`Research article`

The Association Analysis between ACE and ACTN3 Genes Polymorphisms and Endurance Capacity in Young Cross-Country Skiers: Longitudinal Study

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Abstract

Endurance performance depends on the integration of several phenotypic traits influenced by multiple environmental and genetic factors. Objectives of the study were: (1) to examine the genotypic frequencies of the *ACE* I/D, *ACTN3* R577X polymorphisms and endurance performance-related phenotypes, (2) to evaluate the dynamics of endurance performance parameters during a 5-year period in relation to *ACE* I/D and *ACTN3* R577X genotypes in Estonian young skiers. Determination of VO_{2peak} was performed in 58 skiers aged 15-19 years (41 males, 17 females) during a 5-year period. The control group consisted of 322 healthy non-athletic subjects (145 males, 177 females). The study groups were genotyped for the *ACE* I/D and *ACTN3* R577X variants. Frequencies of the *ACE* ID and *ACTN3* RR genotypes were significantly higher (p = 0.047 and p = 0.003, respectively) and the RX genotype was lower (p = 0.008) in young male skiers compared with controls. A significant relationship was found between change (Δ) of training volume and ΔVO_{2peak} (mL·kg^{-1}·min^{-1}) (r = 0.475, p = 0.002). No significant main effect was detected between VO_{2peak} (mL·kg^{-1}·min^{-1}) dynamics (comparison with the previous age group data) and *ACE* I/D and *ACTN3* R577X genotypes interactions (F = 0.571, p = 0.770 and F = 0.650 and p = 0.705, respectively) in all young skiers. Study results indicated a significantly higher frequency of the *ACE* ID and *ACTN3* RR genotypes among Estonian young male skiers compared with the male control group. Significant genotype-related differences in dynamics of VO_{2peak} during a 5-year period were not found. In the future, longitudinal research including different gene variants may contribute to a better understanding of the nature of endurance performance.

Key words: Genes polymorphisms, endurance capacity, skiing, longitudinal study.

Introduction

Genetic predisposition has a substantial role in the development of athletic physical performance and is characterized by a large number of genes polymorphisms while the influence of multiple environmental factors is essential on gene expression (MacArthur and North, 2005). Endurance performance depends mainly on the integration of cardiorespiratory fitness, metabolic processes in skeletal muscles and neurological factors, but biomechanical, psycho-logical, physical, nutritional and other parameters are important as well (Joyner and Coyle, 2008; Rusko, 2003). The maximal oxygen uptake (VO_{2max}) is recognized as one of the major quantitative traits in sports physiology and the most common parameter to demonstrate training effects. VO_{2max} is influenced by different environmental and genetic factors (Rankinen et al., 2000). The maximal heritability for VO_{2max} (adjusted for age, sex and body mass) in the sedentary cohort of the HERITAGE Family Study was observed to be up to 50% (Bouchard et al., 1998). On average, women's VO_{2max} values are 10% lower compared to men due to higher body fat mass and lower levels of haemoglobin (Pate et al., 1987; Durstine et al., 1987). During the growth and maturation period in 11-17-year-old humans, the increase of VO_{2peak} is affected primarily by body lean mass (Armstrong and Welsman, 2001).

The angiotensin-converting enzyme gene insertion/deletion (*ACE* I/D) polymorphism (rs4340) and the α-actinin-3 gene *(ACTN3)* R577X polymorphism (rs1815739) are two of the most extensively studied genes in recent years in association with human physical performance. As part of the renin-angiotensin system, ACE (angiotensin-converting enzyme) has an essential role in the regulation of cardiovascular function and metabolic processes in muscles. ACE catalyses the conversion of angiotensin I to physiologically active angiotensin II, which causes vasoconstriction and through the release of aldosterone influences the regulation of electrolytes and water. In addition, ACE reduces the vasodilatory effect of bradykinin causing an increase in blood pressure (Thompson and Binder-Macleod, 2006). The functional polymorphism of human *ACE* I/D is related to the presence (insertion, I allele) or absence (deletion, D allele) of a 287-basepair (bp) segment in intron 16. Alvarez et al. (2000) have reported that the activity of ACE enzyme in the DD homozygotes is 3-4 times higher than in the II homozygotes. The I allele has been associated with improved endurance performance and the D allele with higher ACE activity and enhanced strength and sprint performance (Myerson et al., 1999; Thompson and Binder-Macleod, 2006). On the contrary to the previous researches, there are studies not showing the correlation between the *ACE* I/D poly-

