The *MTHFR* 1298A>C (rs1801131) polymorphism is associated with speed and strength sports: a study with two ethnic groups

Aleksandra Zarebska[1], Ildus I. Ahmetov[2,3], Stanislaw Sawczyn[1], Alexandra S. Weiner[4], Mariusz Kaczmarczyk[5,6], Krzysztof Ficek[6], Agnieszka Maciejewska-Karlowska[6], Marek Sawczuk[6], Agata Leonska-Duniec[1,6], Tomasz Klocek[7], Elena N. Voronina[4], Uljana A. Boyarskikh[4], Maksim L. Filipenko[4], Pawel Cieszczyk[1,6]

[1]Academy of Physical Education and Sport, Department of Sport Education, Gdansk, Poland

[2]Kazan State Medical University, Laboratory of Molecular Genetics, Kazan, Russia

[3]Volga Region State Academy of Physical Culture Sport Technology Education Research Laboratory, Sport and Tourism, Kazan, Russia

[4]Russian Academy of Sciences Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Novosibirsk, Russia

[5]Pomeranian Medical University, Department of Clinical and Molecular Biochemistry, Szczecin, Poland

[6]University of Szczecin, Department of Physical Culture and Health Promotion, Szczecin, Poland

[7]Academy of Sport Education, Department of Physical Education, Krakow

Correspondence:

Mariusz Kaczmarczyk, MD, PhD

Pomeranian Medical University, Department of Clinical and Molecular Biochemistry, Szczecin, Poland

ul. Powstancow Wlkp. 72

70-110 Szczecin, POLAND

phone: + 48 91 466 1494

fax: + 48 91 466 1492

e-mail: mariush@pum.edu.pl

Abstract

It has been suggested that DNA hypomethylation due to poorer efficiency of the 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme may induce muscular growth. We hypothesized that the common, functional 1298A>C polymorphism in the *MTHFR* gene may be associated with athletic status. To test this hypothesis, we investigated the distribution of the 1298A>C variant in Polish (n=302) and Russian (n=842) athletes divided into four groups: endurance, strength-endurance, sprint-strength and strength-endurance according to the energy requirements of their disciplines, as well as in 1540 control subjects. We found significantly different genotype and allele distributions amongsprint-strength athletes and strength athletes compared with the groups of sedentary controls in each cohort (Polish, Russian and combined cohort). The results of the initial and repetition studies as well as the combined analysis suggest that the functional 1298A>C polymorphism in the *MTHFR* gene is associated with athletic status. The presence of the C allele seems to be beneficial in sprint-strength and strength athletes. It needs to be established whether and to what extent this effect is mediated by alteration in DNA methylation status.

Keywords: DNA methylation, athletic ability, MTHFR, single nucleotide polymorphism

INTRODUCTION

A 5,10-methylenetetrahydrofolate reductase (MTHFR), one of the key regulatory enzymes in one-carbon metabolism, catalyses the biologically irreversible reduction of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyl-tetrahydrofolate (5-methyl-THF) (Bailey and Gregory, 1999). One-carbon metabolism reactions include purine and pyrimidine (thymidine) synthesis and methylation of homocysteine (Hcy) to form methionine, much of which is converted to S-adenosylmethionine (SAM), a universal donor of methyl groups (Selhub, 2002).

DNA methylation is one of the most investigated epigenetic mechanisms (García-Giménez et al., 2012). The term epigenetics refers to modifications in gene expression caused by hereditable, but possibly reversible, alterations in chromatin structure or DNA methylation without introducing changes in the DNA sequence (McCaughan et al., 2012). . Since the late 1970's (McGhee and Ginder, 1979, Taylor and Jones, 1979), there has been growing evidence that the methylation of DNA is involved in the regulation of gene expression and it has been found to be associated with a repressed chromatin state and inhibition of gene expression (Klose and Bird, 2006).

