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ACTN3 Genotype Association with Motoric Performance of Roma Children

J. Bernasovska, I. Boronova, J. Poracova, M. Mydlarova Blascakova, V. Szabadosova, P. Ruzbarsky, E. Petrejcikova, I. Bernasovsky

***Abstract*—**The paper presents the results of the molecular genetics analysis in sports research, with special emphasis to use genetic information in diagnosing of motoric predispositions in Roma boys from East Slovakia. The ability and move are the basic characteristics of all living organisms. The phenotypes are influenced by a combination of genetic and environmental factors. Genetic tests differ in principle from the traditional motoric tests, because the DNA of an individual does not change during life. The aim of the presented study was to examine motion abilities and to determine the frequency of ACTN3 (R577X) gene in Roma children. Genotype data were obtained from 138 Roma and 155 Slovak boys from 7 to 15 years old. Children were investigated on physical performance level in association with their genotype. Biological material for genetic analyses comprised samples of buccal swabs. Genotypes were determined using Real Time High resolution melting PCR method (Rotor-Gene 6000 Corbett and Light Cycler 480 Roche). The software allows creating reports of any analysis, where information of the specific analysis, normalized and differential graphs and many information of the samples are shown. Roma children of analyzed group legged to non-Romany children at the same age in all the compared tests. The % distribution of R and X alleles in Roma children was different from controls. The frequency of XX genotype was 9.26%, RX 46.33% and RR was 44.41%. The frequency of XX genotype was 9.26% which is comparable to a frequency of an Indian population. Data were analyzed with the ANOVA test.

***Keywords*—**ACTN3 gene, R577X polymorphism, Roma children, Slovakia, sports performance.

I. Introduction

ALPHA-ACTININS are a family of actin-binding proteins which are major structural components of the sarcomeric Z-discs in skeletal muscle [1]. They are believed to be responsible for anchoring the actin-containing filaments and maintenance of the spatial arrangement of thin filaments within the sarcomere, as well as the interaction of the cytoskeleton with the sarcolemma [2]. One of the α-actinin isoforms, α-actinin-3, is expressed solely in type II (fast twitch) muscle fibers which are the predominant muscle fibers involved in sprint and power activities [3]. The *ACTN3* gene, located on 11q13.1, is expressed only in fast-twitch fibers, which trigger faster and generate more force than alfa-actinin-2. A common genetic variation in the *ACTN3* gene that results in the replacement of an arginine (R) with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified [3]. Homozygosity for the nonsense mutation, 577X, within *ACTN3* results in the deficiency of α-actinin-3 but do not result in an abnormal muscular phenotype. This suggests that this protein is functionally redundant in humans. The finding that approximately 16% of the world population has a congenital deficiency of α-actinin-3 further corroborates this theory. Since this gene has been conserved through evolution, it must fulfill an as yet unidentified function [4].

Physical fitness is a very complex phenotype influenced by many genetic and environmental factors that contribute to inter-individual variability not only in athletes but also in the general population [5]. Athletic performance is determined by many factors, with genetic compound accounting for 20 - 80% [6]. The human genetic map of performance and health consists of more than 150 genes and gene regions associated with athletic performance and fitness. R577X polymorphism of the ACTN3 gene for alpha-actinin-3 is a potential precondition contributing to differences in the structure and function of muscle performance [2]. It is a substitution of cytosine for thymine at the position 1747 in exon 16, which results in the change of codon for arginine (R) at position 577 to a premature stop codon (X) [4]. It causes premature transcription termination, subsequently shorter mRNA molecule, and dysfunctional protein [7]. This variation results in two versions of human ACTN3 protein. Allele 577R is functional, while 577X is dysfunctional allele. This polymorphism generates three possible genotypes: XX, RX, and RR. Genotype XX: R577X variant is present in both copies of the ACTN3 gene. This genotype is associated with a natural predisposition for endurance disciplines (triathlon, swimming ≥ 400m). Genotype RR: R577X variant is not present in any copy of the gene ACTN3. This absence is associated with a natural predisposition for sprint/power discipline (running ≤ 800 m, swimming ≤ 200m, track cycling for short distances, rugby, bodybuilding and weight lifting). Genotype RX: R577X variant is located in one of the two copies of the ACTN3 gene. This combination is suitable for sports, where the power and speed are needed and as well as endurance (football, handball, tennis or basketball).

The specific analysis of ACTN3 gene may be helpful in choosing the appropriate sports activities. The results of sports

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Jarmila Bernasovska, Iveta Boronova, Janka Poracova, Marta Mydlarova Blascakova, Viktoria Szabadosova, Eva Petrejcikova are with the Department of Biology, Faculty of Human and Natural Sciences, Prešov University in Prešov, 080 01 Prešov, Slovak Republic (phone: +421 51 75 70 640; e-mail: jarmilly2@gmail.com). Pavel Ruzbarsky is with Faculty of Sport, Prešov University in Prešov, 080 01 Prešov, Slovak Republic (e-mail: pavel.ruzbarsky@unipo.sk) and Ivan Bernasovsky is with the Institute of Romany Studies, Prešov University in Prešov, Slovak Republic (e-mail: bernaky@unipo.sk).

performance genetic research are useful for precise profiling of athletic training and the creation of individual training plans for elite athletes. The genetic test can provide athletes with the relevant information that can help them to decide which kind of sport to pursue, in order to achieve the best utilization of their abilities.

