Lack of replication of associations between multiple genetic polymorphisms and endurance athlete status in Japanese population

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Introduction
Elite athletic status is a complex trait resulting from the interaction of numerous factors including training methods, socio-economic aspects, psychology, technology, injury history or diet, and genetic endowment is one of the many factors that affect athletic endurance performance. Many studies have attempted to identify genetic

Abstract
The aim of this study was to examine a polygenic profile related to endurance performance, based on current knowledge, in the Japanese population. We analyzed 21 genetic polymorphisms that have been reported to be associated with endurance performance and its related phenotypes in 175 endurance runners (60 international-, 94 national-, and 21 regional-level) and 649 controls in the Japanese population. Then, we calculated the total genotype score (TGS) (maximum value of 100 for the theoretically optimum polygenic score) for endurance performance. There was no association between the TGS and endurance athlete status (Control: 49.0 ± 7.6, Regional: 47.3 ± 7.6, National: 49.1 ± 5.7, and International: 48.2 ± 7.0, P = 0.626). These results suggested that TGSs based on the 21 previously published endurance performance-associated polymorphisms do not influence endurance running performance in the Japanese population. Nevertheless, some marginal tendencies have to be noted: the frequencies of the ACTN3 R577X rs1815739 RR+RX genotype and the GNB3 rs5443 CC+CT genotype were higher in international athletes than in controls (85% vs. 73.6%, P = 0.042 and 90% vs. 76%, P = 0.007, respectively), but not significantly different after Bonferroni correction.
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polymorphisms associated with elite endurance athlete status and physical performance traits such as maximum oxygen uptake, energetic metabolism, and muscle strength or mass (Ahmetov and Fedotovskaya 2015; Loos et al. 2015). The number of these potential polymorphisms is increasing each year, and it is now accepted that physical performance is highly polygenic (Miyamoto-Mikami et al. 2016). Therefore, several studies have attempted to identify polygenic profiles that could affect the possibility to become an elite endurance athlete (Eynon et al. 2011; Ruiz et al. 2009; Williams et al. 2008). Nevertheless, most of these studies were conducted in Caucasian subjects. Since genetic background differs among different ethnicities, the effects of the previously studied polymorphisms on physical performance in Asian populations remain unclear. In particular, little information regarding polygenic profiles and endurance performance is available for Asian populations. Thus, the purpose of this study was to examine the association between a polygenic profile based on current knowledge and endurance athlete status in the Japanese population.

**Methods**

**Subjects**

This study included 175 Japanese endurance track-and-field athletes (65 women): 152 long-distance runners (≥3000 m) and 23 middle-distance runners (800–1500 m). The athletes were assigned to three groups according to their competitive achievement, as follows: (1) 60 international athletes, who participated at major international competitions, such as the Olympic Games or World and Asian Championships, and included several medalists at these international competitions; (2) 94 national athletes, who participated in Japanese national competitions; and (3) 21 regional athletes, with at least 3 years of competitive experience. The control group consisted of 649 nonathletic healthy Japanese (465 women) from the Tokyo area. Written informed consent was obtained from all subjects, and the study was approved by the ethics committees of the Juntendo University, the Japan Institute of Sports Sciences, and the National Institute of Health and Nutrition.

**Candidate gene polymorphisms**

We searched for genetic polymorphisms that were associated with endurance performance-related phenotypes (endurance performance, maximal oxygen consumption, lactate threshold, and trainability of these parameters) using PubMed.

Exclusion criteria were: (1) polymorphism presenting unknown or less than 5% minor-allele frequency in Japanese populations; (2) presence of other polymorphism within two bases; (3) linkage disequilibrium with other target polymorphism; (4) unknown rs number; and (5) length polymorphisms. Finally, 22 polymorphisms were selected for the analysis (PPARGCB rs7732671 was excluded from analysis, not respecting Hardy–Weinberg equilibrium [HWE]); these are listed in Table 1.