Two common non-synonymous coding region polymorphisms (677C>T (Frosst et al., 1995) and 1298A>C (van der Put et al., 1998)) in the *MTHFR* gene have been identified. The adenine to cytosine transversion at position 1298 (1298A>C, rs1801131) changes a glutamate into alanine residue within the C-terminal regulatory domain of MTHFR protein (van der Put et al., 1998). Both variants were shown to confer reduced enzyme activity (van der Put et al., 1998, Castro et al., 2003, Weisberg et al., 1998), leading to a decreased pool of 5-methyl-THF, the methyl donor for the remethylation of Hcy to methionine (Castro et al., 2004, Bailey and Gregory, 1999). Unlike the 677C>T polymorphism, which has well been established as an important genetic determinant of elevated Hcy (Frosst et al., 1995, Castro et al., 2003), the effect of the 1298A>C polymorphism on plasma concentrations of homocysteine remains controversial. Some studies (Castro et al., 2003, Castro et al., 2004), but not all (van der Put et al., 1998, Friedman et al., 1999, Weisberg et al., 1998), reported a significant effect of 1298A>C on plasma Hcy levels with CC homozygotes exhibiting higher Hcy plasma concentration. Both *MTHFR* polymorphisms were, however, shown to affect

DNA methylation status. Compared with 677C>T, the effect was smaller for 1298A>C, the homozygotes for the C allele displaying lower DNA methylation status (Castro et al., 2004). This may be explained by reduced MTHFR activity that causes an accumulation of homocysteine, disrupting DNA methylation pattern by favouring the synthesis of S-adenosylhomocysteine (SAH), an inhibitor of DNMT (Castro et al., 2004), and secondly, a decreased availability of methyl-THF, which affects the synthesis of S-adenosylmethionine (SAM), the molecule primarily responsible for methylation of DNA (Weinstein et al., 2006, Friso et al., 2005).

Adaptive response of skeletal muscle to exercise is a complex process involving a variety of primary and secondary messengers and specific signalling pathways, which result in alterations in exercise-induced gene expression and protein synthesis or degradation (Coffey and Hawley, 2007). DNA hypomethylation has recently been demonstrated to be an important factor in the exercise-induced adaptation in skeletal muscles (Barres and Zierath, 2011, Terruzzi et al., 2011). Terruzzi et al. (Terruzzi et al., 2011) have shown that the expression of myogenic regulatory factors and the myosin heavy chain (*MHC) gene* as well as the myotube length and diameter increase were largely induced by hypomethylation of DNA (Terruzzi et al., 2011). Additionally, genetic polymorphisms of enzymes involved in one-carbon metabolism were investigated in a group of elite athletes and it was found that three polymorphisms, *MTHFR* 1298A>C*,* methionine synthase (*MTR*) 2756A>G and methionine synthase reductase (*MTRR*) 66A>G, were significantly associated with elite athlete status (Terruzzi et al., 2011). The authors speculated that reduced enzyme activity due to genetic variants in the corresponding genes depletes cellular 5-methyl-THF pool, thereby inducing DNA hypomethylation and muscle-specific gene expression (*MHC*), leading to myogenic and hypertrophic effects exerted by physical exercise (Terruzzi et al., 2011).

It has been hypothesized that in athletes, reduced activity of important enzymes in one-carbon metabolism – 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), methionine synthase reductase (MSR), due to *MTHFR* 1298A>C*,* methionine synthase (*MTR*) 2756A>G and methionine synthase reductase (*MTRR*) 66A>G gene polymorphisms, results in decreased availability of methyl group donors (5-methyl-THF, SAM), DNA hypomethylation and consequently, an increase of muscle specific gene

expression and muscle hypertrophy in response to physical exercise (Terruzzi et al., 2011). Muscle hypertrophy/strength is of great importance in explosive and impulse sports requiring a high rate of force development (Eynon et al., 2011).

As DNA methylation has been suggested to be one of the regulatory mechanisms controlling genes involved in muscle hypertrophy (Terruzzi et al., 2011) and, inter alia, mitochondrial biogenesis (Barrès et al., 2012), we aimed to investigate the distribution of the *MTHFR* 1298A>C polymorphism in two independent Polish and Russian case-control studies, where athletes were categorized into four groups according to the energy requirements of their disciplines, from more endurance-oriented sports to more strength-oriented ones.

METHODS

The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants, and the study was approved by the ethics committees of the Pomeranian Medical University Ethics Committee in Szczecin and The Kazan State Medical University Ethics Committee.

