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THE OCCURENCE OF R577X POLYMORPHISM OF *ACTN3* GENE IN A SELECTED GROUP OF ELITE FENCERS

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ABSTRACT

The presented study is focused at identifying the genetic predispositions for anaerobic performance in a group of elite fencers of the Czech Republic. Based on theoretical findings and previous studies, we assume that the identification of genetic coding can help revealing sport talents or recommend to a person interested in sport a suitable branch considering his qualifications. The study concentrates on the comparison of speed-strenght abilities determined by Wingate test performed in the Biomedical laboratory of FTVS at Charles University in Prague and specific speed tests for fencing with R577X polymorphism of *ACTN3* gene in elite fencers of the Czech Republic. By the analysis of buccal smear, we found that 80% of our sample of fencers contains in their genotype at least one R allele of the *ACTN3* gene of the R577X polymorphism and 27% of the whole are homozygotes with RR genotype.

Keywords: speed abilities; fencing; polymorphism R577X *ACTN3*; genetic predispositions; genotype

INTRODUCTION

Sport performance is a broad term covering many agents who are directly or indirectly participating in its level. Environmental factors, such as training and nutrition, are important for the development of the elite athlete, however these factors alone are not enough and most of us can never achieve an elite athlete status no matter how hard we try. Elite sport performance is a complex phenotype of physical capability defined by genetic potential (MacArthur & North, 2005). Phenotype of an individual, which may have a genetic basis for endurance skills, muscle strenght, physiological capacity to

repeat a series in high-intensity or ability of tendons and ligaments to withstand damage, provides us information important for the selection of sport branch (Lippi, Longo, & Maffulli, 2009). Current scientific information suggest that the genetic information in form of several genotypes is an inseparable part of influencing the sport level of every sportsman, and because motoric skills are determined to a greater or lesser extent genetically, it is necessary to take the new molecular genetic research into consideration and involve them in the well-established methods used for predicting of movement abilities (Měkota & Novosad, 2005). Genotype differences, i.e. changes in DNA, can affect a concrete protein function and thus positively or negatively influence some abilities of a given individual. Ahmetov et al. (2012a) in his study states that the most common types of polymorphism variant of DNA sequence are insertion or deletion (I/D) of individual nucleotides. Lippi, Longo, & Maffulli (2009) in their study states that the most commontypes of polymorphism are SNP's (single-nucleotide polymorphism).

Genetic differences can thus influence the amount and the structure of mRNA or protein and therefore may represent major share of genetic factors in the variability of human phenotype. Bouchard, Malina, & Pérussé (1997) in their publication mention another important role of nutritional genomics, in which the normal diet contains a number of bio-active substances which can activate or modulate the transcription of target genes through receptors or directly cause a change of chromatin structure. They represent the next possibility which could be used in the future supplement of the training plans, regeneration methods or convalescence therapies.

According to the existing body of knowledge, the best sportsmen of different specialisations have different share of fibers (Dovalil et al., 2009). The influence of genetic load for a specific physical performance is estimated around 40–50%. The anaerobic performance, in the form of short-term anaerobic action to 10 s, is estimated to be higher than 50% (Bouchard, Malina, & Pérusse, 1997). Měkota and Novosad (2005) reported that the strongest is genetically determined maximal anaerobic alactate performance, which is crucial for the realization of speed-strenght movements. The same argument is also supported by Grasgruber and Cacek (2008), who report as significantly genetically influenced components of sport performance the composition of muscle proteins, blood flow in heart and lungs, as well as the activity of of key enzymes participating in energy production. Havlíčková (2004) states that the speed capabilities are genetically influenced from 65–80%. The simple movement speed is affected the least and we can reach the greatest development of speed capabilities in our school age.

Fencing is a speed-strenght sport, whose energy coverage takes place mainly in the glycidic energy substrate zone. The maximum heart rate during the fight rises on the level of 70–90% of its maximum value. This discipline makes demands on the coordination abilities, dexterity and speed. The basic precondition of a successfull fencer is the prevalence of fast muscle fibers and therefore preconditions to speed abilities rather than endurance movement elements (Jirka, 1995). Factors that may affect the preconditions of the performance in fencing can be observed in the area of anaerobic metabolism measured by Wingate test. The important area for assumption of sport performance in fencing is the level of complex psychomotoric state, such as speed, accuracy and adaptability in motoric learning (Borysiuk & Waskiewicz, 2008). The duration of each fight is influenced by a number of interruptions caused by a referee, that can be used by the fencers as a resting

interval for recovery of the organism. Owing to this fact, the fencers can perform most of the actions which last on average 5–15 s (depending on the type of weapon), in high-intensity load (Lavoie, Léger, Pitre, & Marini, 1985).

