropometrical and performance traits in middle school-age Russian children and to replicate the findings of previous studies. We have therefore tested the hypothesis that the ACE D, ACTN3 R and PPARA C alleles would be associated with better results of handgrip strength and standing long-jump testing.

Materials and methods

Subjects

Four hundred and fifty-seven healthy physically active pupils (219 boys and 238 girls; aged 11 ± 0.4 years) from five different schools were studied. All subjects were Caucasians, and unrelated citizens of Naberezhnye Chelny, Russia. The Russian Federal Agency for Physical Culture and Sports approved the study and written informed consent was obtained from each participant's parents.

Phenotyping

All measurements were carried out by two well-trained investigators. Children's strength-related traits were assessed with two performance tests, including handgrip

strength and standing long-jump test [30]. The hand dynamometer (DK-140; Russia) was used for the handgrip strength testing. The strength of both the left and right hands was measured thrice each in a standing position (with the arm in complete extension without touching any part of the body with the dynamometer), and the best score of the dominant hand (kg) was used in the analysis. When performing the standing long-jump test, the subject was instructed to push off vigorously and jump as far as possible trying to land with both feet together. The score (cm) was the distance from the take-off line to the point where the back of the heel nearest to the take-off line lands on the mat (measured with a 5-m tape). The subjects were allowed to perform three trials in each test (the best result was chosen for analysis). Furthermore, the subjects underwent anthropometry [height (cm), weight (kg) and body mass index (BMI; kg/m²)].

Genotyping

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells using a DNK-sorb-A sorbent kit according to the manufacturer's instructions (Central Research Institute of Epidemiology, Moscow, Russia). Genotyping for the *ACE* I/D, *ACTN3* R577X and *PPARA* intron 7 G/C polymorphisms was performed by polymerase chain reaction (PCR) on a Tercyk thermal cycler (DNA Technology, Moscow, Russia) according to the previously described methods [31–33]. Briefly, PCR primers for the *ACE* I/D polymorphism were forward CTGGAGACCACTCCCATCCTTTCT and reverse GATGTGGCCATCACATTCGTCAGAT. D and I alleles of the *ACE* gene were determined by the presence of 190- or 490-bp fragments, respectively. PCR primers for the *ACTN3* R577X polymorphism were forward CTGTTGCCTGTGGTAAGTGGG and reverse TGGTCACAGTATGCAGGAGGG, generating a fragment of 290 bp. PCR products were further digested with BstDEI (SibEnzyme, Russia) for 12 h at 60 °C. PCR primers for the *PPARA* intron 7 G/C polymorphism were forward ACAATCACTCCTTAAATATGGTGG and reverse AAGTAGGGACAGACAGGACCAGTA, generating a fragment of 266 bp. PCR products were further digested with TaqI (SibEnzyme, Russia) for 12 h at 65 °C. All PCR and restriction products were separated by 8 % polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in UV light. All genotyping analyses were conducted blind to subject identity.

Statistical analysis

Genotype distribution and allele frequencies between different groups of children were compared using χ^2 tests.

Differences in performance phenotypes (handgrip strength, standing long-jump test) between groups with different genotypes (or combinations of genotypes) were analyzed using ANOVA (when three genotypes were compared) or unpaired *t* tests (when two genotypes were compared). All values are means (standard deviation; SD). *P* values <0.05 were considered statistically significant. Bonferroni's correction for multiple testing was performed by dividing the *P* value (0.05) with the number of tests (overall 35 tests). The squared correlation coefficient R^2 was used as a measure of explained variance. Statistical analyses were conducted using GraphPad InStat software.

Results

ACE, *ACTN3* and *PPARA* genotype distributions amongst subjects were in Hardy–Weinberg equilibrium. No significant differences were found in genotype and allele frequencies between boys and girls (Tables 1, 2). *ACE* D, *ACTN3* R, *PPARA* C alleles' frequencies in boys and girls were 48.4, 61.0 and 16.7, and 53.6, 59.3 and 13.2 %, respectively.

Girls were significantly taller [147.6 (6.7) vs. 146.0 (7.1) cm; *P* = 0.018], but not heavier [37.4 (7.6) vs. 36.9 (8.6) kg; *P* = 0.56] than boys. On the other hand, boys had better results of handgrip strength testing [15.1 (4.1) vs. 12.7 (3.7) kg; *P* < 0.0001] and standing long-jump results [152.3 (17.7) vs. 136.7 (16.5) cm; *P* < 0.0001] than girls. We therefore performed all analyses separately for each sex group.