Participants

The initial study was done on a group of 302 Polish athletes (221 males and 81 females, age 27.8±7.1) of the highest nationally competitive level. The athletes were prospectively assigned to one of four groups according to their relative aerobic/anaerobic energy system contribution, time of competitive exercise performance, and intensity of exertion in each sport. The group of Polish athletes has been previously described in detail (Maciejewska et al., 2012). The first group (endurance athletes) consisted of individuals (n=26) with predominantly aerobic energy production. The second group, designated as strength-endurance athletes (n=66), comprised athletes whose sports utilize mixed anaerobic/aerobic energy production. The third group, sprint-strength athletes (n=110), also included athletes with mixed energy production, but compared to strength-endurance athletes, their competitive exercise time was shorter. The fourth group (n=110), the strength athletes, included individuals with predominantly anaerobic energy production. Control participants were recruited from 684 unrelated volunteers (students of the University of Szczecin, aged 19-23 years). All athletes and controls were Caucasians to minimize the

issues arising from population stratification. The replication study was conducted on 842 Russian athletes (459 males and 383 females, age 25.6±0.2) of a nationally competitive standard. All athletes were classified into one of four groups according to the criteria established for the initial study (see (Maciejewska et al., 2012) for details). The group of endurance athletes (n=123) included biathletes (n=39), triathletes (n=14), 15-50 km cross-country skiers (n=49), 5-25 km swimmers (n=21). The group of strength-endurance athletes (n=83) included rowers (n=68), 3-10 km runners (n=5), 800-1500 m swimmers (n=10). The third group, sprint-strength athletes (n=486) consisted of kayakers (n=23), canoers (n=11), 800-1500 m runners (n=2), 200-400 m swimmers (n=17), 1.5-3 km speed skaters (n=14), middle distance track cyclists (n=10), boxers (n=24), bobsleighers (n=15), wrestlers (n=117), alpine skiers (=19), divers (n=17), artistic gymnasts (n=59), 1.5-3 km short track skaters (n=9), synchronized swimmers (n=27), fencers (n=50) and figure skaters (n=72). The group of strength athletes consisted of 100-400 m runners (n=9), 500-1000 m speed skaters (n=11), 500-1000m short track skaters (n=13), 50-100 m swimmers (n=46), weightlifters (n=34), throwers (n=5), sprint track cyclists (n=16), jumpers (n=8) and heptathlon/decathlon athletes (n=8).

The Russian controls were 856 healthy unrelated citizens of Moscow and Novosibirsk (478 males and 378 females, age 29.1±8.4).

Genotyping

Polish study

Genomic DNA was extracted from the oral epithelial cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to manufacturer's protocol. Allelic discrimination of the *MTHFR* 1298A>C (rs1801131) polymorphic site was performed using a TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, USA), including primers and fluorescently labelled (FAM and VIC) MGB probes for the detection of alleles. All samples were genotyped on a Rotor-Gene real-time polymerase chain reaction (PCR) instrument (Corbett, Australia). Thermal cycler conditions were as follows: an initial step at 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 15 s and anneal/extend at 60°C for 1 min.

Russian study

Genomic DNA was isolated from leukocytes in venous blood by proteinase K (Serva) digestion followed by phenol/chloroform extraction and ethanol precipitation. Genotyping of the *MTHFR* 1298A>C was carried out by real-time PCR allelic discrimination with TaqMan probes at iQ5 PCR Thermal Cycler (Bio-Rad). PCR was performed in 25 µl reaction volumes containing 20–100 ng of genomic DNA, 65 mMTris-HCl (pH 8.9), 23 mM ammonium sulphate, 3 mM MgCl2, 0.05% Tween 20, 0.2 mMdNTP, 0.3 µM of each primer (forward AGGAGCTGCTGAAGATGTG and reverse ATCACTCACTTTGTGACCATTC), 0.1 µM of each probe (FAM-CCAGTGAAGAAAGTGTCTTTG-BHQ and R6G-CCAGTGAAGCAAGTGTCTTTG-BHQ) and 1.0 U of Taq polymerase. PCR thermal cycling conditions were as follows: denaturation for 2 min at 95°C followed by 49 cycles each for 8 s at 95°C and 40 s at 58°C. dNTP, TaqMan probes, oligonucleotide primers and Taq polymerase were synthesized by the Institute of Chemical Biology and Fundamental Medicine (Siberian Branch, Russian Academy of Sciences).

Statistical analysis

The STATISTICA software package, version 9.0, was used to perform statistical analyses. Allele frequencies were calculated from genotype counts. A χ^2 test and Fisher exact test were used to compare the *MTHFR* 1298A>C genotype and allele frequencies between athletes and controls. The odds ratios for the 1298C allele with 95% confidence intervals (95%CI) were estimated using a logistic regression procedure. Statistical significance was set at $p<0.05$.

