The gene polymophisms pattern of Hungarian elite athletes

Abstract of the PhD Thesis

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INTRODUCTION

Without considering the genetic background, the image built by sport sciences is not complete. It is necessary to analyse and expound the interaction of the stabile genetic definiteness and the dynamic environmental influence, and to examine the key factors in the conformation of genetical program during evolution. Sportgenomics is a young scientifical discipline concentrating on the genome's organization and functions of elite athletes. This is one of the most promising tool on the field of sport selection, individual traning plans, sporttraumathology and genedoping. The performance related genes alone might not be enough to express concrete attributes, but can be predictive on the quality of sport performance. By the development of the complex traits, a few or numerous genes' paralell or cumulative functions influence the trait's exhibition, where every gene itself explains a small part of the whole progress. It is well known, that poligenetic definiteness is usually more sensitive to environmental factors. Physical activity is a complex human behaviour, which makes the process even more complicated.

The learning of the movements, the physical environment, the information of the outworld can highly influence the expression of any genes in connenction with physical activity. Knowing the aforementioned facts, specifying the gene polymorphisms associated with physical performance is very limited.

Mostly elite athletes have been studied on the field of sportgenomics. The highest proportion of researches were executed in connenction of endurance performance, however, the examination of power and sprint performance is also frequent. Many studies focused on individual gene polymorphisms, which provided a good take-off to further, more complex researches, like configuration a ploygenetic profile for definite abilities (endurance, sprint etc.).

The insertion and deletion polymorphisms of the angiotensin-converting enzyme gene (ACE)

ACE polymorphisms effect the serum and tissue leveles of ACE enzyme. The plasma and tissue ACE levels are significantly higher in the presence of DD genotype, comparing that to the heterozygote (ID) and II genotypes. In case of the II genotype the ACE activity level is the half of those who are carrying the DD genotype, while in heterozygotes the activity is moderate. Lower serum ACE level and activity are characteristic for the I allele, which cause lower vasoconstruction, and lead to more

oxygenized blood flow to the muscles. According to aforementioned findings the I allele is the preferable variation, and it is more advantageous for the endurance athletes, because in their case the suppliance of oxygene demand is essential. The D allele of the ACE gene comes with higher angiozensin-converting ezyme activity. The activity of circulating ACE correlates significantly with the isometric and isokinetic muscle power of the quadriceps.

The R577X polymorphism of the alpha-actinin-3 gene (ACTN3)

The ACTN3 gene expression takes place only in muscle-fibres type II, which are responsible for the fast and powerful muscle contraction. The α-actinin-3 deficiency is typical for the normal population which is caused by the homozygote stop codon in the ACTN3 (R577X) gene. The α-actinin-3 protein is functionally not redundant, the isoform 2 cannot compensate the absence of ACTN3. The presence or absence of the α-actinin-3 influences the striated muscle's performance. The R allele is preferable in power and sprint performances, while the XX genotype causes the deficit of the protein and influences muscle efficiency.

The bradykinin receptor B2 +9/−9 polymophism (BDKRB2)

The polymophism is induced by the presence (+9) or absence (−9) of a nine-base-pair sequence. The activation of the bradykinin receptor B2 stimulates the skeleton muscle's glucose uptake during load and improves the muscle's circulation. The −9 variant increases the gene transcription and the receptor mRNA expression is higher. This polymorphism is mostly studied in connection with physical activity.

The combination of ACE and ACTN3

The ACE DD genotype is preferable in short-term performances, the D allele is associated to power and sprint performance. The ACTN3 R allele and RR genotype are in connection with the power-dominant performances of elite athletes, R allele is associated to higher efficiency of the muscle contraction. The combination of ACE D and ACTN3 R alleles can be advantegous in sprint and power sports, while the ACE I and ACTN3 X alleles in endurance sports.

The combination of ACE and BDKRB2

The connection of the two polymorphisms can be described by the ACE's role in the renine-angiotensin and the kinin–kallikrein systems. The angiotensin-converting enyme catalyses the alteration of angiotensin I to the vasoconstuctive angiotensin II, and splits

the vasodilatator bradykinin to inactive metabolits. The presence of ACE I allele decreases the circulating angiotensin activity, which results in limited vasonconstruction, and also decreases the dissimilation of the vasoprotective bradykinin. The −9 polymophism of the bradykinin receptor B2 increases the gene transcription hence as the effect of the activation the BDKRB2 raises futher the glucose uptake and circulation in muscles. Based on the aforementioned findings the ACE I and BDKRB2 −9 alleles together make preferable conditions for endurance performances.