**Genotyping**

Total DNA was isolated from venous blood or saliva, as previously described (Kikuchi et al. 2016). All polymorphisms were genotyped using TaqMan SNP Genotyping Assays and StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA). Custom primers were used for the MT-ND2 polymorphism as follow: forward primer: 5-GCCCCCTTTTACTTCTGTAGT-3; reverse primer: 5-GGGCTAGTTTTGTCAAGTGAG-3; A-allele probe, 5-CAAGGCCTGCTC-3; G-allele probe, 5-CCAGGACCCCTG-3. Genotype calling was conducted using StepOne™ Software v2.1 (Applied Biosystems, Foster City, CA). rs4341 being in complete linkage-equilibrium with rs4340 in Asian populations (Tanaka et al. 2003), ACE I/D genotypes were calculated as follows: rs4341 G/G as D/D, C/G as I/D, and C/C as I/I.

**Total genotype score**

Total genotype score (TGS) was calculated from the selected polymorphisms following the procedure previously described (Miyamoto-Mikami et al. 2016; Williams et al. 2008). Each genotype was scored based on literature information (Table 1). We assigned a genotype score (GS) of 2, 1, and 0 to “optimal”, “intermediate”, and “less optimal” genotypes, respectively. Then, we summed the GSs and transformed the sum to a scale of 0–100 for easier interpretation. The TGS formula is as follows:

\[
\text{TGS} = (\text{GS}_{ACE} + \text{GS}_{ACTN3} + \text{GS}_{ADRA2A} + \text{GS}_{ADRB2} + \text{GS}_{ADRB3} + \text{GS}_{APOE} + \text{GS}_{CKM} + \text{GS}_{COLS1A1} + \text{GS}_{CYP2J1} + \text{GS}_{DKB} + \text{GS}_{DFTC4} + \text{GS}_{PPARD} + \text{GS}_{PPARGC1A} + \text{GS}_{SLC6A1} + \text{GS}_{TFAM} + \text{GS}_{UCP2} + \text{GS}_{UCP3} + \text{GS}_{TRHR} + \text{GS}_{VDR})
\]

In the above formula, 40 is the result of multiplying 20 (number of analyzed polymorphisms) by 2, which is the score given to the optimal genotype.

**Statistical analysis**

Hardy–Weinberg equilibrium was determined for each polymorphism by the \(\chi^2\) test. Genotypic association with elite athlete status was analyzed by logistic regression.
Table 1. Studied polymorphisms for endurance performance.