RESULTS

The *MTHFR* 1298A>C genotypes conformed to Hardy-Weinberg equilibrium in both athletes (including sub-groups) and controls in the two populations studied (Polish and Russian). The distribution of the *MTHFR* 1298A>C genotypes in Polish and Russian athletes compared to control subjects is depicted in Table 1 and Table 2, respectively.

Polish (initial) study

The 1298A>C genotype frequency differed significantly between athletes and sedentary control subjects (Table 1). Specifically, significant differences in genotype distribution were observed in the group of sprint-strength athletes (χ^2=6.04, p=0.049), the group of strength athletes (χ^2=6.75, p=0.034), as well as in all athletes combined (χ^2=8.03,

p=0.018) compared to controls. The frequency of the 1298C allele was significantly greater in all athletes combined (37.7%, χ^2=4.24, OR 1.23 [1.00-1.51], p=0.039) and in sprint-strength athletes (40.0%, χ^2=4.18, OR 1.36 [1.00-1.83], p=0.041) as compared to controls (33.0%). The difference in the 1298C allele frequency between strength athletes (39.5%) and control subjects was insignificant (χ^2=3.33, OR 1.33 [0.97-1.82], p=0.068).

****Table 1 near here****

Russian (replication) study

In the replication study (table 2) similar differences in genotype distributions were observed. Genotypes frequencies were significantly different in sprint-strength athletes (χ^2=7.33, p=0.026) and strength athletes (χ^2=9.51, p=0.009), as well as in all athletes combined (χ^2=10.98, p=0.004) as compared with control sedentary subjects. As in the initial study, the frequency of the 1298C allele was also greater in all athletes (35.0%, χ^2=5.67, OR 1.19 [1.03-1.38], p=0.017) and in the strength athletes (38.0%, χ^2=5.52, OR 1.36 [1.04-1.76], p=0.019) compared to controls (31.1%).

****Table 2 near here****

Combined study

Taking the results of the initial and replication study together, significant differences in genotype frequencies were observed in the sprint-strength (χ^2=11.17, p=0.004) and strength athlete groups (χ^2=16.05, p=0.0003), as well as among all athletes (χ^2=17.48, p=0.0002) compared to controls. The 1298C allele was over-represented in the whole cohort of Polish and Russians athletes when compared to controls (35.7% *versus* 31.9%, χ^2=8.32, OR 1.18 [1.05-1.33], p=0.004), as well as in sprint-strength and strength athletes, in which the allelic frequencies for the 1298C variant were 35.6% (χ^2=5.10, OR 1.18 [1.02-1.36], p=0.024) and 38.6% (χ^2=8.63, OR 1.34 [1.10-1.64], p=0.003), respectively.

****Figure 1 near here****

DISCUSSION

In the current study we examined the distribution of the 1298A>C polymorphism in two independently ascertained cohorts. When athletes were stratified into endurance, strength-endurance, sprint-strength and strength-oriented groups, according to their relative aerobic/anaerobic energy system contribution, time and intensity of exertion, we found a significantly different genotype distribution among sprint-strength athletes and strength athletes compared with sedentary control participants in each cohort (Polish, Russian). Combining the datasets of the two cohorts, similar results were obtained. Although the over-representation of the C allele in Polish strength and Russian sprint-strength athletes was insignificant ($p=0.068$ for both comparisons), in combined analysis, the C allele frequency was significantly more frequent in both sprint-strength and strength athletes than in sedentary control subjects. No differences in genotype and allele frequencies were observed between endurance athletes and controls.

Terruzzi et al. (Terruzzi et al., 2011) hypothesised that the athletes possessing specific variants of genes involved in one-carbon metabolism may be predisposed to decreased global methylation levels. It is therefore likely that the exercise-induced demethylation in athletes may be easier and more rapid, thereby resulting in increased gene expression specific to the mode of exercise (resistance or endurance). Our results partially support the hypothesis proposed by Terruzzi et al. (Terruzzi et al., 2011), as the over-representation of the C allele associated with decreased DNA methylation was found in sprint and sprint-strength athletes, but not in endurance or strength-endurance athletes. It must be noted, however, that as in the study by Terruzzi et al. (Terruzzi et al., 2011), the genotype distribution in the athletes, sprint-strength and strength in particular, exhibited a pattern of overdominance or heterozygous advantage. A heterozygote advantage refers to the fitness superiority of heterozygotes over homozygotes (McClelland et al., 2003). Although the difference between sprint-strength or strength athletes and control individuals in the frequency of AC genotype was rather small (7.3%-13% depending on the sub-group and cohort), it merits attention. The heterozygote advantage for the *MTHFR* gene has already been reported in neural tube defects (Weitkamp et al., 1998) and disorders of cognitive