morphism and enhanced physical performance (Rankinen et al., 2000; Taylor et al., 1999).

Another widely investigated gene influencing athletic performance is the α-actinin-3 *(ACTN3)*. The *ACTN3* gene encodes an actinin-binding protein in the sarcomeric Z line of fast-twitch muscle fibres of skeletal muscle (North et al., 1999; Mills et al., 2001). A common genetic variation in the *ACTN3* gene is the replacement of the arginine (R) with a stop codon (X) at amino acid 577 (C→T transition at position 1747 in exon 16) (North et al., 1999). The R577X polymorphism of the human *ACTN3* gene results in two variants of alleles: a functional R allele and a non-functional X allele (North et al., 1999; Mills et al., 2001). Yang et al. (2003) detected a significant association between the *ACTN3* R577X polymorphism and athletic performance. They found significantly higher frequencies of the R allele in elite sprint athletes than in controls. The non-functional X allele, which leads to the α-actinin-3 deficiency, supports better endurance ability. Eynon et al. (2012) reported that the *ACTN3* XX genotype had a higher frequency in endurance athletes, and it was more prevalent in world-class endurance athletes compared to national level athletes. On the contrary, in the Genathlete study on Caucasian male elite endurance athletes conducted by Döring et al. (2010), no association between endurance performance and the *ACTN3* R577X polymorphism was found. Furthermore, a study on Russian endurance athletes revealed that the α-actinin-3 deficiency may have a negative effect on performance in endurance athletes (Ahmetov et al., 2010). Ahmetov et al. (2010) disclosed that the XX genotype was significantly underrepresented among Russian endurance athletes compared with the control group. They pointed out that in addition to endurance capability, power and speed components are required as well to be successful in different endurance-oriented sports events.

Meta-analysis, which analyses relationships between the *ACE* I/D, the *ACTN3* R577X genotypes and athletic performance conducted by Ma et al. (2013), revealed significant associations of the *ACE* II genotype with enhanced endurance performance and the *ACTN3* R allele with better power performance.

Altogether, numerous previous studies have associated the *ACE* I/D and *ACTN3* R577X polymorphisms with enhanced physical performance, although outcomes have been contradictory across investigations. Secondly, there are relatively few longitudinal studies observing the association of dynamics in training-induced changes in endurance performance with gene polymorphisms.

The primary aim of the present study was to examine the prevalence of the *ACE* I/D and *ACTN3* R577X genotypes in Estonian young skiers compared to a control group. The secondary purpose was to evaluate the dynamics of endurance performance parameters during a 5-year period in relation to the *ACE* I/D and *ACTN3* R577X genotypes in Estonian young skiers.

Methods

Participants

Participants in the present study were young cross-country and biathlon skiers (hereafter young skiers). The total number of subjects was 58 (41 males and 17 females). 52% of young skiers were current or former Estonian junior national team members. The database of the electronic records (eHL) of Tartu University Hospital from the period 2002-2013 was used. All study participants had five consecutive pre-participation health evaluations (PHE) between the ages 15 to 19 years. Study inclusion criteria for young skiers were regular specific cross-country skiing training during the observed age period, and the PHE had to include training characteristics and an exercise test on a treadmill determining the maximal oxygen uptake peak (VO_{2peak}). The control group consisted of 322 healthy sedentary subjects without previous athletic training (145 males and 177 females). Male controls' mean age was 33.9 ± 13.2 years (height 1.81 ± 0.07 m, weight 80.0 ± 13.0 kg) and female controls' mean age was 37.1 ± 13.6 years (height 1.67 ± 0.06 m, weight 65.2 ± 11.0 kg). The ethnic origin of all participants was Caucasian.