| Gene symbol | Gene name                                      | rs number | Polymorphism (Function)         | Reference                             | Genotype score |
|-------------|------------------------------------------------|-----------|---------------------------------|---------------------------------------|----------------|
| ACE         | angiotensin I converting enzyme                | rs4340    | ID (Intron)                     | Myerson et al. (1999)                 | II = 2, ID = 1, DD = 0 |
| ACTN3       | actinin, alpha 3                               | rs1815739 | C>T (Arg577Ter)                 | Yang et al. (2003)                    | TT = 2, CT = 1, CC = 0 |
| ADRA2A      | adrenoceptor alpha 2A                          | rs553668  | T>C (3′-UTR)                    | Wulfarth et al. (2001)                | CC = 2, CT = 1, TT = 0 |
| ADRB2       | adrenoceptor beta 2                            | rs1042713 | C>G (Gln27Glu)                  | Moore et al. (2001)                   | CC = 2, CG = 1, GG = 0 |
| ADRB3       | adrenoceptor beta 3                            | rs4994    | T>C (Trp64Arg)                  | Santiago et al. (2011)                | CC = 2, CT = 1, TT = 0 |
| APOE1       | apolipoprotein E                               | rs429358  | T>C (Cys112Arg)                 | Thompson et al. (2004)                | E3E4 (E4E4,E4E2) = 2 |
| CKM         | creatine kinase, muscle                        | rs8111989 | ΔΔG (3′-near gene)              | Rivera et al. (1997)                  | AA = 2, AG = 1, GG = 0 |
| COL5A1      | collagen, type V, alpha 1                      | rs12722   | ΔΔG (3′-UTR)                    | Posthumus et al. (2011)               | TT = 2, CT = 1, CC = 0 |
| GABPB1      | GA-binding protein transcription factor, beta subunit 1 | rs7181866 | A>G (Intron)                     | Eynon et al. (2009)                   | GG = 2, AG = 1, AA = 0 |
| GNB3        | guanine nucleotide-binding protein (G protein), beta polypeptide 3 | rs5443    | C>T (Synonymous)                | Eynon et al. (2009)                   | TT = 2, CT = 1, CC = 0 |
| KDR         | kinase insert domain receptor                  | rs1870377 | ΔΔG (Gly472His)                 | Ahmetov et al. (2009a)                | AA = 2, AT = 1, TT = 0 |
| NFATC4      | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4 | rs2229309 | G>C (Gly160Ala)                 | Ahmetov et al. (2009b)                | GG = 2, GC = 1, CC = 0 |
| PPARD       | peroxisome proliferator-activated receptor delta | rs2016520 | C>T (Gly162Ser)                 | Hautala et al. (2007)                 | TT = 2, CT = 1, CC = 0 |
| PPARGC1A    | peroxisome proliferator-activated receptor gamma, coactivator 1 alpha | rs8192678 | G>A (Gly482Ser)                 | Lucia et al. (2005)                   | GG = 2, AG = 1, AA = 0 |
| PPARGC1B    | peroxisome proliferator-activated receptor gamma, coactivator 1 beta | rs7732671 | G>C (Ala203Pro)                 | Ahmetov et al. (2009b)                | not included |
| SLC16A1     | solute carrier family 16 (monocarboxylate transporter), member 1 | rs1049434 | T>A (Asp490Glu)                 | Cupheiro et al. (2012)                | AA = 2, AT = 1, TT = 0 |
| TFAM        | transcription factor A, mitochondrial           | rs1937    | G>C (Ser12Thr)                  | Ahmetov et al. (2009b)                | CC = 2, GC = 1, GG = 0 |
| UCP2        | uncoupling protein 2                           | rs660339  | C>T (Ala55Val)                  | Ahmetov et al. (2009b)                | TT = 2, CT = 1, CC = 0 |
| UCP3        | uncoupling protein 3                           | rs1800849 | C>T (G5′-UTR)                   | Ahmetov et al. (2009b)                | TT = 2, CT = 1, CC = 0 |
| UCPD2       | mitochondrially encoded NADH dehydrogenase 2   | m.4833    | A>G (Thr122Ala)                 | Mikami et al. (2011)                  | G = 2, A = 0 |

Phenotype-associated alleles in previous studies (optimal alleles) are underlined. Ala: alanine, Arg: arginine, Asp: aspartic acid, Cys: cysteine, Gln: glutamine, Glu: glutamic acid, Gly: glycine, His: histidine, Pro: proline, Ser: serine, Ter: termination codon, Thr: threonine, Trp: tryptophan, UTR: untranslated region, and Val: valine. 1 GS of APOE was assigned by a combination of rs429358 and rs7412 genotypes based on Thompson et al. (2004) (PMID: 14767871): the E4 and E3 alleles, respectively, determined as “optimal” and “less optimal” for endurance phenotypes.
Significance threshold was set after Bonferroni correction for multiple comparison at $P < 0.002$ ($=0.050/21$). The group variances being unequal (Levene's test), Welch's one-way ANOVA was used to compare means of TGSs among the four groups (control, regional, national, and international). All tests were performed using SNPstats software (http://bioinfo.iconcologia.net/SNPstats) (Solé et al. 2006) and the Statistical Package for Social Sciences (SPSS, v. 20. For Windows; SPSS Inc., Chicago, Illinois).