functions (Tsai et al., 2011). The reason AC heterozygotes are fitter than CC homozygotes has not been ascertained. If the C allele has both detrimental and beneficial effects on speed or strength phenotypes, one could offer a reasonable explanation as to why the AC heterozygotes are more frequent among these athletes than CC homozygotes. A detrimental effect of the C allele may be mediated by homocysteine. The CC homozygotes tend to have greater homocysteine plasma concentrations (Castro et al., 2003, Castro et al., 2004) and high homocysteine levels are associated with decreased physical activity (Dankner et al., 2007) and quadriceps strength (Kuo et al., 2007). The protein homocysteinylation is a possible mechanism of homocysteine-related protein damage and may thus affect muscle strength (Kuo et al., 2007).

Improved endurance is associated with the increase in mitochondrial content and volume (mitochondrial biogenesis) (Irrcher et al., 2003). Transcription factors and transcriptional co-activators seem to represent critical regulators of mitochondrial biogenesis (Coffey and Hawley, 2007). The upregulation of *PGC-1α*, an important regulator of mitochondrial content was shown to be associated with dynamic change in DNA methylation in skeletal muscle (Barrès et al., 2012). Recently, Wang et al. (Wang et al., 2011) demonstrated that combined endurance and resistance training (concurrent training) amplifies the adaptive signalling response of mitochondrial biogenesis compared with single-mode endurance exercise. However, neither endurance (exposed to predominantly single-mode endurance training) nor strength-endurance athletes (exposed to a program of concurrent training) exhibited any significant differences in genotype and allele frequencies of 1298A>C polymorphism.

Our study has several limitations. DNA methylation does not exclusively control exercise-induced gene expression (Barrès et al., 2012), as the complex transduction network with numerous signalling pathways is regulated at different levels and cross-communication occurs (Coffey and Hawley, 2007). Moreover, some other factors may also modify the DNA methylation level. Many environmental factors, potential modifiers of methylation status, have been described (Handy et al., 2011, Barres and Zierath, 2011). Toxins such as heavy metals, oestrogenic and antiandrogenic toxins, and dietary modifications – folate restriction or folic acid supplementation in particular, might have a profound effect on DNA methylation

(Friso et al., 2005, Barres and Zierath, 2011, Zhang et al., 2011).

The enzyme betaine-homocysteine methyltransferase (BHMT) uses betaine as a methyl donor and provides an alternative pathway of homocysteine remethylation into methionine (Heil et al., 2000). This enzyme could theoretically compensate for a deficiency in MTHFR activity, but its expression is limited mainly to liver and kidney and skeletal muscle, which are devoid of BHMT mRNA (Sunden et al., 1997).

In addition, Friedman et al. found that CC homozygotes for the 1298A>C polymorphism had lower levels of homocysteine, which would suggest increased MTHFR enzyme activity (Friedman et al., 1999). However, despite this incongruity, 1298A>C has been reported to regulate DNA methylation. Indeed, Castro et al. (Castro et al., 2004) concluded that both *MTHFR* polymorphisms, 677C>T and 1298A>C, when in a homozygous state (TT and CC, respectively), have effects on DNA methylation, although the hypomethylation effect is slightly less pronounced for 1298A>C. Therefore, further studies may be warranted to examine whether the association between the 1298A>C polymorphism in the *MTHFR* gene and athlete status is related to DNA hypomethylation or some other mechanisms are involved. For instance, MTHFR activity is critical to both DNA methylation and DNA synthesis (Lin et al., 2004). Indeed, reduced activity of MTHFR due to the 1298A>C polymorphism could facilitate entry into S-phase of DNA replication and DNA synthesis, stimulated by physical exercise promoting hypertrophic responses (Terruzzi et al., 2011). There has, however, been considerable debate concerning whether satellite addition is obligatory or not for skeletal muscle hypertrophy (McCarthy and Esser, 2007). Interestingly, contractile activity has been shown to induce hypertrophy of skeletal muscle in the absence of increases in DNA content (Wong and Booth, 1990b, Wong and Booth, 1990a).