Ahmetov, Vinogradova, and Williams (2012b) reported that the abilities to perform aerobic and anaerobic exercise differs significantly depending on the composition of muscle fibres. Muscle fibers are classified as type I and type II fibres with subgroups IIA and IIX (IIB). These fibre types differ in maximum shortening speed. Type I fibres show the slowest contractions but high fatigue resistance, and type II fibres, especially type IIX, show the fastest contractions, hence higher maximum shortening speed, larger cross-section area diameter but low fatigue resistance. That means that they can produce substantially greater maximum performance. The data based on metabolic profile of muscle fibers show that type I fibres are rich in oxidative enzymes and suitable for endurance performance. Type IIX fibres are rich in glycolytic enzymatic activity and are adjusted to short exploding high speed and performance. Type IIA fibres have middle activity of enzymatic function and are better adapted for medium anaerobic exercise. Melichna (2004) states that the degree of genetic interdependence limits the range of adaptive plasticity of a given character and thereby limits also the influence of sport training. The smaller the influence of the training is, the higher will be the value of heritability coefficient. This coefficient can be up to 99.6% for the percentage share of fast or slow muscle fibres. This is relatively high genetic interdependence of a character. Conversely metabolic potential of the muscles participating in releasing the energy for muscle function is apparently not unambiguously genetically dependent.

A significant protein, which is present only in fast muscle fibres (fast twitch – FT fibres, or type IIA and IIX fibres), and to which this contribution is dedicated, is actin-binding protein (alpha-actinin-3 = *ACTN3*). *ACTN3* is always present among the best performative athletes-sprinters (Lippi, Longo, & Maffulli, 2009). Human sarcomeric α -actinin isoforms (α -actinin-2, α -actinin-3) contain a predominant protein part of sarcomeric Z-line, where it produces a latticed structure, which binds together thin actin filaments and stabilizes muscle contractile apparatus (MacArthur & North, 2004). According to Sovičová (2010), the isoforms of α -actinin are divided into two groups based on the ability to bind calcium. Muscle α -actinins (*ACTN2*, *ACTN3*) are not dependent on calcium to bind aktin. Non-muscle isoforms (*ACTN1*, *ACTN4*) bind actin via calcium. The expression of *ACTN3* gene is limited to a subgroup of two fast muscle fibres. The change of the basis occurs in nucleotide 1747 in exon 16 of *ACTN3* gene, which results in the emergence of R577X polymorphism. Ahmetov et al. (2012) adds that 577X allele contains modified sequences, which can completely prevent the production of a functional protein α -aktinin-3. Sovičová (2010) further states that the presence of *ACTN3* has a beneficial effect on the function of skeletal muscles in creating strong contractions in high speed and thus provides evolutionary advantage in sprint disciplines. Ahmetov et al. (2012b) mention several studies which state that the *ACTN3* RR genotype is over-represented or that *ACTN3* XX genotype is under-represented in strenght and speed athletes compared with control group. Eyon et al. (2009) mention functional polymorphism, which was identified in *ACTN3* gene and can affect the speed performance. This polymorphism encodes the muscle isoform of alpha-actinin-3, which leads to the substitution of arginin (R) with premature stop-codon (X) at 577 amino acid. 577R allele and thus also 577RR genotype

from polymorphism *ACTN3* R577X was found in connection with the highest level of sprinters in wide selection of ethnic groups. Ahmetov et al. (2012a) reported that to date more than 20 genetic variants were connected with strength and performance-related phenotypes, of which gene polymorphisms *ACE* (angiotensin-converting enzyme), *ACTN3* and *PPARA* (peroxisome proliferator-activated receptor α) has been the most studied for the time being. Our study is based on the knowledge of fencing and genetic encoding of speed abilities and thus we presuppose certain connection between genetic encoding of speed abilities, i.e. R allele *ACTN3* R577X gene polymorphism, and performed tests in a group of elite fencers.

PURPOSE

The aim of this work is to determine the occurrence frequency of R577X gene polymorphism in the group of elite fencers and compare these results with applied tests.

METHODS

Fifteen elite and subelite fencers of the average age of 24.9 (\pm 6.6) participated in the research investigation. The criterion for the selection of the subjects was placing of the fencers in the first twenty percent of the total number of starters in current season 2011/2012. The subjects were instructed to perform all measured attempts at maximum possible speed. The measurement was conducted during May and June, which was the peak of the season.