**Results**

All the polymorphisms were in HWE, excepted $PPARGC1B$ rs7732671 polymorphism, which was excluded from further analysis. No significant difference in TGSs was found among the four groups (Control: $49.0 \pm 7.6$, Regional: $47.3 \pm 7.6$, National: $49.1 \pm 5.7$, and International: $48.2 \pm 7.0$, $P = 0.626$, Fig. 1). Even when the endurance athletes were divided into middle-distance runners (Control: $49.0 \pm 7.6$, Regional: $48.4 \pm 5.6$, and National: $47.3 \pm 8.1$, $P = 0.871$) and long-distance runners (Control: $49.0 \pm 7.6$, Regional: $47.8 \pm 6.0$, National: $49.2 \pm 5.7$, and International: $48.3 \pm 6.9$, $P = 0.765$), there were no significant differences in TGSs among the four groups. Furthermore, even when the endurance runners limited to the five outlier athletes who were world record holders and medalists at Olympics and/or World championships, their TGS were $47.1 \pm 7.3$ (Range: $35.0 – 57.5$). Nevertheless, the polymorphisms $ACTN3$ rs1815739 and $GNB3$ rs5443 have been shown to be linked with international athlete status (Table 2). The frequencies of the $ACTN3$ R577X rs1815739

![Figure 1. Total Genotype Score, based on 21 polymorphisms related with endurance performance, in the four studied groups ($P = 0.626$). The horizontal bars represent the mean values with standard deviations.](image)

![Table 2. Allele and genotype frequencies of the polymorphisms presenting significant results between international athletes and controls.](image)
### Table 3. Genotype frequencies of 20 polymorphisms in all groups.

