The relationship between CA repeat polymorphism of the IGF-1 gene and the structure of motor skills in young athletes

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INTRODUCTION

Human physical fitness, which is an indicator of self-development and health, is still the main focus of many studies concerning physical activity (Gronek & Holdys, 2013). Investigations aimed at determining the characteristics of body conditioning that allow the achievement of high levels of sport performance are of key importance in the general training process (Tucker & Collins, 2012). In the recruitment and selection process, the level of so-called prognostic features was once a criterion for inclusion in sports teams and rejection of those less adapted to the later stages of the training process (Vaeyens et al., 2008). Nowadays, these studies have gained more importance in planning, especially in the determination of training regimes based on the regularities of biological development (Ben-Zaken et al., 2013; Eliakim et al., 1996).

The map of candidate genes that can potentially affect physical fitness becomes larger every year, and they are associated with such aspects as respiratory and cardiovascular stability; body build and composition – especially muscle mass and strength; carbohydrate and lipid metabolism; response to training; and exercise intolerance. The aim of this study was to analyze the relationship between the CA repeat polymorphism of the P1 promoter of the IGF1 gene and the structure of motor skills in the two groups of Polish young athletes in 2011–2013. In this study, 350 young sportsmen representing different sports disciplines were examined (age = 15.5 ± 0.5 years), by genotyping the IGF1 gene and determining the structure of motor skills using the International Physical Fitness Test (IPFT) battery. The multiple stepwise regression was used to determine the impact of the investigated motor skills on the indicator of the overall physical fitness, measured by the total score of the International Physical Fitness Test (IPFT). The analysis showed some regularity related to the character of the IGF1 gene polymorphism. It can be concluded that the two groups of young boys athletes practicing various sports disciplines (kinds of physical exercise) displayed similar associations between CA repeat polymorphism of the P1 promoter of the IGF1 gene and the level of motor effects. Our results suggest that this polymorphism may be a genetic marker of the physical performance phenotype. We demonstrated that CA repeat polymorphism of the P1 promoter of the IGF1 gene was associated with strength predispositions in the homozygous and non-carriers groups. In the group who were heterozygous it was speed-strength aptitudes.

Key words: genetic variations, motor skills, young athletes

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Abbreviations: IGF-1, insulin-like growth factor-1; IPFT, international physical fitness test; BMI, body mass index; ACTN3, alpha-actinin 3 gene; PPAR delta, peroxisome proliferator-activated receptor delta; ACE, angiotensin convertase enzyme

The reaction of the human body to training loads is determined by diverse gene expression (Thompson & Binder-Macleod, 2006; Rupert, 2003). Much of the scientific interest in this area is focused on insulin-like growth factor (IGF-I), which has a wide spectrum of functions in the human body (Eliakim & Nemet, 2010). The IGF-I protein plays a key role in regulating many cellular actions, including proliferation, differentiation, and apoptosis. IGF-I actions also involve metabolic functions, which have an impact on adaptation to physical activity (Fílhus & Zdrojewicz, 2014). The association of IGF-I with physical exertion was investigated in terms of its impact on skeletal muscle. During physical exercise, IGF-I exhibits strong anabolic effects via stimulation of amino acid transport to skeletal muscle cells and activation of skeletal muscle protein synthesis (Kim et al., 1991).

Many researchers demonstrated that strength training elevates the level of IGF1 gene expression in skeletal muscles, which is connected with an increased concentration of the IGF-I protein and muscle hypertrophy (Bamman et al., 2001; Hamed et al., 2003; Philippou et al., 2007; Singh et al., 1999).

Expression of the IGF1 gene depends on both environmental and genetic factors, such as the type of tissue, level of hormones, kinds of amino acids in-
gested with food, developmental age, and the time and intensity of the physical exercise (Jernstrom et al., 2001). It is worth mentioning that the level of circulating IGF-I protein shows large variations among individuals. It was estimated that up to 60% of interindividual differences in concentration of IGF-I are hereditary (Harrela et al., 1996). These variations reflect the expression pattern of the IGF1 gene, which can be modulated by polymorphic sequences in the promoter of this gene. The IGF1 CA repeat polymorphism is located ~ 1 kb upstream from the transcription start site of the IGF1 gene. This region contains regulatory sequences that have an impact on P1 promoter activity. In the Caucasian population, the most common is the (CA)19 repeat allele, which is 192 bps and is called the wild-type allele (Weber & May, 1989). It was suggested that this polymorphism is related to the differences in IGF1 expression and IGF-I protein concentration in the blood. Considerable differences in skeletal muscle IGF-I concentration observed between individuals may be associated with CA repeat polymorphism of the P1 promoter of the IGF1 gene (Hand et al., 2007).