The combination of ACTN3 and BDKRB2

Considering the physiological effects of the two gene variants from the aspect of endurance sports, ACTN3 X and the BDKRB2 -9, while from the view of the muscle power the ACTN3 R and the BDKRB2 +9 combinations are preferable.

The connection of the ACE gene and the relative aerob capacity (relVO_2max)

The examination of the connections of the ACE I/D polymorphism and the different exercise-physiological parameters are evident after knowing the physiological effects expressed by the gene's allele. The connenction of the I allel and the higher maximal and relative aerob capacity was confirmed in many studies.

The connection of the BDKRB2 gene and the relative aerob capacity (relVO_2max)

There have been no studies executed about this possible connection yet, however, according to the physiological effects of the genevariants it is practical to examine the trend of the relative aerob capacity related to the BDKRB2 genotypes.

The HindIII (rs1800247) polymorphism of the osteocalcin gene

The alleles are definiated by the presence (h) or the absence (H) of the *Hind*III restriction fragment. The osteocalcin is activated by the binding in of a carboxil group and helps to built in the calcium into the bones, nevertheless this process can only be accomplished with the presence of vitamin K. The *Hind*III polymorphism of the osteocalcin influences the bone mineral content and the bone built. If the H allele is present than there is a negative influence on the bone.

OBJECTIVES OF THE STUDY

The aim of the study was the mapping of the sportgenetical background of Hungarian elite athletes.

- Introduction, comparison and differences of allele and genotype frequencies in athletes and control groups.
- Analysis of the differences of allele and genotype frequencies among endurance, non-endurance and ball games groups.
- Comparison the allele and genotype frequencies among the different sportgroups: combat sports, endurance sports, kayak-canoe/rowing, ball games, waterpolo and the control group.
- Examination the allele and genotype frequencies between sports and control groups separated by genders.
- Analysis of the allele and genotype frequencies among the different sportgroups by genders.
- Comparison of the results to predominantly caucasian samples and to other studies. Displaying the similarities and differences to other results, portray the national characteristics.
- Comparison of the genotype combinations between the athletes and the control group.
- Analysis of genotype combinations in different sportgroups.
- Examination the genotype combinations between the athletes and the control group separated by genders.

- Analysis the relationship between the relative aerob capacity and the ACE and the BDKRB2 gene polymorphisms and their combinations.
- Study the relation of osteocalcin gene variations and bone ultrasound parameters, humanbiological characteristics in Hungarian elite athletes.

According to our aims we have determined the following hypothesises before examination:

- Similarly to international results the ratio of ACE, ACTN3 and BDKRB2 gene polymorphisms will differ between sport and control groups.

- ❖ Frequency correlation can be found between the ACE+ACTN3, ACE+BDKRB2 and ACTN3+BDKRB2 gene polymorphism combinations and proper sport groups based on the gene polymorphism combinations' physiological benefits.
- ❖ Compared to the other two gene variants the ACE I and BDKRB2 –9 gene polymorphisms result higher relative aerobic capacity.
- ❖ There is a connection between the *Hind*III polymorphism of osteocalcin gene and bone parameters.

MATERIALS AND METHODS

Subjects

The blood samples were collected between 2008 and 2011. In this period there were 449 sampling (176 women, 273 men). In some cases genotyping was unsuccessful, which causes the different sample sizes by genes (Table 1.).

Table 1: Subjects involved in the study

Gene	Total	Men	Woman	Athlete	Control
ACE	430	261	169	335	95
ACTN3	434	265	169	338	96
BDKRB2	161	67	94	161	0
ACE+ACTN3	391	236	155	298	93
ACE+BDKRB2	161	67	94	161	0
ACTN3+BDKRB2	161	67	94	161	0
ACE+relVO$_2$max	182	110	72	182	0
BDKRB2+relVO$_2$max	105	38	67	105	0
Osteocalcin	302	203	99	214	88

All subjects were Hungarian, their average age was 32±12,7 years. 63 of them took part on bone ultrasound measurement, 17 atheletes and 47 control people. The main aspect of the selection of the control group (n=97, men=58, women=39) was the hypoactive lifestyle, with no sport experience at all. All participants matched the age of the athletes, and all of them were healthy. The main aspects of selection the athletes were the sportsmen's results (Olympic, World, European and national champions or

placed in the first 8 places). Rowing, kayak-canoe, cycling, short track, triathlon, long distance running formed the group of endurance sports. Combat sports and gymnastics belonged to the non-endurance group. The ball games group have been created with players from handball, basketball, waterpolo and football. After the sport specific grouping we separated the dry-landed ball games from the waterpolo, moreover, the group of combat sports has also been created.