The level of maximum anaerobic performance and general dispositions for speed were conducted by Wingate load test on MONARK (Sweden) bicycle ergometer in Biomedical laboratory of FTVS of Charles University (Prague). The test started after cca 5 minute warm-up without a load, when the subject reached above the level of 130 beats per minute. The value of heart frequency was monitored by sport-tester POLAR. The actual test was carried out for 30 s with cca 10% resistance of the actual body weight of each subject. Given the focus of our study, we used the maximum number of revolutions (RPT – number of revolutions per 30 s) and the maximum value of power (P_{\max}) expressed in watts to compare the results from genetic inquiry.

Another method used was determination of the speed of extension at the elbow joint. The tested subjects had to hit as quickly as possible a hitting target, which was a part of the Fitosword device for visual stimulus. The target was placed at a height of the tested person's breastbone (*processus xiphoideus os sternum*) in the guard position. After lightin up the red LED diodes, the guard of the used sword left (Uhlmann, Germany) the highly sensitive horizontal obstacle on which was the sword placed in the guard position. Software Sword evaluated the movement time of the hit alone, and thus we excluded the negative intervention of reaction time, which was not the subject of our investigation. The horizontal barrier was placed 125 cm from the target. For the direct hit inquiry we came out of the Williams and Walmsley study (2000). Fitosword device was used again for detection of the lunge speed. In this case, we proceeded as in the previous experiment,

where we separated the reaction time from the overall response time. The lunge's movement distance was determined as the distance between the vertical axis intersecting the vertical line of the centre of the hitting target and the floor. From this point, we measured individual distance considering the height of each subject. The height of the tested person in centimetres was multiplied by 1.5 coefficient (Williams & Walmsley, 2000) and the resulting value was transferred to the floor.

To detect the strength preconditions, we used digital dynamometer in Biomedical Laboratory of FTVS of Charles University, Prague. The tested subject was in the sitting position and the arm, which held the dynamometer, was freely lowered along the body to the ground. In this position, the test subjects performed three handgrips. The resulting values were averaged.

Another test used was specific shuttle test (Iglesias & Rodriguez, 2008; Tsolakis & Vagenas, 2010). The subjects were to move between two lines that were spaced 5 m, as quickly as possible using the advance and backward, so that the total distance of the shuttle test would be 30 m. The actual test was launched from the guard position on the baseline by START command. The test was terminated as soon as the subject overstepped the start line with his front leg and overcame the mentioned 30 m. The test was conducted three times, with a 30 minute rest interval. The average values of all three tests are shown in the results.

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells using the isolation kits provided by the commercial laboratories Genomac International, s. r. o. (Prague, CR). The smears were made by a special sterile brushes that were after the swabbing put into test tubes with lids. The obtained samples were thoroughly described and taken into the genetic laboratory. The genotypic analysis of R577X *ACTN3* was conducted the above mentioned laboratory.

RESULTS

The results of our study are presented below in Table 1. Due to a low number of tested individuals, we did not statistically analyze the results. The resulting values of single variables are always expressed in arithmetic average and standard deviation.

The Table 1 shows that R allele occurs 12× at the investigated group, of which 4× it is in a homozygous form. The complete absence of the observed allele appeared only in 3 subjects. Certain connection was found between the relevant genotype and the direct lunge movement time, the maximum performance value in Wingate test (Pmax) and the number of revolutions in the maximum 30 second Wingate test (RPT). Subjects with RR genotype, i.e. homozygotes, had the best results in the above mentioned tests. Subjects with XX genotype had the weakest results and the subjects with RX genotype, i.e. heterozygotes, achieved a little weaker results than the individuals with RR genotype.

In the applied tests, in which we investigated lunge movement time, specific shuttle test and handgrip strength, the resulting values did not meet our assumptions.

It is obvious from Table 1, that R allele, i.e. speed allele, occurs in 80% of monitored fencers.