As previously noted, insulin-like growth factor-I (IGF-I) plays a key role in exercise-associated muscle growth and development (Ben-Zaken et al., 2013). The regulatory region of the promoter of the IGF-I gene is labile, but changes in this region were studied mostly in the elderly and in relation to pathological states, but rarely studied in athletes (Bamman et al., 2001; Arends et al., 2002; Fletcher et al., 2005; Hernandez et al., 2007; Rotwein et al., 1993; Widdowson et al., 2009). Studies also indicated that single nucleotide polymorphism of the P1 promoter region of the IGF1 gene is more frequent in athletes than in non-athletes (Ben-Zaken et al., 2013; Eliakim et al., 2010). Therefore, it seems important and reasonable to consider carrying out tests to determine the presence of the gene polymorphisms and their correlation with the effects of motor skills among athletes in relation to different sports (types of physical exertion).

In light of the foregoing, the main aim of this study was the analysis of the influence of CA repeat polymorphism of the P1 promoter of the IGF1 gene on the structure of motor skills in two groups of young Polish athletes representing different sports disciplines (endurance sports, team games).

MATERIAL AND METHODS

Subjects. Data were collected over a three-year period (2007–2009) on a group of talented young athletes from the province of Greater Poland, all of who were competing in the Youth Sports System program as members of their local Provincial Junior Team. The analysis included a total of 350 boys (mean ± S.D.: age=15.5 ± 0.5 years; height=177.8 ± 7.98 cm; weight=66.8 ± 10.29 kg). Participants represented the following disciplines: athletics (n=38), canoeing (n=61), cycling (n=57), handball (n=35), and volleyball (n=39).

All examined athletes were divided into two groups according to the Farfel’s concept of motor coordination level (second and third level) (Farfel, 1960). Farfel (1960) divided the categories into three levels of varying complexity. This concept was illustrated and expanded by Starosta (1987; 1993).

The first level – has a spatial accuracy of movement activities carried out under standard conditions without a time limit.

The second level - determines the spatial precision of movement in a limited or strictly defined time, and in a standard conditions.

The third level – characterized by motor tasks performed accurately, quickly and with adjusting to changing external conditions.

The validity of this concept was confirmed by additional studies (Rutkowska-Kucharska & Bober, 1986).

The first group consisted of athletes representing endurance sports of the second level of coordination, which involves the accuracy of movements performed in the shortest possible time or for a pre-defined time in constant and relatively invariable conditions (canoeing, cycling, athletics, rowing). The second group was players practicing team games (the third level of coordination), which requires performing the shortest possible movements while maintaining spatial accuracy under constantly changing conditions resulting from opponents’ activities (basketball, field hockey, handball, and volleyball).

The measurements were made every year in the second half of March in the facilities of the university. These specific dates were due to the temporal structure of training for young athletes. They were preparing for the finals of the National Youth Olympic Games.

The research presented in this paper was conducted in accordance with the ethical standards of the Declaration of Helsinki (Ethical Principles for Research Involving Human Subjects). Participants and their parents or caregivers were informed of the testing procedures and gave their written informed consent. The study was approved by the local Research Ethics Committee (Karol Marcinkowski Medical University in Poznań, Poland, resolution numbers 712/05 and 519/07).