The strength of the study lies in the remarkable similarity of genotype distributions between different athletic sub-groups of the Polish and Russian cohorts. The replication (Russian) study was conducted in an independent data-set with a greater sample size than the initial (Polish) study: 1698 subjects (842 athletes and 856 controls) *versus* 986 subjects (302 athletes and 684 controls). In addition, both studies analysed the same phenotypes in comparable populations – all Polish and Russian participants were Caucasians. Although

sport disciplines differed slightly across Polish and Russian cohorts, they were assigned to one of the four athlete groups according to the same criteria in each sport (relative aerobic/anaerobic energy system contribution, time of competitive exercise performance, and intensity of exertion). Therefore, each group of athletes was relatively homogenous in terms of the type, time and intensity of exertion. It must be noted, however, that some authors suggest that only single sporting discipline studies should be considered and a study of a mixed cohort of athletes from various sporting disciplines may result in failing case-control association studies (Nazarov et al., 2001). Finally, statistical significance was obtained using the same genetic model reported in the initial study and joint analyses (combined Polish and Russian participants) led to a smaller p value (for genotype comparisons) than that seen in the initial report (Chanock et al., 2007). Therefore, by fulfilling most of the considerations suggested for a replication study, we believe that our study validates the credibility of the findings.

CONCLUSIONS

The results of the initial (Polish) and repetition (Russian) studies as well as the combined analysis suggest that the functional 1298A>C polymorphism in the *MTHFR* gene is associated with athletic status. The presence of the C allele seems to be beneficial in sprint-strength and strength sports such as sprinting, power-lifting, weight-lifting, throwing and jumping events and combat sports. However, whether and to what extent this effect is mediated by alteration in methylation status and tissue-specific expression of genes essential for athletes' skeletal muscle growth or other strength phenotypes is not clear. Therefore, studies of the entire genome methylation profile and studies which attempt to compare tissue-specific DNA methylation patterns with respect to athlete status, athletic performance or response to resistance, endurance or concurrent training, should be undertaken.

Acknowledgements

Conflict of interest

The authors have no conflicts of interest to declare

REFERENCES

Bailey, L.B., Gregory, 3rd, J. (1999). Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *J Nutr*, 129, 919–922.

Barrès, R., Yan, J., Egan, B., Treebak, J.T., Rasmussen, M., Fritz, T., Caidahl, K., Krook, A., O'Gorman, D.J., Zierath, J.R. (2012). Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab*, 15, 405–411.

Barres, R., Zierath, J.R. (2011). DNA methylation in metabolic disorders. *Am J Clin Nutr*, 93, 897S–8900.

Castro, R., Rivera, I., Ravasco, P., Camilo, M.E., Jakobs, C., Blom, H.J., de Almeida, I.T. (2004). 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C–>T and 1298A–>C mutations are associated with DNA hypomethylation. *J Med Genet*, 41, 454–458.

Castro, R., Rivera, I., Ravasco, P., Jakobs, C., Blom, H.J., Camilo, M.E., de Almeida, I.T. (2003). 5,10-Methylenetetrahydrofolate reductase 677C–>T and 1298A–>C mutations are genetic determinants of elevated homocysteine. *QJM*, 96, 297–303.

Chanock, S.J., Manolio, T., Boehnke, M., Boerwinkle, E., Hunter, D.J., Thomas, G., Hirschhorn, J.N., Abecasis, G., Altshuler, D., Bailey-Wilson, J.E., Brooks, L.D., Cardon, L.R., Daly, M., Donnelly, P., Fraumeni, Jr, J.F., Freimer, N.B., Gerhard, D.S., Gunter, C., Guttmacher, A.E., Guyer, M.S., Harris, E.L., Hoh, J., Hoover, R., Kong, C.A., Merikangas, K.R., Morton, C.C., Palmer, L.J., Phimister, E.G., Rice, J.P., Roberts, J., Rotimi, C., Tucker, M.A., Vogan, K.J., Wacholder, S., Wijsman, E.M., Winn, D.M., Collins, F.S. (2007). Replicating genotype-phenotype associations. *Nature*, 447, 655–660.

Coffey, V.G., Hawley, J.A. (2007). The molecular bases of training adaptation. *Sports Med*, 37, 737–763.

Dankner, R., Chetrit, A., Dror, G.K., Sela, B.A. (2007). Physical activity is inversely associated with total homocysteine levels, independent of C677T MTHFR genotype and plasma B vitamins. *Age (Dordr)*, 29, 219–227.