Tables 1 and 2 demonstrate the basic results of anthropometric and performance testing in different genotype groups of boys and girls, respectively. Genotype–phenotype associations were shown only for boys. For our genotype combination analysis, we used the genotypic data of 2 (*ACE* + *ACTN3* or *ACE* + *PPARA* or *ACTN3* + *PPARA*) or all 3 genes (Table 3). Due to the small number of individuals in the *PPARA* CC homozygous group, we performed a combined GC + CC versus GG analysis. According to the hypothesis, the male carriers of DD-RR (*n* = 17), DD-GC/CC (*n* = 12), RR-GC/CC (*n* = 25) and DD-RR-GC/CC (*n* = 3) genotype combinations should possess a greater genetic potential for strength and power-oriented performance than carriers of the opposite combinations [II-XX (*n* = 9), II-GG (*n* = 40), XX-GG (*n* = 22) and II-XX-GG (*n* = 8), respectively].

Height

None of the genetic polymorphisms were separately associated with the height of boys and girls. The mean height

Table 1 Physical and physiological characteristics and *ACE*, *ACTN3* and *PPARA* genotypes of male subjects ($n = 219$)

| Trait | <i>ACE</i> genotype | | | <i>ACTN3</i> genotype | | | <i>PPARA</i> genotype | | |
|--------------------------|---------------------|--------------|---------------|-----------------------|--------------|-----------------------------|-----------------------|--------------|-------------------------|
| | II | ID | DD | RR | RX | XX | GG | GC | CC |
| n (%) | 53 (24.2) | 120 (54.8) | 46 (21.0) | 79 (36.2) | 108 (49.6) | 31 (14.2) | 153 (69.9) | 59 (26.9) | 7 (3.2) |
| Height (cm) | 146.0 (7.1) | 145.9 (6.7) | 145.8 (7.8) | 146.4 (7.2) | 146.6 (7.0) | 143.4 (6.4) | 145.8 (6.4) | 146.4 (8.6) | 147.0 (6.3) |
| Weight (kg) | 36.1 (6.9) | 36.5 (7.4) | 37.1 (8.8) | 37.6 (8.4) | 36.9 (8.0) | 33.9 (6.3) ^{&} | 37.0 (7.9) | 36.5 (8.0) | 38.6 (11.1) |
| BMI (kg/m ²) | 16.8 (2.2) | 17.1 (2.8) | 17.3 (3.1) | 17.4 (3.1) | 17.3 (2.9) | 16.4 (2.4) | 17.3 (3.0) | 16.9 (2.4) | 17.6 (3.6) |
| Standing long-jump (cm) | 148.3 (16.3) | 152.6 (19.6) | 158.2 (19.1)* | 152.6 (18.4) | 151.8 (17.6) | 153.9 (16.7) | 152.9 (17.2) | 151.2 (19.0) | 147.9 (20.2) |
| Handgrip strength (kg) | 14.3 (3.6) | 15.0 (3.6) | 15.6 (4.8) | 15.5 (4.3) | 15.2 (4.2) | 14.0 (3.5) | 14.6 (4.0) | 15.8 (4.3) | 14.9 (4.8) [#] |

Values are means (SD)

* $P = 0.037$ differences between male subjects with different *ACE* genotypes (II vs. ID vs. DD)

[&] $P = 0.0215$ differences between male subjects with different *ACTN3* genotypes (RR/RX vs. XX)

[#] $P = 0.048$ differences between male subjects with different *PPARA* genotypes (GG vs. GC/CC)

Table 2 Physical and physiological characteristics and *ACE*, *ACTN3* and *PPARA* genotypes of female subjects ($n = 238$)

| Trait | <i>ACE</i> genotype | | | <i>ACTN3</i> genotype | | | <i>PPARA</i> genotype | | |
|--------------------------|---------------------|--------------|--------------|-----------------------|--------------|--------------|-----------------------|--------------|--------------|
| | II | ID | DD | RR | RX | XX | GG | GC | CC |
| n (%) | 50 (21.0) | 121 (50.8) | 67 (28.2) | 84 (35.4) | 113 (47.7) | 40 (16.9) | 180 (75.6) | 53 (22.3) | 5 (2.1) |
| Height (cm) | 146.8 (6.3) | 147.5 (6.8) | 148.2 (6.8) | 146.8 (6.0) | 148.1 (7.3) | 147.6 (6.4) | 147.5 (6.8) | 147.6 (6.5) | 149.4 (4.2) |
| Weight (kg) | 37.2 (7.4) | 37.2 (7.7) | 37.8 (7.8) | 36.0 (5.6) | 37.9 (8.2) | 38.6 (9.2) | 37.3 (7.8) | 37.3 (7.5) | 40.8 (3.6) |
| BMI (kg/m ²) | 17.2 (2.7) | 17.0 (2.9) | 17.1 (2.7) | 16.6 (2.1) | 17.2 (2.6) | 17.7 (4.1) | 17.1 (2.9) | 17.0 (2.6) | 18.2 (0.9) |
| Standing long-jump (cm) | 138.5 (17.4) | 137.3 (16.6) | 134.2 (15.4) | 136.0 (17.2) | 136.8 (15.5) | 137.9 (17.8) | 136.3 (16.1) | 138.4 (17.6) | 132.4 (15.3) |
| Handgrip strength (kg) | 13.0 (3.0) | 12.8 (3.9) | 12.3 (3.7) | 12.2 (3.2) | 12.9 (4.1) | 13.1 (3.5) | 12.7 (3.6) | 12.9 (3.9) | 10.8 (3.8) |