Procedures. Genomic DNA was isolated from epithelial cells taken from the mouth using a sterile cotton swab and a standard isolation kit (A&A Biotechnologie). IGF1 CA repeat polymorphisms were determined by PCR amplification. PCR was performed using primers designed to amplify the polymorphic (CA)n repeat 1 kb upstream of the human IGF1 gene (dbSNP: RS10665874). The reaction was carried out in a final volume of 15 μl containing 2-3 μl of total DNA sample isolated from the buccal swabs, 3.75 pmol of the forward primer (5’-AAGAAAAACACACTCTGGCGAC-3’) labeled with FAM, 3.75 pmol of the reverse primer (5’-ACACCCTCTGGAGAAAGGTA-3’), 0.1 M mM of deoxy-NTP, 1.5 mM MgCl2, 1x PCR buffer and 0.6 U of HiFi DNA polymerase (Novazym).

Two-step PCR was performed in a 2720 thermal cycler (Applied Biosystems) with the following parameters: 94°C for 4 min; 28 cycles of 5 s at 94°C, 30 s at 60°C and completed with a final extension for 30 min at 65°C. The size of the PCR products was analyzed by means of an automated sequencing apparatus (ABI 3130xl; POP-7 gel, filter set G5, array length 36 cm; GeneMapper software version 3.7). The size of the PCR products was determined by comparing with the internal GS600LIZ size standard (Applied Biosystems). The estimation of CA repeat numbers in each analysed specimen was based on extrapolation of the previously developed specific allelic ladder. The ladder marker consisted of 14 sequenced amplicons representing alleles with 7, 9, 11, and 13–23 CA repeats.

We used the X2 test to determine if IGF1 genotypes were in Hardy-Weinberg equilibrium.
The measurements focused on the level and structure of motor effects in the athletes studied. For this purpose, we used the International Physical Fitness Test (IPFT) battery, which included eight simple tests that were complementary enough to allow a comprehensive evaluation of physical fitness. With its 40–plus-year history, the IPFT was widely used by numerous coaches and researchers who applied use of this non-complex method to evaluate the effective aspect of human motor activity (Szopa et al., 2000). The IPFT battery is also recommended by the Ministry of Sport and Tourism in Poland as a tool for evaluation of physical fitness of young talented athletes.

Measurements of motor skills and flexibility were carried out according to the recommendations of the IPFT battery (Pilicz, 2004; Rosandich, 1999). Speed was evaluated by means of a 50-meter sprint test, also termed a short run (it evaluated locomotor speed). Endurance was evaluated over a distance of 1000 meters (running endurance); agility, a 4 × 10-meter shuttle run with carrying blocks. Strength was tested with a standing long jump test (explosive power of lower limbs) and 30-second sit-ups (strength endurance of trunk muscles). Handgrip strength was tested by means of a handgrip dynamometer, whereas upper limb strength and shoulder girdle strength was evaluated with the bent-arm hang or a pull-up test on a bar. Trunk flexibility is an anatomical trait of a person, with a standing trunk flexion test used to reveal the scope of the vertebral column and hip joint movement.

The results obtained in each attempt of the International Physical Fitness Test were converted on a scale of 0 to 100 points (calculated according to the T scale), depending on the surveyed calendar age, which in the sport of children and young people is the main criterion of qualifications to training groups at different stages of training.

We also measured basic somatic parameters such as body height and weight and calculated BMI (Body Mass Index).

Statistical analyses. We used basic statistical methods for analysis of the data. The following software programs were employed: Statistica 10 PL and Microsoft Office 2010. The statistics calculated included arithmetic mean, standard deviation, and minimum and maximum. The results were normalized with respect to the mean and standard deviations.

The multiple regression method was used to determine whether motor skills correlated with CA repeat polymorphism of the IGF1 gene (Stanisz, 2006).

RESULTS

IGF1 CA genotypes were classified according to the presence of the most frequent allele, 192bp, which contains 19 CA repeats. Athletes were classified as homozygous or heterozygous for the 192-bp allele or non-carriers of the 192-bp allele.

Genotype and alleles distributions were in Hardy-Weinberg equilibrium. The numbers of identified repeats were between 9 and 21.

The genotype distribution for microsatellite repeats (CA)n in athletes from II and III coordination levels is presented in Table 1.

Table 2 presents the statistical summary of the results (average, minimum, maximum). Characteristics concern the division of athletes due to the level of motor coordination.

Figures 1–2 and Tables 3–4 present the results of stepwise multiple regression analysis. The analysis was performed by the level of coordination.