Eynon, N., Ruiz, J.R., Meckel, Y., Santiago, C., Fiuza-Luces, C., Gómez-Gallego, F., Oliveira, J., Lucia, A. (2011). Is the -174 C/G polymorphism of the IL6 gene associated with

elite power performance? A replication study with two different Caucasian cohorts. *Exp Physiol*, 96, 156–162.

Friedman, G., Goldschmidt, N., Friedlander, Y., Ben-Yehuda, A., Selhub, J., Babaey, S., Mendel, M., Kidron, M., Bar-On, H. (1999). A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. *J Nutr*, 129, 1656–1661.

Friso, S., Girelli, D., Trabetti, E., Olivieri, O., Guarini, P., Pignatti, P.F., Corrocher, R., Choi, S.W. (2005). The MTHFR 1298A>C polymorphism and genomic DNA methylation in human lymphocytes. *Cancer Epidemiol Biomarkers Prev*, 14, 938–943.

Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J., den Heijer, M., Kluijtmans, L.A., van den Heuvel, L.P. (1995). A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*, 10, 111–113.

García-Giménez, J.L., Sanchis-Gomar, F., Lippi, G., Mena, S., Ivars, D., Gomez-Cabrera, M.C., Vi?a, J., Pallardó, F.V. (2012). Epigenetic biomarkers: A new perspective in laboratory diagnostics. *Clin Chim Acta*, 413, 1576–1582.

Handy, D.E., Castro, R., Loscalzo, J. (2011). Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation*, 123, 2145–2156.

Heil, S.G., Lievers, K.J., Boers, G.H., Verhoef, P., den Heijer, M., Trijbels, F.J., Blom, H.J. (2000). Betaine-homocysteine methyltransferase (BHMT): genomic sequencing and relevance to hyperhomocysteinemia and vascular disease in humans. *Mol Genet Metab*, 71, 511–519.

Irrcher, I., Adhihetty, P.J., Joseph, A.M., Ljubicic, V., Hood, D.A. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. *Sports Med*, 33, 783–793.

Klose, R.J., Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci*, 31, 89–97.

Kuo, H.K., Liao, K.C., Leveille, S.G., Bean, J.F., Yen, C.J., Chen, J.H., Yu, Y.H., Tai, T.Y. (2007). Relationship of homocysteine levels to quadriceps strength, gait speed, and late-life disability in older adults. *J Gerontol A Biol Sci Med Sci*, 62, 434–439.

Lin, W.Y., Chou, Y.C., Wu, M.H., Huang, H.B., Jeng, Y.L., Wu, C.C., Yu, C.P., Yu, J.C., You,

S.L., Chu, T.Y., Chen, C.J., Sun, C.A. (2004). The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. *Anticancer Res*, 24, 3863–3868.

Maciejewska, A., Sawczuk, M., Cieszczyk, P., Mozhayskaya, I.A., Ahmetov, I.I. (2012). The PPARGC1A gene Gly482Ser in Polish and Russian athletes. *J Sports Sci*, 30, 101–113.

McCarthy, J.J., Esser, K.A. (2007). Counterpoint: Satellite cell addition is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol*, 103, 1100–2; discussion 1102–3.

McCaughan, J.A., McKnight, A.J., Courtney, A.E., Maxwell, A.P. (2012). Epigenetics: time to translate into transplantation. *Transplantation*, 94, 1–7.

McClelland, E.E., Penn, D.J., Potts, W.K. (2003). Major histocompatibility complex heterozygote superiority during coinfection. *Infect Immun*, 71, 2079–2086.

McGhee, J.D., Ginder, G.D. (1979). Specific DNA methylation sites in the vicinity of the chicken beta-globin genes. *Nature*, 280, 419–420.

Nazarov, I.B., Woods, D.R., Montgomery, H.E., Shneider, O.V., Kazakov, V.I., Tomilin, N.V., Rogozkin, V.A. (2001). The angiotensin converting enzyme I/D polymorphism in Russian athletes. *Eur J Hum Genet*, 9, 797–801.

Selhub, J. (2002). Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J Nutr Health Aging*, 6, 39–42.

Sunden, S.L., Renduchintala, M.S., Park, E.I., Miklasz, S.D., Garrow, T.A. (1997). Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. *Arch Biochem Biophys*, 345, 171–174.

Taylor, S.M., Jones, P.A. (1979). Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. *Cell*, 17, 771–779.