II. Materials and Methods

Genotype data were obtained from 293 children - 138 Roma and 155 Slovak boys from 7 to 15 years old. Biological material for genetic analyses comprised samples of buccal swabs which were extracted by available commercial kits according to the standard protocol and concentration of DNA samples was measured by a spectrophotometer Nanodrop ND 2000. Genotypes for the *ACTN3* rs1815739 (R577X) polymorphism were detected by polymerase chain reaction (PCR) followed by high resolution melting (HRM) analysis using Rotor Gene 6000 Corbett and LightCycler ® 480 Real-Time PCR System (Roche). Amplification of the fragments was performed using the forward primer 5'-TCAGTTCAAGGCAACACTGC-3' and reverse primer 5'-CTTCTGGATCTCACCCTGGA-3'. A 35-cycle PCR was carried out with the following conditions: denaturation of the template DNA for the first cycle of 94°C for 120 s, denaturation of 94°C for 20 s, annealing of 60°C for 20 s, and extension of 72°C for 20 s. In the HRM phase, Rotor-Gene 6000 Corbert measured the fluorescence in each 0.1°C temperature increase in the range of 70-94°C. Melting curves were generated by the decrease in fluorescence with the increase in the temperature; nucleotide changes resulting from different curve patterns were analyzed and genotyped. Children were investigated on physical performance level in association with their genotype.

III. Results

The functional allele (577R) of ACTN3, which encodes human alpha-actinin-3, has been reported to be associated with elite athletic status and with response to resistance training, while the nonfunctional allele (577X) has been proposed as a candidate metabolically thrifty allele. Genotyping of buccal cell DNA was successful in 293 children (155 Slovak boys, 138 Roma boys). R577X genotype distributions (RR=44.41%, RX=46.33%, XX=9.26%) were similar to those previously reported in a Caucasian population and were consistent with Hardy–Weinberg equilibrium (chi^2 [df=1]=0.364; P=0.612); allele frequencies in the whole sample were f(R)=0.59 and f(X)=0.41.

Genotypic associations between the R577X polymorphism and measures of physical and performance-related phenotypes were assessed by ANOVA. Slovak and Roma boys were analyzed separately. Our analyses are presented in Table I. We expected improving performance with increasing allele R and our expectation was confirmed. Slovak and Roma boys with both RR and RX genotypes had faster 50 m and 400 m run times than boys with the XX genotype, but the difference between RR and RX means was not significant. We observed a slower time in each discipline of Roma boys. RR homozygotes were over-represented (OR=1.5; 95%CI 1.3–2.4; P=0.137) and XX homozygotes under-represented (OR=0.5; 95%CI 0.1–0.5; P=0.118). Calculated values of Body Mass Index were lower in Roma boys in compare with Slovak children (Tables II and III). We observed no association with measures of body composition (BMI), which would have indicated a possible 'thrifty' effect of the polymorphism on metabolism.

TABLE I
ANOVA Analysis of Association between R577X Genotype and BMI, 50 M SPRINT, 400 M Run

	Slovak boys n=138	Roma boys n=155
Body composition BMI (kgm^{-2})	RR=17.5 (16.7–18.9)	RR=16.3 (15.2–16.9)
	RX=17.1 (16.8–17.5)	RX=15.8 (15.4–16.7)
	XX=16.6 (16.2–17.9)	XX=16.1 (15.1–17.2)
	V=0.2%, P=0.782	V=0.4%, P=0.355
50 m sprint (s)	RR=10.9 (9.8–11.6)	RR=11.3 (10.6–11.8)
	RX=10.2 (9.9–11.8)	RX=10.7 (10.3–11.3)
	XX= 9.8 (9.5–10.3)	XX=10.4 (9.9–11.0)
	V=0.4%, P=0.026	V=0.3%, P=0.641
400 m run (s)	RR=115.3 (112.6–116.0)	RR=117.9 (116.5–119.1)
	RX=114.7 (113.5–117.2)	RX=116.8 (116.0–118.2)
	XX=112.9 (111.3–116.6)	XX=117.3 117.1–118.8)
	V=0.5%, P=0.421	V=0.3%, P=0.618

RR, RX, XX–genotypes; P-statistical significance, V-percentage variance and probability

TABLE II
Body Mass Index in Slovak Boys (BMI)

Years	BMI min	BMI mean	BMI max
7	8.95	15.69	28.51
8	8.81	15.86	28.32
9	9.21	16.45	31.63
10	11.78	17.12	29.52
11	12.15	17.52	30.66
12	12.22	17.96	27.28
13	12.38	19.37	28.76
14	12.38	20.14	35.24
15	13.26	20.42	33.45

Min–minimum value, max–maximum value, average mean value.