Table 1. Allele distribution of the IGF1 (CA)n genotype in athletes.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Coordination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II n = 206</td>
</tr>
<tr>
<td>192/192</td>
<td>192 (44.18%)</td>
</tr>
<tr>
<td>192/–</td>
<td>25 (12.14%)</td>
</tr>
<tr>
<td>–/–</td>
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</tr>
</tbody>
</table>

Legend: 192/192 genotype homozygous for the IGF1 192-bp allele; 192/– genotypes heterozygous for the IGF1 192-bp allele; –/– genotypes of non-carriers of the IGF1 192-bp allele

Table 2. Statistical characterization of the results (based on the level of coordination).

<table>
<thead>
<tr>
<th>Athletes – level II of motor coordination</th>
<th>Athletes – level III of motor coordination</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 206</td>
<td>n = 144</td>
</tr>
<tr>
<td>Min.</td>
<td>Min.</td>
</tr>
<tr>
<td>Max.</td>
<td>Max.</td>
</tr>
<tr>
<td>Body height</td>
<td>cm 178</td>
</tr>
<tr>
<td>Body mass</td>
<td>kg 68</td>
</tr>
<tr>
<td>Bmi</td>
<td>kg/m² 21</td>
</tr>
<tr>
<td>Speed</td>
<td>pts. 55</td>
</tr>
<tr>
<td>Leg strength</td>
<td>pts. 55</td>
</tr>
<tr>
<td>Endurance</td>
<td>pts. 59</td>
</tr>
<tr>
<td>Shoulders strength</td>
<td>pts. 55</td>
</tr>
<tr>
<td>Agility</td>
<td>pts. 56</td>
</tr>
<tr>
<td>Strength endurance</td>
<td>pts. 57</td>
</tr>
<tr>
<td>Flexibility</td>
<td>pts. 55</td>
</tr>
<tr>
<td>Sum of ipft</td>
<td>pts. 455</td>
</tr>
</tbody>
</table>

Legend: 192/192 genotype homozygous for the IGF1 192-bp allele; 192/– genotypes heterozygous for the IGF1 192-bp allele; –/– genotypes of non-carriers of the IGF1 192-bp allele
In the group of endurance athletes (the second level of coordination), determinants of the overall physical fitness, measured by scores of the IPFT, were similar to the group of all studied athletes. Physical fitness of the athletes homozygous for the 192-bp allele in 81% of individuals, was conditioned by the leg power and shoulder strength. Similar results as in the group of homozygous athletes were found for sportsmen who were non-carriers of the 192-bp allele. In heterozygous athletes, the physical fitness in 77% of individuals, was conditioned by speed predispositions and shoulder strength (Fig. 1, Table 3).

A similar result was shown in athletes of team games (the third level of coordination). In homozygous athletes, the greatest extent (85%) of the level of motor effects was determined by shoulder and hand strength as well as speed predispositions. In athletes who were heterozygous for the 192-bp allele, the most important determinants of the overall physical fitness were speed predispositions. In the group of non-carriers of the 192-bp allele, the dependent variable was determined by shoulder and hand strength as well as by agility predispositions (Fig. 2, Table 4).

**DISCUSSION**

The way in which an organism adapts to physical exertion depends, among others, on the regulation of many genes’ expression e.g. *IGF1, ACTN3, PPAR delta, ACE* (Karlowatz et al., 2011; Jung et al., 2016; Maciejewska-Karłowska, 2013; Orsyska et al., 2015; Holdys et al., 2011). Their polymorphism turned out to be an important factor that influences the level of physical performance (Scheinowitz et al., 2003). Among those genes, *IGF1* plays a remarkable role because of the broad spectrum and specificity of its action. It is known that the regulatory region of the P1 promoter of this gene is labile (Mukherjee et al., 2016).

This manifests itself by a significant degree of single nucleotides variation and by the presence of the different number of microsatellite repeats (CA) (Wong et al., 2005). These changes were analyzed in relation to various pathological states (Fletcher et al., 2005; Hernandez et al., 2007; Zhai et al., 2004), but rarely were studied in athletes (Ben-Zaken et al., 2013; Ben-Zaken et al., 2014).