Table 1. Identified variables in various tests

ACTN3	Genotype	Subject	PČV [ms]	PČPB [ms]	SČT [s]	HG D [N]	RPT/30s	P _{max} [W]
	XX	Subjekt 1	512	207	11.83	55.5	52	938
		Subjekt 2	540	213	10.81	55.1	53	1081
		Subjekt 3	580	197	11.48	46.1	47	749
		Diameter	544	205.7	11.4	52.2	50.7	922.7
		SD	34.2	8.1	0.5	5.3	3.2	166.5
	RX	Subjekt 4	528	178	11.85	41.7	51	876
		Subjekt 5	608	179	12.8	50.3	52	946
		Subjekt 6	453	192	12.51	46.8	45	828
		Subjekt 7	522	174	11.21	42.1	60	1015
Subjekt 8		455	180	11.92	37.1	54	947	
Subjekt 9		508	141	11.25	41	53	948	
Subjekt 10		483	194	12.08	56.8	50	1115	
Subjekt 11		499	179	12.51	50.4	47	985	
Diameter		507	177.1	12.0	45.8	51.5	957.5	
SD	49.5	16.2	0.6	6.5	4.6	86.7		
RR	Subjekt 12	519	174	10.75	57.5	57	1107	
	Subjekt 13	537	184	12.45	34	50	788	
	Subjekt 14	552	185	11.85	57.2	50	1072	
	Subjekt 15	552	142	11.12	56.1	50	1102	
	Diameter	540.0	171.3	11.5	51.2	51.8	1017.3	
	SD	15.7	20.1	0.8	11.5	3.5	153.6	

Legend: PČV – lunge movement times; PČPB – direct lunge movement time; SČT – specific shuttle test; HG D – dominant limb handgrip; RPT/30 s – maximum number of revolutions in 30 s during Wingate testu; P_{max} – maximum value in Wingate test

DISCUSSION

The aim of our study was to determine the occurrence frequency of the R577X polymorphism of *ACTN3* gene in a group of elite fencers and to compare these results with the tests used.

For the analysis of speed capabilities, we were looking for suitable tests that would bring specific physical skills and abilities closer to the laboratory conditions. For this reason, we chose a simple movement maneuver in the form of direct hit, lunge and specific shuttle test, in which we examined the movement time or duration of the activity. After the evaluation of the results we discovered that the easiest movement test in the form of direct hit was the most suitable for our research. For tests, such as specific shuttle test and movement time of the lunge, the skill level of tested people, which is associated with learned movement patterns, could have negatively affected the results.

Although fencing is a speed sport where an attack may last only a few seconds, some actions at a high intensity can sometimes during a fight last up to 30 s. These circumstances led us to use Wingate test in determining the performance level of fencers. We selected only the value of the maximum power (P_{\max}) and the number of revolutions achieved in 30 s (RPT) from the obtained data.

Due to the fact that the fencers have a weapon in hand during the whole fight which weighs at the most 700 g, we also involved in our study the testing of hand grip strength (so-called handgrip). The results of this testing contained great differences, in which we found that subjects younger than 24 years had significantly lower values.

The results of our study also proved that individuals with XX genotype, i.e. without the presence of “speed allele” had worse values in some tests and in others excelled. We believe that further similar genetic investigations should be directed at a larger number of genes that affect athletic performance and try to give more complex overview of the possible linkages of certain genes and movement skills.

CONCLUSION

Fencing is a speed-strength sport that makes demands on coordination skills, dexterity and speed (Jirka, 1995). In the second half of the 20th century occurred, due to the existence of signaling devices which made decisions easier, an acceleration of the course of the fight. This put greater demands on speed and complex actions became redundant (Trohař, 1973). From this point of view, we decided to perform a study in which we tested 15 elite fencers in several speed tests and DNA tests, where we monitored the frequency of the R577X polymorphism of the *ACTN3* gene. After studying the relevant studies, we assumed that our selected group will have higher occurrence of R allele genotype (RR, RX).

The results of genetic investigations were compared with values from the speed-power tests. We concluded that the simple movement actions are more suitable for determination of the speed level than the complex actions, where the specific and longer-lasting training and final performance with individual skills of the tested individuals must precede.

By the analysis of buccal smear (Genomac International, s. r. o., Prague), we found that 80% of our sample of fencers contains in their genotype at least one R allele of the *ACTN3* gene of the R577X polymorphism and 27% of the whole are homozygotes with RR genotype. Only 20% of our sample has XX genotype. After the comparison with the performative tests, we found that the group with RR and RX genotype had better results than subjects with XX genotype in performative tests, such as the lunge movement time, maximum number of revolutions during Wingate test and the maximum power at Wingate test. In other tests, we found no association between the genotype and the results. We are aware of the low number of observed probands which should be extended in future studies. Yet it is clear from our study that R allele genotype supporting speed capabilities occurs in the group of elite fencers and in the next investigation we want to concentrate on the other possibilities of genetic encoding.

We believe that for the fencing performance are undoubtedly important speed abilities, whose usability during the game is complemented by a composition of other factors (technical, tactical, fitness, somatic, psychological, and so on), which are in mutual interaction (Barth & Beck, 2007; Roi & Bianchedi, 2008). For this reason it is necessary to monitor

the genetic predispositions in other components of sport performance and thus contribute towards the completion of the knowledge of genetic encoding of movement skills.

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