TABLE III
Body Mass Index in Roma Boys (BMI)

Years	BMI min	BMI mean	BMI max
7	8.87	15.18	28.11
8	8.54	15.56	27.58
9	8.89	16.25	28.13
10	10.66	16.34	28.44
11	11.20	16.27	29.34
12	11.65	17.36	29.52
13	12.52	18.58	28.97
14	12.48	19.66	31.21
15	13.05	20.14	31.26

Min–minimum value, max–maximum value, mean average value.

IV. Discussion

In the presented paper examined motoric performance in Roma and Slovak boys in school age. Given the problems of

Roma children and youth, the phenomenon of sports has been sufficiently employed for the overall socialization of Roma population and for their integration into society. We present the results of the application of molecular genetics methods for diagnosing of motoric predispositions for the sake of identifying talented sportsmen. While classical methods of motoric performance testing within the diagnostics of motoric predispositions may seem to be in retreat due to the ever-growing emphasis on the application of modern molecular-genetic methods it may be expected that the two approaches will be complementary in the years to come. At present, they are aimed at integrated measurements of the effects of various genes and environmental effects on one´s phenotype. Genetic tests differ in principle from the traditional motoric tests, because the DNA of an individual does not change during his/her life. Genetic information advises us of an individual´s genetic predisposition in his/her early childhood.

Genetics plays an important role in determining the capacity of an individual to go in sports at the top professional level. The question in which genetic elements influence motion abilities and what is their respective significance. Furthermore, it is necessary to know the related genes as well as the mechanisms and metabolic paths and their influence. These questions may be expected to be answered after genomics, and bio information science has been introduced into the analysis of genetic effect upon physical predisposition of individuals. A genetic test can inform trainers and sportsmen whether one´s genotype is of endurance or of speed nature. This sort of information may be combined with results of the "classical" motoric tests in developing individual training programs and in discovering talented children.

The functions of actinin-3 are likely to include a structural role in the maintenance of muscle mechanical integrity and other functions related to muscle signaling and metabolism, and *ACTN3* R577X polymorphism leads to its deficiency [8]. Furthermore, R577X has been studied in the general population, and it has been reported as one of many genetic factors that may influence muscle function [9]. We have shown that the ACTN3 R577X polymorphism is associated with a complex phenotypic characteristic in an unselected population, namely sprinting and running ability. This finding is consistent with previous studies that have reported an association between the ACTN3 577R allele and elite sprint athlete status. The prevalence of allele X associated with endurance performance was demonstrated in all studied populations in the world [4]. XX genotype is common in humans (14-20%) and causes reducing of strength, muscle mass, and diameter, but increases the metabolic efficiency of skeletal muscle and the proportion of slow-twitch muscle fibers [10]-[11]. The lowest frequency of X allele has been described in Kenya, Nigeria and South Africa (8-11%). Caucasian population from Australia, Spanish people of European origin and Japanese have the highest frequencies of X allele (44-54%). The frequency of X allele higher than 50% is typical for Japanese. The frequency of genotype XX varies from 25% in the Asian population for <1% of the population of the African tribe Bantu. In European population it is approximately 18% [12] but 9% in Finland [13]. The frequency XX genotype observed in Roma boys is lower than in Northern India (17% vs. 9.3%) [14]. Reference [15] found higher frequencies of R allele and RR genotype in sprinters compared with athletes and control population. Our results are in contrast with recent studies reporting no association of the R577X polymorphism with endurance performance [16], [17]. The most comprehensive study of the association of ACTN3 gene with a sports performance in Caucasoid population by [1] followed a set of professional athletes and compared them with a common population. Significant variations in the frequency of ACTN3 genotypes among different groups of athletes were observed. RR genotype was present in higher frequency in the group of speed athletes, while the presence of genotype XX was higher in the group of endurance athletes [1]. The 577R allele and 577RR genotype have both previously been shown to be associated with stronger performance in sprinting or power based events. On the other hand, it has been suggested that the 577X allele and 577XX genotype, which results in a deficiency of α-actinin 3, would be associated with better performance in an endurance event. If, as suggested by [18], the R577X polymorphism is acted upon at the extremes of human performance it would be expected that any association of the 577X allele or the 577XX genotype with endurance performance should become evident in athletes competing in extended endurance events such as ultramarathons and Ironman triathlons. The prevalence of the mutated X-allele is lower among power/sprint oriented athletes compared with controls, indicating that the lack of α-actinin-3 is detrimental in these sports, but a mechanistic link has not been established. Results of reference [19] suggest that *ACTN3* genotype-associated differences in muscle mass and glycogen utilization provide a mechanistic explanation for the modulation of human performance by the *ACTN3* genotype. Another study associated *ACTN3 XX* with low testosterone levels of soccer players immediately after eccentric training [20]. Several studies have confirmed that testosterone increases muscle mass, strength, and endurance it may be favorable for power and strength sports [21]. Since the *ACTN3* R allele is associated with higher testosterone levels, increased muscle mass [22], [23]. In the Human Gene Map for Performance and Health-related Fitness Phenotypes [24], it is clear that athletic ability is a polygenic multifactorial phenotype, which involves numerous physiological systems and is influenced by many gene-gene and gene-environment interactions.

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