*IGF-I* affects morpho-functional parameters of the human body, which are essential for gaining high sports performance (Bamman et al., 2001; Philippou et al., 2007; Hand et al., 2007; Koska et al., 2000; Neri Serneri et al., 2001). The *IGF-I* concentration is determined by *IGF1* gene expression, which may be modulated by polymorphic sequences in the P1 promoter region (Rosen et al., 1998). It is known that the sequence of the P1 promoter of the *IGF1* gene is highly variable (Vaessen et al., 2001). However, better adaptation to physical exercise was associated rather with C-1245T (rs35767 C/T) (Ben-Zaken et al., 2013) and rs1464430 A/C polymorphism (Ben-Zaken et al., 2015) of this gene than in P1 promoter. The applied approach is unique because in searching for connections between polymorphism of the *IGF1* gene and physical exertion, scientists focused mainly on the impact of physical exercise on the *IGF1* concentration in the blood (Monnier et al., 2000).
This provides indirect evidence that CA polymorphism of the IGF1 gene, which is more frequent in athletes than non-athletes (Ben-Zaken et al., 2013; Krych-Garsztka et al., 2011), may have a very significant impact on achievements in many sports, by defining its occurrence and relationship with level of body adaptation to specific physical efforts. This is a particularly important issue in the case of young athletes, who, on the one hand, fight an intense biological development; and on the other hand, they are often exposed to excessive training loads (Gronek et al., 2015).

A significant number of studies were published in the last decade indicating that genetic variants have an influence on human physical performance and/or elite athlete status (Bray et al., 2009). The available research results constitute a large database which must be systematized before implementing genetic profiling in sport (Ruth et al., 2014). Therefore, it seems justified to conduct this type of research among young athletes representing various sports disciplines.

Analysis of the multiple stepwise regression used to determine the impact of the investigated motor skills on the indicator of the overall physical fitness, measured by the total score of the International Physical Fitness Test (IPFT), showed some regularity related to the character of the IGF1 gene polymorphism.

| Table 3. The results of multiple regression for endurance sports – II level of coordination (n = 206). |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Genotype | Independent Variables | Summary of stepwise regression; Dependent variable: total pts. IPFT | | | |
| | | Step | Multiple Spearman | Multiple R-squared | R-squared change of | |
| HOM | Leg strength | 1 | 0.682 | 0.465 | 0.465 | |
| | Shoulder strength | 2 | 0.897 | 0.804 | 0.338 | |
| | Endurance | 3 | 0.923 | 0.851 | 0.048 | |
| | Flexibility | 4 | 0.941 | 0.885 | 0.024 | |
| | Speed | 5 | 0.959 | 0.919 | 0.034 | |
| | Hand strength | 6 | 0.977 | 0.954 | 0.035 | |
| | Agility | 7 | 0.988 | 0.977 | 0.023 | |
| | Strength endurance | 8 | 1.000 | 1.000 | 0.023 | |
| HET | Speed | 1 | 0.660 | 0.436 | 0.436 | |
| | Shoulder strength | 2 | 0.879 | 0.773 | 0.337 | |
| | Flexibility | 3 | 0.921 | 0.848 | 0.075 | |
| | Strength endurance | 4 | 0.945 | 0.893 | 0.045 | |
| | Hand strength | 5 | 0.968 | 0.936 | 0.043 | |
| | Leg strength | 6 | 0.980 | 0.960 | 0.024 | |
| | Endurance | 7 | 0.990 | 0.979 | 0.019 | |
| | Agility | 8 | 1.000 | 1.000 | 0.021 | |
| NON | Leg strength | 1 | 0.782 | 0.611 | 0.611 | |
| | Shoulder strength | 2 | 0.879 | 0.772 | 0.161 | |
| | Speed | 3 | 0.931 | 0.867 | 0.095 | |
| | Strength endurance | 4 | 0.954 | 0.911 | 0.044 | |
| | Agility | 5 | 0.970 | 0.940 | 0.029 | |
| | Endurance | 6 | 0.980 | 0.960 | 0.020 | |
| | Flexibility | 7 | 0.991 | 0.982 | 0.022 | |
| | Hand strength | 8 | 1.000 | 1.000 | 0.018 | |

It can be concluded that in the case of two groups of young male athletes (15-16 years) practicing various sports disciplines (kinds of physical exercise) there is a similar association between CA repeat polymorphism of the P1 promoter of the IGF1 gene and the level of motor effects.