Research Ethics Committee of the University of Tartu (protocol No. 196/M-30, and No. 207/M-9) approved the study protocol. Written informed consent was obtained from each participant.

Anthropometric measurements

Subjects' height (m) was measured using a metal anthropometer (± 0.1 cm) and body mass (kg) was determined by using clinical scales (± 0.05 kg). The body mass index (BMI) was calculated.

Cardiopulmonary exercise test

All incremental continuous exercise tests were performed on the treadmill (Telineyhtymä, Kotka, Finland). A standardized stress test protocol for cross-country skiers increasing the treadmill inclination and speed was used. All tests were performed to volitional exhaustion to obtain the parameters of maximal aerobic endurance. It has been found that not all individuals achieve the maximum oxygen consumption plateau during maximal exertion (Bassett and Howley, 2000). In this case, the highest level of oxygen uptake (VO_{2peak}) attained in an exercise test to exhaustion should be evaluated. VO_{2peak} is considered to be an indicator in assessing the aerobic capacity of children and adolescents (Armstrong and Welsman, 2001). VO_{2peak} (absolute, $L \cdot min^{-1}$ and relative, $mL \cdot kg^{-1} \cdot min^{-1}$) was determined by the direct breath-by-breath method using a gas analyzer (Oxycon Pro version 5.2, Hoechberg, Germany). The absolute value of VO_{2peak} is influenced by age-related overall growth in body size and increase in body mass (Bongers et al., 2014). To exclude this main phenomenon, we used the relative value of VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$) to assess the dynamics of VO_{2peak} related to the training capacity and investigated genes variants. In addition, the duration of the standardized exercise test (min) and maximal heart rate (HR_{max}, $beats \cdot min^{-1}$) were recorded, and exercise tolerance ($W \cdot kg^{-1}$) was calculated. Heart rate was recorded by using a sport-tester (Polar Electro, Finland). Gas analysis data were automatically calculated for every 30-second period. VO_{2peak} was considered as the highest VO_2 rate achieved within 30 se-

conds at the end of the exercise test. The criteria for maximal exercise test were the following: (1) respiratory exchange ratio (RER) equalled at least 1.1; (2) $HR_{max} >$ 90% of the age-predicted maximum calculated according to the formula 220 – age (Davis, 2006).

The change in VO_{2peak} (ΔVO_{2peak}; absolute, $L \cdot min^{-1}$; relative $mL \cdot kg^{-1} \cdot min^{-1}$) was obtained by calculating the differences of VO_{2peak} between 15 and 19 year-old age groups of female and male skiers. The change in exercise test duration (Δ duration of exercise test, min) and the training volume per week (Δ training hours, $h \cdot week^{1}$) were calculated similarly. Besides, the dynamics of VO_{2peak}, duration of exercise test and training volume were observed in each age group separately compared with the previous age group.