Methods

DNA isolation

3 ml venal blood have been collected from the athletes and control people. The isolation of the genomic DNA was made by Fujifilm Quickgene Mini80 system using the adequate protocol. After the cleaning we defined the DNA concentration with NanoDrop 2000 spectrophotometer.

Genotyping

ACTN3, osteocalcin

The genotyping was processed with the conspiration of Avidin Kft. During the nanocapillar QRT-PCR one reaction proceeds in 33 nl volumen. For the polymorphism analysis we used with fluorescent paint signed (VIC, FAM) TaqMan probes. We gave OpenArray SNP MasterMixet to the genomic DNA, and filled the reactionfields of the nanocapillar chip with OpenArray™ Autoloader.

The OpenArray™ chips were placed and fixed into an oil filled glass holder, then those were put into the Perkin Elmer 1000 Plate PCR, where a 10 minutes 92°C activation was followed by the process held 50 cycles (97°C-1 min, 51°C-20 sec, 53°C-20 sec, 55°C-10 sec). Afterwards the chips were placed into the BioTrove OpenArray™ NT Cycler system, which detected automatically the fluorescent intensity.

ACE

The determination of the ACE I/D variants was made also by the OpenArray system, after an advanced validation. The GenoID Laboratory made the genotyping with the conventional PCR methode, form which 96 random selected samples were analyzed with the OpenArray system for the ACE rs4343 ploymorphism, because this plymorphism is the best proxy for the Alu I/D variant (Daya and Gaunta 2008, Glenn et al. 2009). The results matched, so henceforth we used the OpenArray method.

BDKRB2

The genotyping of the bradykinin receptor B2 was made through the GenoID Laboratory using the protocol of Williams et al. The gene polymorphisms were defined by using PCR technology.

Anthropometry

The necessary body dimensions was measured according to the suggestions of the International Biological Program. A total of 24 parameters were recorded, from which the body height, the body weight, the elbow and knee girths and the bone percentage were displayed.

Quantitative bone ultrasound measurement

Calcaneal quantitative ultrasound parameters were registered with a Sonost 3000 bone densitometer. The analysis included 3 dimensions:

- SOS − Speed Of Sound (m/s), informs about the bone quality,
- BUA − Broadband Ultrasound Attenuation (dB/MHz), shows information about the microstructure of the bone;
- BQI index − Bone Quality Index, the calculated bone quantity index (BQI=αSOS+βBUA, αβ: temperature corrections).

Exercise physiology test

Some of the athletes took part in a treadmill test as well, which was executed with a Schiller ergometry system. The vita maxima test lasted till full exhaustion. We used the maximal oxigen uptake results, and calculated with the relative aerob capacity (relVO_2max, ml/ttkg/min).

Statistical analysis

GraphPad Prism and Microsoft Excel was used for statistical analysis and graphical presentation. In the statistical analysis Pearson's chi-squared test was used to check whether the variants were in Hardy–Weinberg equilibrium, like at the comparism the genotype, allele and polymorphism combination frequency differences among the groups. Differences between the two groups separated by the presence or absence of the H allele were investigated using an independent samples t-test. We used the One-way Anova test to compare the ultrasound results and bone percentage values of the different groups by genotype, furthermore we used One-way Anova test at the analisys of the

relVO_2max results as well. We calculated the correction of multiple testing with Benjamini-Hochberg and Bonferroni tests. Differences of $p<0.05$ were considered statistically significant.

RESULTS

ACE

The heterozygote genotype's frequency was 50,47% in the total sample, while the frequency of DD (25,58%) was higher than that of the II (23,95%). The ACE II genotype's presence was much more lower in the control group (15,79%), than that in the athletes (26,27%). There was no difference in the frequencies of either the genotypes or the alleles between the athletes and the control group. The proportion of the II in the endurance athletes was lower, while the frequency of the DD was higher than those in the non-endurance group, but there was no difference between the two groups.

ACTN3

The genotype and allelefrequencies did not differ between the athletes and the control group. There was no significant difference in the aforementioned frequencies between the endurance and the non-endurance groups and between the non-endurance vs. control groups either. The RR frequency was higher in the endurance athletes and in the ball games group (37,00% and 38,68%) than those in the non-endurance athletes (28,30%), but there was no significant difference.