It was found that in the group of endurance athletes (the second level of coordination) who were homozygous for the 192-bp allele, the level of general physical fitness was determined by strength predispositions, above all, leg and shoulder strength.

In athletes of the team games (the third level of coordination) who were homozygous, the most important features that determined the overall physical fitness were strength predispositions, hand and shoulder strength and speed predispositions. In athletes who were heterozygous for the 192-bp allele, the most important determinants of the overall physical fitness were speed predispositions and shoulder strength. This situation concerns endurance athletes and the athletes of team games.

In the groups of athletes that were non-carriers of the 192-bp allele, it was shown that the level of the overall physical fitness was determined as in the case of homozygous athletes, by the leg and shoulder strength.

Increasingly high performance among athletes today causes the need for coaches to meet increasingly com-
plex and more comprehensive requirements. Facing these challenges calls for the identification of solutions aimed at reasonable and effective preparation of the athletes. Striving for champion-level achievement led to shifting the emphasis in coaching to the period of childhood and youth (Karpowicz et al., 2015).

The targeted stage of sports training is a specific period in human development, which coincides with high sensitivity of the human body to external stimuli. Therefore, modern auxology and training methodology put a great emphasis on the necessity of systematic control of the physical development of children and young people. The problem of the assessment of this development and its components (harmony and level) becomes especially important in the context of scientific investigations of the determinants of physical activity on different stages of training and motor development (Karpowicz & Karpowicz, 2013). The morphological characteristic is a commonly used measure of development evaluation. Considering sport teams, the assessment of biological maturity based on the characteristics, which are often a criterion for admission to various levels of locomotor activity, is not relevant to the actual biological picture of the human body (Karpowicz et al., 2015).

Our results revealed the possibility of using analysis of the IGF1 gene sequence in sports. Application of molecular biology techniques may allow the identification of predispositions resulting from individual characteristics of the gene code.

Nevertheless, there still is a need for further studies to elucidate the mechanism of the functional significance of CA polymorphism in the promoter region of the IGF1 gene in regulating its expression and determining the level of the motor effects.

**REFERENCES**


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**Table 4. The results of multiple regression for team games – III level of coordination (n = 144).**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Independent Variables</th>
<th>Summary of stepwise regression; Dependent variable: total pts. IPFT</th>
<th>Step</th>
<th>Multiple R-squared</th>
<th>Multiple R-squared change of</th>
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<tbody>
<tr>
<td></td>
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<td>change of</td>
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<tr>
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<tr>
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<td>Speed</td>
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<tr>
<td></td>
<td>Agility</td>
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<td>Leg strength</td>
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<tr>
<td></td>
<td>Endurance</td>
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<td>0.954</td>
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<tr>
<td></td>
<td>Flexibility</td>
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<td>7</td>
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<td>0.972</td>
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<tr>
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<td>Strength endurance</td>
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<td>8</td>
<td>1.000</td>
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<tr>
<td>HET</td>
<td>Speed</td>
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<tr>
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<tr>
<td>NON</td>
<td>Shoulder strength</td>
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<td>1</td>
<td>0.723</td>
<td>0.523</td>
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<tr>
<td></td>
<td>Hand strength</td>
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<td>0.836</td>
<td>0.723</td>
</tr>
<tr>
<td></td>
<td>Agility</td>
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<td>3</td>
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<td>0.899</td>
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<tr>
<td></td>
<td>Leg strength</td>
<td></td>
<td>4</td>
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</tr>
<tr>
<td></td>
<td>Flexibility</td>
<td></td>
<td>5</td>
<td>0.980</td>
<td>0.961</td>
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<td>Strength endurance</td>
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<td>0.993</td>
<td>0.985</td>
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<td>Speed</td>
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<tr>
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</tr>
</tbody>
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35 -

36 -

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