Genotyping

Peripheral venous blood samples for DNA extraction were collected from all study subjects and controls during the period 2010-2013. The DNA was extracted from peripheral venous blood samples in 9 ml EDTA containing vacuettes by using the standard salting-out method (Miller et al., 1988). The EDTA tubes were stored at -20°C until DNA extraction. Isolated DNA was dissolved in Tris-EDTA (TE) buffer. The purity and concentrations of the DNA were measured by a spectrophotometer (NanoDrop, ND-1000). The DNA samples were stored at -80°C until usage. Genotyping of rs4343 (*ACE*, C_11942562_20) and rs1815739 (*ACTN3*, C_590093_1) was carried out by using the TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA), which is a multiplex endpoint assay that detects variants of a single nucleic acid sequence. PCR reactions were run on the ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA) by using the following cycling parameters: after the first step at 95°C for 10 min, 40 cycles of denaturation at 92°C for 15 s and extension at 60°C for 1 min. Genomic DNA (20ng/μl) was amplified in a total volume of 5μl containing 1x Amplification Master Mix (Applied Biosystems, Foster City, CA, USA) and 1x probe. Genotypes were analysed by using the allelic discrimination function of the system.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 22.0 for WINDOWS). Descriptive data are presented as means (x), standard deviations (SD) and percentages (%). The chi-square (χ2-test) test was used to determine significant differences between the frequencies of the *ACE* I/D and the *ACTN3* R577X genotypes. The dynamics of VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$) and the duration of the exercise test (min) as the change between five time points were analysed with repeated analysis of variance (dynamics as a factor of five levels). Two-factor ANOVA was used to determine the interaction between VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$) dynamics (five levels), the *ACE* I/D genotypes (three levels) and the *ACTN3* R577X genotypes (three levels), respectively. The Pearson's correlation coefficient (r) was applied to determine the relationships between variables. P values of ≤ 0.05 were set as statistically significant.

Results

Anthropometrical, physical performance and training characteristics of young skiers by age and sex are presented in Table 1. Among young male skiers, a remarkable increase of the training volume ($h \cdot week^{1}$) was observed at the age of 16 years (t = -3.059, p = 0.007). In female skiers, no significant changes in the dynamics in training hours per week were found up to 18 years, whereas a significant increase was observed at the age of 19 (t = -2.882, p = 0.024). A significant improvement in the duration of the standardized exercise test performance (min) was detected in the 16- and 17-year age groups (t = -2.317, p = 0.030; t = -2.508, p = 0.018, respectively) among young male skiers and in young female skiers of 18 years in comparison with the previous age group (t = -3.977, p = 0.002). A statistically significant improvement of the absolute VO_{2peak} was found in males at the age of 16 (t = -3.849, p = 0.001), 17 (t = -3.232, p = 0.003) and 19 (t = -2.503, p = 0.020) compared with the previous age group. However, after correcting the differences for body size, the dynamics of relative VO_{2peak} was not significant in comparison across all age groups in young male skiers. We found a significant positive trend of absolute VO_{2peak} as well as relative VO_{2peak} in young female skiers only in the 17-year-old age group compared with the previous age group (t = -3.535, p = 0.008 and t = -2.386, p = 0.044, respectively).

Changes in performance parameters in young female and male skiers between the 15 and 19 year-old groups are shown in Table 2.

There were no significant differences in the distribution of the *ACE* I/D genotypes between young female skiers and controls although the prevalence of the *ACE* II genotype compared with controls was higher, 39% and 23%, respectively (t = 2.177, p = 0.140) and the *ACE* ID

Table 1. Characteristics of young skiers by age and sex groups. All data are presented as means (±SD).

Age group (yrs)	15 years		16 years		17 years		18 years		19 years	
Gender	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Weight (kg)	63.2 (8.0)	58.4 (8.6)	67.2 (8.4)[c]	60.2 (6.8)[b]	69.7 (7.8)[c]	60.6 (5.2)[a]	71.4 (7.6)[c]	62.3 (4.4)	72.5 (8.2)[c]	61.8 (5.0)
Height (m)	1.77 (.06)	1.67 (.04)	1.80 (.06)[c]	1.70 (.04)[c]	1.81 (.06)[c]	1.69 (.04)	1.82 (5.5)[b]	1.71 (.04)	1.82 (.06)	1.69 (.04)
BMI (kg·m⁻²)	20.2 (1.9)	20.8 (2.3)	20.8 (2.0)	21.0 (1.9)	21.3 (1.8)	21.3 (1.6)	21.5 (1.6)	21.4 (1.5)	21.9 (1.6)	21.6 (1.3)
TrVol (h·week⁻¹)	8.5 (2.5)	7.0 (1.4)	9.9 (2.7)[b]	7.6 (2.4)	11.3 (2.9)	9.3 (2.1)	12.4 (2.9)	10.9 (2.9)	13.4 (2.9)	13.2 (3.1)[a]
ET duration (min)	24.6 (5.1)	20.5 (3.4)	27.0 (2.2)[a]	21.1 (2.8)	27.9 (2.0)[a]	21.9 (2.4)	28.3 (2.2)	23.4 (1.7)[b]	29.3 (1.6)	24.6 (2.6)
VO$_{2peak}$ (L·min⁻¹)	4.0 (.6)	3.0 (.3)	4.4 (.5)[c]	3.1 (.1)	4.7 (.6)[b]	3.4 (.3)[b]	4.8 (.7)	3.4 (.3)	5.0 (.5)[a]	3.5 (.4)
VO$_{2peak}$ (mL·min⁻¹·kg⁻¹)	64.1 (6.1)	53.9 (5.9)	66.1 (4.7)	53.0 (5.2)	67.0 (4.5)	55.4 (5.5)[a]	67.6 (5.7)	54.9 (5.0)	68.1 (5.0)	57.1 (5.2)