Values are means (SD). No statistically significant differences in the measurements were found in girls with different genotypes

Table 3 Physical and physiological characteristics in male carriers of different genotype combinations

| Trait | <i>ACE</i> - <i>ACTN3</i> genotype combinations | | <i>ACE</i> - <i>PPARA</i> genotype combinations | | <i>ACTN3</i> - <i>PPARA</i> genotype combinations | | <i>ACE</i> - <i>ACTN3</i> - <i>PPARA</i> genotype combinations | |
|--------------------------|---|---------------|---|--------------|---|---------------|--|---------------|
| | DD-RR | II-XX | DD-GC/CC | II-GG | RR-GC/CC | XX-GG | DD-RR-GC/CC | II-XX-GG |
| n (%) | 17 (7.8) | 9 (4.1) | 12 (5.5) | 40 (18.3) | 25 (11.4) | 22 (10.0) | 3 (1.4) | 8 (3.7) |
| Height (cm) | 145.8 (9.4) | 140.6 (7.2) | 150.8 (11.4) | 146.2 (6.5) | 148.4 (8.1) | 142.1 (6.1)** | 159.3 (8.4) | 140.6 (7.2)** |
| Weight (kg) | 36.0 (9.5) | 31.1 (4.5) | 42.3 (12.2) | 36.9 (6.5)* | 38.6 (8.6) | 32.8 (5.4)** | 50.0 (10.1) | 31.1 (4.5)** |
| BMI (kg/m ²) | 16.7 (2.9) | 15.7 (1.7) | 18.3 (3.9) | 17.2 (2.1) | 17.4 (2.6) | 16.2 (2.3) | 19.8 (4.8) | 15.7 (1.7)* |
| Standing long-jump (cm) | 161.8 (17.0) | 144.8 (15.6)* | 154.4 (20.2) | 150.4 (15.8) | 151.4 (22.1) | 154.1 (18.7) | 154.8 (26.3) | 142.9 (15.5) |
| Handgrip strength (kg) | 17.3 (5.7) | 12.1 (1.7)* | 17.3 (5.7) | 12.1 (1.7)* | 15.9 (4.3) | 13.1 (2.7)* | 22.0 (2.6) | 12.1 (1.8)*** |

Values are means (SD)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, statistically significant differences between male subjects with different genotype combinations

values in the group of boys that carry DD-RR-GC/CC combination (associated with a greater hypertrophic potential) were higher than in II-XX-GG combination carriers [159.3 (8.4) vs. 140.6 (7.2) cm; $P = 0.0049$]. The

similar association was found when the RR-GC/CC combinations carriers were compared with the XX-GG combination carriers [148.4 (8.1) vs. 142.1 (6.1) cm; $P = 0.0048$].

Body weight

The *ACTN3* R allele demonstrated an association with the body weight value in boys [XX 33.9 (6.3) kg, RR/RX 37.5 (8.1) kg; $P = 0.0215$]. Furthermore, the DD-RR-GC/CC combination carriers had greater body weight values than the carriers of the II-XX-GG combination [50.0 (10.1) vs. 31.1 (4.5) kg; $P = 0.0015$]. In addition, an association was established between the groups of carriers of the DD-GC/CC and II-GG combinations [42.3 (12.2) vs. 36.9 (6.5) kg; $P = 0.048$], and also between the groups of carriers of RR-GC/CC and XX-GG combinations [38.6 (8.6) vs. 32.8 (5.4) kg; $P = 0.009$].

Body mass index

The average BMI was higher in carriers of the DD-RR-GC/CC combinations (boys only) as compared with the II-XX-GG combination carriers [19.8 (4.8) vs. 15.7 (1.7) kg/m²; $P = 0.045$].