BDKRB2

There was no difference comparing the different groups' allele and genotype frequencies. The BDKRB2 –9 alleles' proportion was higher in the endurance and non-endurance groups as well, the –9/–9 genotype frequency was in both groups higher than that of the +9/+9 genotype. In the non-endurance group the –9/–9 genotype's proportion was almost with 10% higher than that in the endurance group (36,36% and 27,87%).

ACE, ACTN3 and BDKRB2 genotype and allelefrequencies in the sport specific groups

There was significant difference between the waterpolo and control groups' ACE I/D allelefrequencies. Between the men kayak-canoe/rowing and combat sports groups there

was significant difference in the ACTN3 allelefrequencies. The difference was significant in women between athletes and the control group in the ACE genotype proportions.

With the exclusion of the kayak-canoe/rowing group from the analysis in women, in case of the remaining 5 group the ACE genotype frequencies differed significantly from each other.

Between the waterpolo and control group there was significant difference in the ACE allelefrequencies.

The ACE I/D and the ACTN3 R/X combinations

There was a significant difference between the athletes and the control group in the proportion of the ACTN3 RR and ACE II combination compared to the other possible combinations, which is preferable from the aspect of the physical percformance.

The difference was significant comparing the 6 sport specific groups' ACTN3 RR+ACE II/ID/DD combinations, and between the combat sports/kayak-canoe/rowing/ endurance/ waterpolo vs. control group. There was also a significant difference in the ACE DD and the ACTN3 RR/RX/XX combinations' proportion between the endurance athletes and the control group.

For the combat sport the preferable combination was the ACTN3 RR + ACE DD/ID genotype pair. Comparing the proportions to the endurance athletes there was no difference, but the difference was significant between combat sports and the control group.

Combinations of the ACE I/D and the BDKRB2 +9/–9 polymorphisms

In the endurance group the most frequent genotype pair was the double heterozygote combination, while in the combat sports this pair is the least frequent one. There was no difference in the proportions of the combinations between women and men.

Combinations of the ACTN3 R/X and the BDKRB2 +9/–9 polymorphisms

There was no difference in the genotype combinations comparing the athletes by gender.

The ACE I/D and the BDKRB2 +9/–9 polymorphisms and the relative aerob capacity

There was significant difference in the total sample between men and women in the relVO_2max results. Grouping the sample by ACE and BDKRB2 genotypes and

comparing the relative aerob capacity of the groups, no difference have been found among them.

We have compared the two groups, that is the most (ACE II és BDKRB2 –9/–9) and the least (ACE DD és BDKRB2 +9/+9) preferred from the aspect of aeob/endurance performance, but no significant difference have been found. The combinations of the two genotypes have been modified to the common presence of a particular genotype and allele. We have examined the rel. aerob capacity in groups with the presence of ACE II and BDKRB2 –9 allele vs. the ACE DD and BDKRB +9 allele, however, no significant difference have been found, which result is also true in case of BDKRB2 –9/–9 and ACE I allele vs. BDKRB2 +9/+9 and ACE D allele combinations. RelVO_2max results differed among the groups, but there were no difference between groups in case of neighter the genotypes and allele frequencies of ACE, nor that of BDKRB2.

Considering the results of relative aerob capacity athletes have been divided into upper and lower groups based on their average ± 1SD in order to examine and compare their gene polymorphism. No significant difference have been found in genotypes and allele frequency neighter in case of ACE, nor in case of BDKRB2.

Analysis of osteocalcin HindIII polymorphism, bone parameteres and human biology

47 control group member (out of 88) participated in bone ultrasound measurement, which has also been executed on 16 subjects from athletes group. No significant result could be seen between the two groups separated by the presence or the absence H allele in the humanbiological and the bone ultrasound characteristics.

All bone parameters have been examined by genotypes, and no significance based on osteocalcin genotypes could be seen in case of BUA, SOS and BQI.

Athletes had significantly higher bone ultrasound values compared to that of control group. There was no difference between the allele frequencies of *Hind*III polymorphism, the presence of h allele was more frequent than that of H allele in both the athletes and control groups.

CONCLUSIONS

The aim of this study was to analyze the genotype characteristics of the highest possible ratio of Hungarian elite athletes, starting from the polymrphisms of unique

genes to the combinations, moreover to reveal their connections with exercise physiology and human biology data.

Presence of ACE II genotype and ACE I allele was higher among athletes than in control group, however, there have been no difference found. This confirms the low-keyed tendency of connection between sport performance and polymorphisms, which has been discribed by Montgomery et al first. The ratio of ACE genotype and allelefrequency were similar in both endurance and non-endurance groups, which does not enable author to declare the connection between I allele and endurance performance among Hungarian athletes.