[a] $p \leq 0.05$; [b] $p \leq 0.01$; [c] $p \leq 0.001$ compared to the previous age group; TrVol = training volume; ET = exercise test
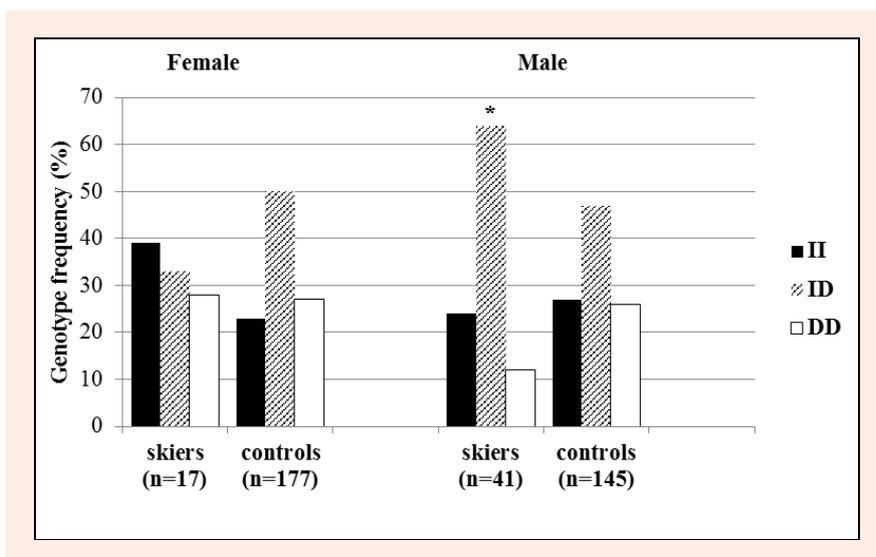
Figure 1. Genotypic frequencies of the *ACE* I/D polymorphism (rs4340) in young skiers and non-athletic controls.
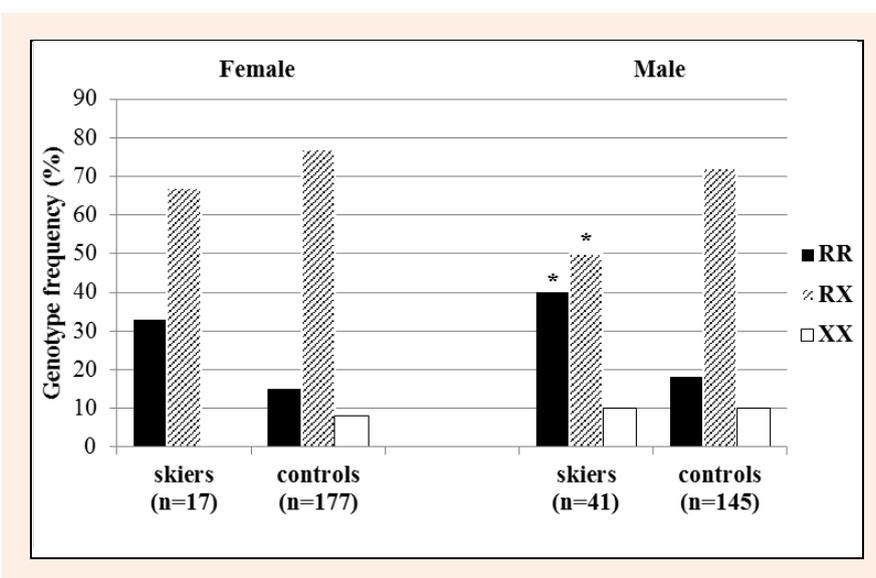* $p < 0.05$ in comparison with controls (χ2-test).