Handgrip strength

The *PPARA* gene C allele demonstrated an association with the results of handgrip strength testing in boys [GG 14.6 (4.0) kg, GC/CC 15.7 (4.3) kg; $P = 0.048$]. Since body weight of boys was positively correlated with their handgrip strength ($r = 0.48$, $P < 0.0001$), we also performed a relative to body weight analysis. The association of the *PPARA* gene C allele with higher results of handgrip strength testing remained significant after adjustment for body weight ($P = 0.037$). In addition, any combination of genotypes of similar (strength and power) effect associated with the handgrip strength of boys: 17.3 (5.7) kg versus 12.1 (1.7) kg ($P = 0.016$) in a comparison of DD-RR and II-XX combinations; 17.3 (5.7) kg versus 12.1 (1.7) kg ($P = 0.016$) in a comparison of DD-GC/CC and II-GG combinations; 15.9 (4.3) kg versus 13.1 (2.7) kg ($P = 0.016$) in a comparison of RR-GC/CC and XX-GG combinations, and 22.0 (2.6) versus 12.1 (1.8) kg ($P < 0.0001$; after correction for multiple testing, this finding remained significant) in comparison of DD-RR-GC/CC and II-XX-GG combinations. The *ACE-ACTN3-PPARA* genotype combination explained 61.7 % of the variation in handgrip strength of boys.

Standing long-jump test

The *ACE* D allele demonstrated an association with the standing long-jump results in boys [II 148.3 (16.3) cm, ID 152.6 (19.6) cm, DD 158.2 (19.1) cm; $P = 0.037$]. The *ACTN3* R allele was associated with high results of standing long-jump test only in combination with *ACE*

gene: 161.8 (17.0) cm versus 144.8 (15.6) cm ($P = 0.021$) in a comparison of DD-RR and II-XX combinations.

Discussion

Physical performance is a complex phenotype influenced by multiple environmental and genetic factors. The question is no longer whether or not there is a genetic component to athletic potential, and endurance and strength trainability, but exactly which genes are involved and by which mechanisms and pathways they exert their effect. Our current progress towards answering these questions still represents only the first steps towards a complete understanding of the genetic factors that influence human physical performance.

It becomes evident that the level of physical performance is inherited by a number of polymorphous genes, and each of them, taken separately, modestly contributes to the overall development of human performance-related traits. This phenomenon may be one of the possible explanations for the negative results of some studies that failed to find the influence of individual genes on human physical performance. Therefore, the influence of gene polymorphisms on physical performance phenotypes should be investigated separately as well as in combination. Of note, it is necessary to consider the impact of combinations of genotypes with homogeneous effects (i.e. those genotypes associated with strength/power or endurance performance).

Our data support the hypothesis that the components of physical performance are inherited by a number of polymorphous genes, and each of them, taken separately, modestly or insignificantly contributes to the overall development of human performance-related traits. The discovered associations concerned strength-related variables (handgrip strength as a measure of maximal isometric muscle strength and standing long-jump as a measure of explosive strength), being in agreement with the generally observed data: the *ACE* D, *ACTN3* R and *PPARA* C alleles and their different combinations were associated with the strength/power performance [4, 7–11, 15, 17–22, 24]. Our findings confirm the polygenic nature of strength performance, a classic complex trait, and demonstrate that the likelihood of showing the best results in handgrip strength and standing long-jump depends on the number of strength-related alleles an individual possesses (additive genetic effect). We also revealed an association between the combination of the aforementioned alleles and higher values of height, weight and BMI which is in accordance with the data regarding the *ACE* genotype [29]. However, it should be noted that, after correction for multiple testing, only one finding remained statistically significant (that is,

higher results of handgrip strength testing in the carriers of DD-RR-GC/CC genotype combinations in comparison with II-XX-GG).

As is evident from the presented data, the association between gene polymorphisms and phenotyping data was discovered only in boys. Sex-specific associations were also reported in Greek adolescents, that is the *ACE* gene I/D polymorphism was associated with handgrip strength and vertical jump in females [28], and the *ACTN3* gene R577X polymorphism was associated with 40-m sprint time in males [21].

The limitation of the study was that the tested children were possibly at different stages of maturation (despite the same chronological age) which could influence the results of the association study. It is known that puberty begins at the age of 11 in the majority of girls (and at the age of 13 in boys) and causes drastic reconstitution of the organism. Therefore, one might anticipate that the absence of genotype–phenotype associations in girls is related to the ontogenetic heterogeneity of the cohort: some of the girls had still not entered puberty, while in others it proceeded at different stages. However, for ethical and practical reasons, it was not possible to assess the stage of pubertal development of our subjects. Further investigations are required to clarify the role of genetic and environmental factors in the development of anthropometric and performance traits in girls. Another limitation of the study was the use of handgrip strength testing as a measure of muscle strength. The results of handgrip strength testing might be influenced by various anatomical and biomechanical factors such as different angle of shoulder, elbow, forearm, and wrist, posture and grip span [30]. However, this type of testing remains one of the most accessible methods of strength estimation in mass screening.

In conclusion, the *ACE*, *ACTN3* and *PPARA* gene variants are associated with strength-related traits in physically active middle school-age boys.

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Conflict of interest The authors declare that they have no conflict of interest.

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