Terruzzi, I., Senesi, P., Montesano, A., La Torre, A., Alberti, G., Benedini, S., Caumo, A., Fermo, I., Luzi, L. (2011). Genetic polymorphisms of the enzymes involved in DNA methylation and synthesis in elite athletes. *Physiol Genomics*, 43, 965–973.

Tsai, S.J., Hong, C.J., Yeh, H.L., Liou, Y.J., Yang, A.C., Liu, M.E., Hwang, J.P. (2011). Heterozygote advantage of the MTHFR C677T polymorphism on specific cognitive performance in elderly Chinese males without dementia. *Dement Geriatr Cogn Disord*, 32, 159–163.

van der Put, N.M., Gabreëls, F., Stevens, E.M., Smeitink, J.A., Trijbels, F.J., Eskes, T.K., van den Heuvel, L.P., Blom, H.J. (1998). A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet*, 62, 1044–1051.

Wang, L., Mascher, H., Psilander, N., Blomstrand, E., Sahlin, K. (2011). Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle. *J Appl Physiol*, 111, 1335–1344.

Weinstein, S.J., Stolzenberg-Solomon, R., Pietinen, P., Taylor, P.R., Virtamo, J., Albanes, D. (2006). Dietary factors of one-carbon metabolism and prostate cancer risk. *Am J Clin Nutr*, 84, 929–935.

Weisberg, I., Tran, P., Christensen, B., Sibani, S., Rozen, R. (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*, 64, 169–172.

Weitkamp, L.R., Tackels, D.C., Hunter, A.G., Holmes, L.B., Schwartz, C.E. (1998). Heterozygote advantage of the MTHFR gene in patients with neural-tube defect and their relatives. *Lancet*, 351, 1554–1555.

Wong, T.S., Booth, F.W. (1990). Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. *J Appl Physiol*, 69, 1709–1717.

Wong, T.S., Booth, F.W. (1990). Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *J Appl Physiol*, 69, 1718–1724.

Zhang, F.F., Cardarelli, R., Carroll, J., Zhang, S., Fulda, K.G., Gonzalez, K., Vishwanatha, J.K., Morabia, A., Santella, R.M. (2011). Physical activity and global genomic DNA methylation in a cancer-free population. *Epigenetics*, 6, 293–299.

Table 1. *MTHFR* genotype distribution in Polish athletes (stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport) versus control subjects – initial study

	1298A>C genotypes			p*	p†
	AA n (%)	AC n (%)	CC n (%)		
Endurance athletes (n=26)	10 (38.5)	14 (53.8)	2 (7.7)	0.463	0.335
Strength-endurance athletes (n=66)	31 (47.0)	27 (40.9)	8 (12.1)	0.986	0.558
Sprint-strength athletes (n=110)	37 (33.6)	58 (52.7)	15 (13.6)	0.049	0.301
Strength athletes (n=100)	33 (33.0)	55 (55.0)	12 (12.0)	0.034	0.132
All Polish athletes (n=302)	111 (36.8)	154 (51.0)	37 (12.3)	0.018	0.140
Polish controls (n=684)	315 (46.1)	287 (42.0)	82 (12.0)	-	0.185

* χ^2 test *versus* sedentary control individuals; † Chi-square Hardy-Weinberg equilibrium test

Table 2. *MTHFR* genotype distribution in Russian athletes (stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport) versus control subjects - replication study

	1298A>C genotypes			p*	p†
	AA n (%)	AC n (%)	CC n (%)		
Endurance athletes (n=123)	51 (41.5)	56 (45.5)	16 (13.0)	0.333	0.920
Strength-endurance athletes (n=83)	39 (47.0)	37 (44.6)	7 (8.4)	0.710	0.663
Sprint-strength athletes (n=486)	201 (41.4)	234 (48.1)	51 (10.5)	0.026	0.156
Strength athletes (n=150)	53 (35.3)	80 (53.3)	17 (11.3)	0.009	0.106
All Russian athletes (n=842)	344 (40.9)	407 (48.3)	91 (10.8)	0.004	0.069
Russian controls (n=856)	415 (48.5)	349 (40.8)	92 (10.7)	-	0.150

* χ^2 test *versus* sedentary control individuals; † Chi-square Hardy-Weinberg equilibrium test

Figure 1. *MTHFR* genotype distribution in Polish and Russian athletes (stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport) versus control subjects - combined study