Figure 2. Genotypic frequencies of the *ACTN3* R577X polymorphism (rs1815739) in young skiers and non-athletic controls.
* $p < 0.05$ in comparison with controls (χ2-test).

Table 2. Changes in performance parameters (Δ) between 15 and 19 year-old age groups of female and male skiers. All data are presented as means ($\pm$SD).

	Young female skiers (n=17)	Young male skiers (n=41)
Δ TrVol (h·week^{-1})	6.0 (3.6)	5.1 (3.5)
Δ ET duration (min)	3.5 (4.2)	3.1 (2.9)
Δ VO$_{2peak}$ (L·min^{-1})	.5 (.4)	.9 (.6)
Δ VO$_{2peak}$ (mL·kg^{-1}·min^{-1})	3.6 (4.6)	4.0 (5.2)

ET = exercise test; TrVol = training volume

genotype was lower, 33% and 50%, respectively (t = 1.757, p = 0.185). However, the frequency of the *ACE* ID genotype in young male skiers compared with controls was statistically higher (t = 3.940, p = 0.047). There was a non-significant lower trend of the *ACE* DD genotype among young male skiers compared to the male control group (t = 3.465, p = 0.063) (Figure 1).

In the distribution of the *ACTN3* genotypes among young female skiers, there were no subjects carrying the *ACTN3* XX genotype in comparison with the group of female controls (n = 13; 7.4%). Although there was a trend towards a higher frequency of the *ACTN3* RR genotype in female skiers (33.3%) in comparison with female controls (15.3%), the difference remained statistically non-significant (t = 3.691, p = 0.055). When analysing the results of the distribution of the *ACTN3* R577X genotypes frequencies in male skiers compared with male controls, the frequency of the RR genotype was statistically higher (t = 9.045, p = 0.003) and the frequency of the RX genotype was lower (t = 7.128, p = 0.008) (Figure 2).

We did not detect any statistically significant relationships between the *ACE* I/D, the *ACTN3* R577X genotypes and the ΔVO$_{2peak}$ (Table 3). However, a considerable increase of the ΔVO$_{2peak}$ was observed in female skiers with the *ACE* ID genotype (6.2 ± 5.7 mL·kg^{-1}·min^{-1}, n = 5) and in male skiers with the *ACTN3* XX genotype (7.6 ±

4.8 mL·kg^{-1}·min^{-1}, n = 4), the increase was statistically nonsignificant.

Table 3. VO$_{2peak}$ changes (Δ VO$_{2peak,}$ mL · kg^{-1} · min^{-1}) according to the *ACE* I/D and the *ACTN3* R577X genotypes between 15 and 19 year-old age groups of female (n =17) and male (n = 41) skiers. All data are presented as means (± SD).