| Gene symbol | Polymorphism (Function or location) | rs number | Genotype frequency, n (%) | Control | All athlete | Regional athlete | National athlete | International athlete |
|-------------|------------------------------------|-----------|---------------------------|---------|-------------|------------------|-------------------|----------------------|
| Nuclear DNA |                                    |           |                           |         |             |                  |                   |                      |
| ACE         | I/D (Intron)                        | rs4340    | II                         | 269 (41.5) | 72 (41.1) | 7 (33.3)        | 39 (41.5)        | 26 (43.3)           |
|             |                                    |           | ID                         | 301 (46.4) | 78 (44.6) | 11 (52.4)       | 40 (42.5)        | 27 (45.0)           |
| ACTN3       | C/T (Arg577Ter)                     | rs1815739 | TT                         | 171 (26.4) | 39 (22.3) | 6 (28.6)        | 24 (25.5)        | 9 (15.0)            |
|             |                                    |           | CT                         | 346 (53.3) | 97 (55.4) | 12 (57.1)       | 50 (53.2)        | 35 (58.3)           |
| ADRA2A      | T>C (3'-UTR)                        | rs553668  | CC                         | 211 (32.5) | 61 (34.9) | 6 (28.6)        | 37 (39.4)        | 18 (30.0)           |
|             |                                    |           | CT                         | 326 (50.2) | 80 (45.7) | 10 (47.6)       | 41 (43.6)        | 29 (48.3)           |
| ADRB2       | A>G (Arg16Gly)                      | rs1042713 | TT                         | 112 (17.3) | 34 (19.4) | 5 (23.8)        | 16 (17.0)        | 13 (21.7)           |
|             |                                    |           | TC                         | 301 (46.4) | 78 (44.6) | 11 (52.4)       | 40 (42.5)        | 27 (45.0)           |
| ADRA2B      | T>C (Trp64Arg)                      | rs4994    | TT                         | 425 (65.5) | 111 (63.4) | 16 (76.2)       | 56 (59.6)        | 39 (65.0)           |
|             |                                    |           | TC                         | 206 (31.7) | 58 (33.1) | 5 (23.8)        | 35 (37.2)        | 18 (30.0)           |
| APOE        | T>C (Cys112Arg)                     | rs429358  | TT                         | 18 (2.8)  | 6 (3.4)    | 0 (0.0)         | 3 (3.2)          | 3 (5.0)             |
|             |                                    |           | TC                         | 128 (19.7) | 38 (21.7) | 3 (14.3)        | 24 (25.5)        | 11 (18.3)           |
| COL5A1      | C>T (3’-near gene)                  | rs8111989 | AA                         | 465 (71.7) | 126 (72.0) | 15 (71.4)       | 66 (70.2)        | 45 (75.0)           |
|             |                                    |           | AG                         | 166 (25.6) | 49 (28.0) | 6 (28.6)        | 28 (29.8)        | 15 (25.0)           |
| GABPB1      | A>G (Cys112Arg)                     | rs429358  | CC                         | 10 (1.5)  | 0 (0.0)    | 0 (0.0)         | 0 (0.0)          | 0 (0.0)             |
|             |                                    |           | CT                         | 99 (15.2)  | 32 (18.3) | 2 (9.5)         | 17 (17.6)        | 55 (91.7)           |
| GNB3        | C>T (Arg158Gly)                     | rs12772   | TT                         | 51 (7.9)  | 14 (8.0)  | 4 (19.1)        | 5 (5.3)          | 5 (8.3)             |
|             |                                    |           | TC                         | 128 (19.7) | 38 (21.7) | 3 (14.3)        | 24 (25.5)        | 11 (18.3)           |
| KDR         | A>T (Gln472His)                     | rs1870377 | TT                         | 18 (2.8)  | 0 (0.0)    | 0 (0.0)         | 0 (0.0)          | 0 (0.0)             |
|             |                                    |           | CC                         | 428 (66.0) | 132 (75.4) | 18 (85.7)       | 73 (77.7)        | 41 (68.3)           |
| NFATC4      | G>C (Gly160Ala)                     | rs12772   | CT                         | 204 (31.4) | 39 (22.3) | 3 (14.3)        | 20 (21.3)        | 16 (26.7)           |
|             |                                    |           | TT                         | 17 (2.6)  | 4 (2.3)    | 0 (0.0)         | 1 (1.1)          | 3 (5.0)             |
| PPARGC1A    | C>T (5’-UTR)                        | rs1229309 | CC                         | 21 (3.2)  | 7 (4.0)    | 0 (0.0)         | 4 (4.3)          | 3 (5.0)             |
|             |                                    |           | CT                         | 203 (31.3) | 58 (33.1) | 13 (7.4)        | 7 (7.5)          | 5 (8.3)             |
| SLC16A1     | T>A (Asp490Glu)                     | rs1815739 | TT                         | 417 (64.2) | 112 (64.0) | 14 (66.7)       | 58 (61.7)        | 19 (32.8)           |
|             |                                    |           | TC                         | 112 (17.3) | 34 (19.4) | 5 (23.8)        | 16 (17.0)        | 13 (21.7)           |
| (Continued) |                                    |           | CC                         | 211 (32.5) | 61 (34.9) | 6 (28.6)        | 37 (39.4)        | 18 (30.0)           |
Ace has recently been found in East-Asian athletes that the haplotype networks between ethnic groups. For example, I/D alleles were associated with elite athlete status, in Caucasian populations, and it is acknowledged that differences exist in genotype frequencies and performance in Caucasian populations, and it is conceivable that the present TGS included, besides ACE I/D, polymorphisms that could also present associations of opposite direction in Asian populations. Furthermore, based on the present findings in controls, the chances of finding a Japanese individual with a “theoretically” perfect TGS was 9.0 × 10^−13. Of course, our lack of significant results could also be explained by methodological errors. Furthermore, functional significance of most of the polymorphisms analyzed remains unclear; therefore, we cannot exclude the possibility that our TGS included polymorphisms that do not influence endurance performance. In addition, it is possible that the studied polymorphisms affect the relevant physiology differently in Caucasian and Japanese populations owing to differences in environmental factors, such as training methods. Furthermore, our genotype score gave all genotypes the same weight; this may not be a true effect of the physiologic/behavioral basis of athlete status. We also did not examine interactions among genes and/or between genes and environment that might affect elite athlete status, because sample size is not enough in this study. Thus, in future, extensive studies are required to consider environmental factors and gene-environment interactions as well as gene–gene interactions.

Two of the studied polymorphisms, namely ACTN3 rs1815739 and GNB3 rs5443, were individually linked with elite endurance athlete status (Table 2), although the statistical significances were not confirmed after multiple-testing corrections. The frequency of ACTN3 577XX genotype was under-represented in international athletes, compared with controls. α-actinin-3 is almost exclusively expressed in fast-twitch muscle fibers, where it acts as a lattice structure that anchors actin-containing thin filaments; this stabilizes the muscle contractile apparatus, thereby conferring a higher capacity for force absorption/transmission compared with slow fibers. Originally, it was thought that the XX genotype presented an advantage for endurance performance. However, considering the loss of functionality due to the XX genotype (Lee et al. 2016), it is presently thought that the R allele and the presence of α-actinin-3 in fast-twitch muscle fibers may be beneficial.