The low-keyed presence of ACTN3 XX genotype and X allele confirms the findings in the scientific literature, which states 18% rate of appearance among European samples. The limited RR frequency experienced in non-endurance group, and the frequent presence of genotype in endurance group confirms the researches that could not find any connection between R allele and power/speed performance.

BDKRB2 –9/–9 is the preferred genotype in case of endurance performance, however it appeared among non-endurance athletes with a higher ratio than among the endurance group, which confirms the results of measurements among Polish and Russian athletes, where no connection have been found between the preferred genotype and endurance performance.

After gender splitting we have found ACE genotype difference between women athletes and control subject which proves the relevace of splitting samples to gender categories.

The difference of ACTN3 allelefrequency between groups of kayak-canoe/rowing and combat sports is highlighted. According to findings of researches the advantages of R allele is beneficial for combat sport athletes, however, it has appeared less than 50% of the Hungarian sample, whereas its 60% ratio in kayak-canoe/rowing group is prominent. The wast majority of kayak-canoe/rowing group have already reached higher ranked results than that of the combat sport members (who are also the best athletes in Hungary), which might be also effected by the lower frequency of R allele, resulting having „only” reached the best 10 in European Championships, and not the top of podium. Besides high demand of endurance power-endurance, maximum force and also explosive power plays a crucial role in kayak-canoe/rowing sports.

BDKRB2 –9/–9 is the most frequent genotype among men endurance athletes. Our study about it confirmed the findings of Saunders et al. with caucasian men triathlonists, Hungarian samples match that of caucasian athletes' results.

With the prominent ACE II genotype frequency among endurance athletes we have confirmed the connection between endurance performance and ACE II genotype among Hungarian women athletes. We have to emphasize the extreme high ACE I allelefrequency among Hungarian water polo players, which might enable them to reach a high level endurance performance.

According to our results we have found the following conclusion about the connection between individual gene polymorphisms and sport groups: partially true.

The differences between groups experienced at combination analysis confirms the relevance of common polymorphism examination. The high ratio of ACE I+ACTN3 X allele combination in combat sports is the opposite of previous findings. Internationally published connection between D and R allele does not apply on Hungarian combat sport athletes. The lower ratio of RR+D combination in combat sports group than in control group also confirms the previous statement. In case of water polo players we have found the mixed, ACE I+ACTN3 R combination the most frequent, which proves that this combination is the best for sports, where wide range of skills is neccessary. Common appearance can be completed with BDKRB2 –9, which also had a frequent presence.

In case of endurance athletes the result of ACE and BDKRB2 combination was found unfavorable regarding the physiological effect of combination, the beneficial combination of two genotype (II and –9/–9) has not appeared in the Hungarian sample, oppositely to the findings of Williams et al. (2004).

According to our results we have found the following conclusion about the connection between gene polymorphism combinations and sport groups: partially true.

The athletes' relative aerob capacity had no connection to the different genotypes, alleles and polymorphism combinations. In this field there are two ways to research: examine other exercise physiology parameters in connection with gene polymorphisms, or extend the number of participants take part in vita maxima test with the aim to broaden the view of possible relations.

According to our results we have found the following conclusion about the connection between ACE I and BDKRB2 –9 gene polymorphism combination and relative aerobic capacity: false.

We have confirmed the fact that better bone quality is connected with physically active lifestyle, which has an indisputable role againast bone atrophy. We could not find any connection between osteocalcin *Hind*III polymorphism and bone characteristics among Hungarian athletes. According to our results osteocalcin gene polymorphism has no effect on Hungarian sample of bone characteristics.

According to our results we have found the following conclusion about the connection between the *Hind*III polymorphism of osteocalcin gene and bone parameters: partially true.

The study proved that examinations shall be executed on elite athletes, because the clearest results will be provided by this subpopulation. Gender grouping is neccessary, since their differences lead to different results. In case proper amount of subjects are available, categories are supposed to be divided by aerobic-anaerobic, or power/speed-endurance character. Analysis shall be executed in case of the extreme sports within the category.

ACE gene polymorphism shall not be considered as the gene of human physical ability, however it might be a modifying marker, which might mean a difference on elite level, depending on the sport.

According to this study one of our aims is to know the national characteristics, since Hungarian sample did not show similarities to that of other populations.

It is very important to emphasize that world class athletes need the best polymorphism combination, since it is a polymorphism characteristic. One gene polymorphism cannot be responsible for sport successes, however, it may modify the answer to physical load, the adaptation ability, the limitations of exercise physiology, which means that it may influence physical performance in many different ways.

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