	Young female skiers Δ VO$_{2\ peak}$	Young male skiers Δ VO$_{2\ peak}$
***ACE* I/D genotypes**		
II	2.0 (2.4) (n = 7)	5.3 (5.5) (n = 10)
ID	6.2 (5.7) (n = 5)	3.2 (5.0) (n = 26)
DD	3.3 (5.5) (n = 5)	5.0 (6.2) (n = 5)
***ACTN3* R577X genotypes**		
RR	3.7 (2.6) (n = 5)	3.8 (4.8) (n = 16)
RX	3.7 (5.3) (n = 12)	3.4 (5.5) (n = 21)
XX	-	7.6 (4.8) (n = 4)

The correlation analysis showed a statistically significant relationship between the Δ training volume and the ΔVO$_{2peak}$ (mL·kg^{-1}·min^{-1}) among young male skiers (r = 0.475, p = 0.002), but there was no significant relationship between these variables among female skiers (r = 0.331, p = 0.247).

According to the two-factor ANOVA analysis, a significant main effect of dynamics of the VO$_{2peak}$ (mL·kg^{-1}·min^{-1}) was detected in the total study group (F = 9.592, p < 0.001), as well as in males (F = 6.933, p < 0.001) and females (F = 5.741, p < 0.001). No significant effect was detected between the dynamics of the *V*O$_{2peak}$ (mL·kg^{-1}·min^{-1}) and the *ACE* I/D genotypes interaction (F = 0.571, p = 0.770) or between the dynamics of the VO$_{2peak}$ (mL·kg^{-1}·min^{-1}) and the *ACTN3* R577X interaction (F = 0.650, p = 0.705) in all young skiers. There were no gender differences in all measured interactions.

Discussion

The results of our study revealed a significantly higher prevalence of the *ACE* ID and the *ACTN3* RR genotypes and lower *ACTN3* RX genotypes in male skiers in comparison with controls. A trend of a lower frequency of the *ACE* DD was observed among young male skiers compared with male controls.

Competitive cross-country skiing belongs to the group of one of the most demanding sports events. In the classification of sports activities by Mitchell et al. (2005), which is based on the type and intensity of exercise performed, cross-country skiing is characterized as a sport with high dynamic and up to moderate static (depending on the technique of skiing) capacities. Regardless of the very different length of race distances − from sprint distances of 1.5 km up to 50 km − all distances require high endurance performance (Rusko, 2003). It has been found in experimental studies that depending on the terrain profile, the proportion of aerobic energy production in longer distances can reach up to 90 - 99%, while during sprint ski-racing it may be 50% on average (Rusko, 2003).

Folland et al. (2000) associated a larger gain in muscle strength and hypertrophy after resistance training with the D allele of the *ACE* gene in their study. Muscle

strength, especially upper body muscle strength plays an important role in addition to endurance capacity in competitive cross-country skiing (Alsobrook and Heil, 2009). Thus, the combination of the I and D allele of the *ACE* I/D polymorphism may be advantageous for good performance in skiing. In our study, it was not possible to distinguish between the sprint and distance skiers because young skiers participated in both distances during the observed age period. In addition, our results did not confirm reliable evidence for the association between the *ACE* II genotype and endurance performance.

Our findings showed a higher prevalence of the *ACTN3* RR genotype among young male skiers, which does not concur with the evidence from meta-analysis by Alfred et al. (2011), where the RR genotype was found more common in sprint and power athletes compared with controls among Europeans (Alfred et al., 2011). However, no evidence was found in this meta-analysis that the X allele gives an advantage in endurance performance (Alfred et al., 2011).

Several studies (Alsobrook and Heil, 2009; Paavolainen et al., 1991) emphasize the importance of muscle mass and explosive power in the speed of skiing. A higher prevalence of the *ACTN3* RR genotype among young male skiers compared with the male control group (p = 0.006) in our study may explain the previous argument. In a study among Russian endurance athletes, researchers found that the *ACTN3* X allele was underrepresented in Russian endurance athletes and the α-actinin-3 deficiency was estimated as one possible limiting factor in improving power and strength in endurance events (Ahmetov et al., 2010). Data of the present study suggest that the distribution of the *ACTN3* R577X genotypes among young male and female skiers was different. Although there was a statistically significant over-representation of the RR genotype in male skiers in comparison with male controls, no significant differences between young female skiers and female controls were found. One reason for this might be a relatively small sample of the female study group (n = 17).