### Table 3. Continued.

| Gene symbol | Polymorphism (Function or location) | Genotype | Control n (%) | All athlete n (%) | Regional athlete n (%) | National athlete n (%) | International athlete n (%) |
|-------------|-----------------------------------|----------|---------------|------------------|-----------------------|------------------------|-----------------------------|
| TFAM        | G>C (Ser12Thr)                    | GG       | 420 (64.7)    | 115 (65.7)       | 15 (71.4)             | 56 (59.6)             | 44 (73.3)                   |
|             |                                   | GC       | 207 (31.9)    | 53 (30.3)        | 6 (28.6)              | 32 (34.0)             | 15 (25.0)                   |
|             |                                   | rs1937   | 22 (3.4)      | 7 (4.0)          | 0 (0.0)               | 6 (4.0)               | 1 (1.7)                     |
| UCP2        | C>T                               | CC       | 165 (25.4)    | 43 (24.6)        | 9 (42.9)              | 21 (22.3)             | 13 (21.7)                   |
|             | (Ala55Val)                        | CC       | 346 (53.3)    | 82 (46.9)        | 7 (33.3)              | 46 (48.9)             | 29 (48.3)                   |
|             |                                   | CT       | 138 (21.3)    | 50 (28.6)        | 5 (23.8)              | 27 (28.7)             | 18 (30.0)                   |
| UCP3        | C>T                               | CC       | 334 (51.5)    | 81 (46.3)        | 13 (61.9)             | 42 (44.7)             | 26 (43.3)                   |
|             | (5’T-UTR)                         | CT       | 257 (39.6)    | 82 (46.9)        | 7 (33.3)              | 45 (47.9)             | 30 (50.0)                   |
|             |                                   | TT       | 58 (8.9)      | 12 (6.9)         | 1 (4.8)               | 7 (7.5)               | 4 (6.7)                     |
| MT-ND2      | A>G                               | A        | 594 (91.5)    | 163 (93.1)       | 20 (95.2)             | 86 (91.5)             | 57 (95.0)                   |
|             | (Thr122Ala)                       | G        | 55 (8.5)      | 12 (6.9)         | 1 (4.8)               | 8 (8.5)               | 3 (5.0)                     |
|             | m.4833                            |          |               |                  |                      |                       |                            |

Gene names of the gene symbols are show in Table 1. All athletes comprise regional-, national-, and international-level athletes.
also to endurance performance (Lee et al. 2016; Kikuchi et al. 2016); this is in accordance with our results.

We also found a possible relation between the GNB3 rs5443 polymorphism and international endurance athlete status. The GNB3 gene encodes the beta subunit of heterotrimeric guanine nucleotide-binding proteins (G protein), which integrate signals between receptors and effector proteins. It is thought to confer an advantage on endurance performance, enhancing glycogen and fatty acid metabolism through the cAMP-insulin receptor pathway (Eynon et al. 2009). Eynon et al. (Eynon et al. 2009) found that the TT genotype frequency was significantly higher in elite Israeli endurance athletes than in controls or sprinters. Our results showed a tendency in the opposite direction: the C allele frequency was higher in international endurance athletes than in controls. However, when Ruiz et al. (2011) conducted a replication of the Eynon et al. study in larger cohorts and other ethnicities (Israeli and Spanish), they could not find significant associations. As we mentioned above, there are several possible explanations justifying these results inconsistency (e.g., ethnicity differences, statistical errors, and/or environmental interactions).

**Practical Applications**

Understanding the genetic of athletic performance is an important point in the development of future methods for talent identification in sport. Obtained data here suggest that the selected multiple genetic effect is not related to endurance performance in Japanese runners, so this fact should be taken into account in the future, especially for Asian athletes. Our nonsignificant results for being an elite runner based on the studied polymorphisms confirm that the possibility of becoming an elite athlete depends on numerous influential factors.

**Conclusions**

In conclusion, our TGS based on 20 polymorphisms related with endurance performance (and related phenotypes), mostly in Caucasian populations, has not been found to be associated with elite endurance athlete status in the Japanese population. These results suggest that most of the polymorphisms analyzed in this study may not influence endurance athlete status in Japanese runners, with the exception of the ACTN3 rs1815739 and GNB3 rs5443 polymorphisms. In order to identify polygenic profiles that allow us to distinguish the potential of someone in the Japanese population to become an international athlete, it seems that future studies should further focus on polymorphisms for which associations have been observed with elite athlete status in Asian populations, and with robust replications.

**Conflict of Interest**

None declared.

**References**

Ahmetov, I. I., and O. N. Fedotovskaya. 2015. Current Progress in Sports Genomics. Adv. Clin. Chem. 70:247–314.

Ahmetov, I. I., A. M. Hakimullina, D. V. Popov, E. V. Lyubaeva, S. S. Missina, O. L. Vinogradova, et al. 2009a. Association of the VEGFR2 gene His472Gln polymorphism with endurance-related phenotypes. Eur. J. Appl. Physiol. 107:95–103.

Ahmetov, I. I., A. G. Williams, D. V. Popov, E. V. Lyubaeva, A. M. Hakimullina, O. N. Fedotovskaya, et al. 2009b. The combined impact of metabolic gene polymorphisms on elite endurance athlete status and related phenotypes. Hum. Genet. 126:751–761.

Cupeiro, R., D. Gonzalez-Lamuno, T. Amigo, A. B. Peinado, J. R. Ruiz, F. B. Ortega, et al. 2012. Influence of the MCT1-T1470A polymorphism (rs1049434) on blood lactate accumulation during different circuit weight trainings in men and women. J. Sci. Med. Sport. 15:541–547.

Eynon, N., J. Oliveira, Y. Meckel, M. Sagiv, C. Yamin, M. Sagiv, et al. 2009. The guanine nucleotide binding protein beta polypeptide 3 gene C825T polymorphism is associated with elite endurance athletes. Exp. Physiol. 94:344–349.

Eynon, N., J. R. Ruiz, Y. Meckel, M. Moran, and A. Lucia. 2011. Mitochondrial biogenesis related endurance genotype score and sports performance in athletes. Mitochondrion 11:64–69.

Hautala, A. J., A. S. Leon, J. S. Skinner, D. C. Rao, C. Bouchard, and T. Rankinen. 2007. Peroxisome proliferator-activated receptor-delta polymorphisms are associated with physical performance and plasma lipids: the HERITAGE Family Study. Am. J. Physiol. Heart Circ. Physiol. 292: H2498–H2505.

Kikuchi, N., E. Miyamoto-Mikami, H. Murakami, T. Nakamura, S. K. Min, M. Mizuno, et al. 2016. ACTN3 R577X genotype and athletic performance in a large cohort of Japanese athletes. Eur. J. Sport Sci. 16:694–701.

Lee, F. X., P. J. Houweling, K. N. North, and K. G. Quinlan. 2016. How does alpha-actinin-3 deficiency alter muscle function? Mechanistic insights into ACTN3, the ‘gene for speed’. Biochim. Biophys. Acta. 1863:686–693.

Loos, R. J., J. M. Hagberg, L. Perusse, S. M. Roth, M. A. Sarzynski, B. Wolfarth, et al. 2015. Advances in exercise, fitness, and performance genomics in 2014. Med. Sci. Sports Exerc. 47:1105–1112.