In our study, we did not find a significant association between the *ACE* I/D, the *ACTN3* R577X genotypes and increase of VO$_{2peak}$ (ΔVO$_{2peak}$, mL·kg^{-1}·min^{-1}) in either the male or female study group. However, a larger increase of the VO$_{2peak}$ (mL·kg^{-1}·min^{-1}) during the 5-year training process can be observed among female skiers with the *ACE* ID genotype, but it remained statistically nonsignificant (t = 0.760, p = 0.472). On the contrary, the ΔVO$_{2peak}$ (mL·kg^{-1}·min^{-1}) in male skiers during the 5-year observation period was not associated with the *ACE* II, ID or DD genotype. We detected a recognizable, although statistically nonsignificant improvement in the ΔVO$_{2peak}$ (mL·kg^{-1}·min^{-1}) among male skiers with the *ACTN3* XX genotype, which is in agreement with the studies, where a higher XX genotype prevalence in endurance sport has been found (Eynon et al., 2012; Yang et al., 2003). However, a limiting factor of the present study is the small sub-group of the *ACTN3* XX genotype − four subjects of 41 male skiers. At the same time, Thompson et al. (2006) did not find significant associations between the dynamics of the VO$_{2max}$ and *ACE* I/D genotypes after a 6-week

training program. In our study, the ΔVO_{2peak} (L·min^{-1}), the ΔVO_{2peak} (mL·kg^{-1}·min^{-1}) and the Δ duration of the standardized exercise test did not show significant associations with the *ACE* I/D and *ACTN3* R577X genotypes. We can assume that the *ACE* II and the *ACTN3* XX genotypes might not provide an advantage in endurance performance phenotypes in our study group. The latter supposition conforms to several previous studies, as conducted by Alfred et al. (2011) and Lucia et al. (2006). We observed the significant correlation between the Δ training volume and the ΔVO_{2peak} (mL·kg^{-1}·min^{-1}), i.e. increase in training hours related positively to the dynamics of the VO_{2peak} (mL·kg^{-1}·min^{-1}). Rusko (1992) has described in his review article that longitudinal studies among skiers have referred to the VO_{2max} increase with age and training during the 15-20-year-old period, and the annual increase may amount up to 1-3 mL·kg^{-1}·min^{-1} per year. In our study, a significant main effect of the VO_{2peak} (mL·kg^{-1}·min^{-1}) was detected in the total study group, but no significant effect was found between the VO_{2peak} (mL·kg^{-1}·min^{-1}) and the *ACE* I/D and *ACTN3* R577X genotypes interactions.

Conclusion

The main result of this study was a significantly higher prevalence of the *ACE* ID and the *ACTN3* RR genotypes among Estonian young male skiers compared with the male control group, which may be an advantage for the explosive speed and power capacity in race skiing. Our study showed a more remarkable trend of increase in the VO_{2peak} (mL·kg^{-1}·min^{-1}) during the 5-year period among male skiers with the *ACTN3* XX genotype and among female skiers with the *ACE* ID genotype. No significant genotype-related associations in the dynamics of the VO_{2peak} during the 5-year period were found.

From a practical viewpoint, the genetic profile combined with physiological and biochemical characteristics could form a strong predictive package, which could be used by athletes and their teams for personalized management, monitoring and better optimization of training processes in the future. As multiple combinations of gene variants influence physical performance phenotypes, future longitudinal research, including different gene variants, may contribute to a better understanding of the nature of physical performance.

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Key points

- Significantly higher prevalence of the *ACE* ID and the *ACTN3* RR genotypes were found among Estonian young male skiers compared with the male control group, which may be an advantage for the explosive speed and power capacity in race skiing.
- A more remarkable trend of increase in VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$) during the 5-year period was observed among male skiers with the *ACTN3* XX genotype and among female skiers with the *ACE* ID genotype.
- No significant genotype-related associations in the dynamics of VO_{2peak} were found during the 5-year period.

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