Lucia, A., F. Gomez-Gallego, I. Barroso, M. Rabadan, F. Bandres, A. F. San Juan, et al. 2005. PPARGC1A genotype
(Gly482Ser) predicts exceptional endurance capacity in European men. J. Appl. Physiol. (1985) 99:344–348.
Mikami, E., N. Fuku, H. Takahashi, N. Ohiwa, R. A. Scott, Y. P. Pitsiladis, et al. 2011. Mitochondrial haplogroups associated with elite Japanese athlete status. Br. J. Sports Med. 45:1179–1183.
Miyamoto-Mikami, E., H. Murakami, H. Tsuchie, H. Takahashi, N. Ohiwa, M. Miyachi, et al. 2016. Lack of association between genotype score and sprint/power performance in the Japanese population. J. Sci. Med. Sport. [in press].
Moore, G. E., A. R. Shuldiner, J. M. Zmuda, R. E. Ferrell, S. D. McCole, and J. M. Hagberg. 2001. Obesity gene variant and elite endurance performance. Metabolism 50:1391–1392.
Myerson, S., H. Hemingway, R. Budget, J. Martin, S. Humphries, and H. Montgomery. 1999. Human angiotensin I-converting enzyme gene and endurance performance. J. Appl. Physiol. (1985) 87:1313–1316.
Posthumus, M., M. P. Schwellnus, and M. Collins. 2011. The COL5A1 gene: a novel marker of endurance running performance. Med. Sci. Sports Exerc. 43:584–589.
Rivera, M. A., F. T. Dionne, J. A. Simoneau, L. Perusse, M. Chagnon, Y. Chagnon, et al. 1997. Muscle-specific creatine kinase gene polymorphism and VO2max in the HERITAGE Family Study. Med. Sci. Sports Exerc. 29:1311–1317.
Ruiz, J. R., F. Gomez-Gallego, C. Santiago, M. Gonzalez-Freire, Z. Verde, C. Foster, et al. 2009. Is there an optimum endurance polygenic profile? J. Physiol. 587:1527–1534.
Ruiz, J. R., N. Eynon, Y. Meckel, C. Fiuza-Luces, C. Santiago, F. Gomez-Gallego, et al. 2011. GNB3 C825T Polymorphism and elite athletic status: a replication study with two ethnic groups. Int. J. Sports Med. 32:151–153.
Santiago, C., J. R. Ruiz, A. Buxens, M. Artieda, D. Arteta, M. Gonzalez-Freire, et al. 2011. Trp64Arg polymorphism in ADRB3 gene is associated with elite endurance performance. Br. J. Sports Med. 45:147–149.
Solé, X., E. Guinó, J. Valls, R. Iniesta, and V. Moreno. 2006. SNPlStats: a web tool for the analysis of association studies. Bioinformatics 22:1928–1929.
Tanaka, C., K. Kamide, S. Takiuchi, Y. Miwa, M. Yoshii, Y. Kawano, et al. 2003. An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. Hypertens. Res. 26:301–306.
Thompson, P. D., G. J. Tsongalis, R. L. Seip, C. Bilbie, M. Miles, R. Zoeller, et al. 2004. Apolipoprotein E genotype and changes in serum lipids and maximal oxygen uptake with exercise training. Metabolism 53:193–202.
Wang, G., E. Mikami, L. L. Chiu, A. De Perini, M. Deason, N. Fuku, et al. 2013. Association analysis of ACE and ACTN3 in elite Caucasian and East Asian swimmers. Med. Sci. Sports Exerc. 45:892–900.
Williams, A. G., and J. P. Folland. 2008. Similarity of polygenic profiles limits the potential for elite human physical performance. J. Physiol. 586:113–121.
Wolfarth, B., B. A. Rivera, J. M. Oppert, M. R. Boulay, F. T. Dionne, M. Chagnon, et al. 2000. A polymorphism in the alpha2a-adrenoceptor gene and endurance athlete status. Med. Sci. Sports Exerc. 32:1709–1712.
Wolfarth, B., T. Rankinen, S. Muhlbauer, J. Scherr, M. R. Boulay, L. Perusse, et al. 2007. Association between a beta2-adrenergic receptor polymorphism and elite endurance performance. Metabolism 56:1649–1651.
Yang, N., D. G. MacArthur, J. P. Gulbin, A. G. Hahn, A. H. Beggs, S. Eastal, et al. 2003. ACTN3 genotype is associated with human elite athletic performance. Am. J. Hum. Genet. 73:627–631.