Whole genome sequencing of elite athletes

AUTHORS: Eugenia A. Boulygina[1], Oleg V. Borisov[2,3], Elena V. Valeeva[4,5], Ekaterina A. Semenova[2,4], Elena S. Kostryukova[2], Nikolay A. Kulemin[2], Andrey K. Larin[2], Roza M. Nabiullina[6], Fanis A. Mavliev[7], Azat M. Akhatov[8], Oleg N. Andryushchenko[9], Liliya B. Andryushchenko[10], Piotr Zmijewski[11], Edward V. Generozov[2], Ildus I. Ahmetov[2,5,10,12]

1   "Omics Technologies" OpenLab, Kazan Federal University, Kazan, Russia
2   Department of Molecular Biology and Genetics, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia
3   Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany
4   Department of Biochemistry, Biotechnology and Pharmacology, Kazan Federal University, Kazan, Russia
5   Laboratory of Molecular Genetics, Central Research Laboratory, Kazan State Medical University, Kazan, Russia
6   Department of Biochemistry, Kazan State Medical University, Kazan, Russia
7   Sport Technology Research Center, Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russia
8   Department of Theory and Methodology of Combat Sports, Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russia
9   Department of Physical Education, Financial University under the Government of the Russian Federation, Moscow, Russia
10 Department of Physical Education, Plekhanov Russian University of Economics, Moscow, Russia
11 Institute of Sport - National Research Institute, Warsaw, Poland
12 Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom

Corresponding author:
Eugenia A. Boulygina
"Omics Technologies" OpenLab
Kazan Federal University
Kazan, Russia
E-mail: boulygina@gmail.com

Ildus I. Ahmetov
Research Institute for Sport and Exercise Sciences
Liverpool John Moores University
Liverpool, United Kingdom
E-mail: i.akhmetov@ljmu.ac.uk

ABSTRACT: Whole genome sequencing (WGS) has great potential to explore all possible DNA variants associated with physical performance, psychological traits and health conditions of athletes. Here we present, for the first time, annotation of genomic variants of elite athletes, based on the WGS of 20 Tatar male wrestlers. The maximum number of high-quality variants per sample was over 3.8 M for single nucleotide polymorphisms (SNPs) and about 0.64 M for indels. The maximum number of nonsense mutations was 148 single nucleotide variants (SNVs) per individual. Athletes' genomes on average contained 18.9 nonsense SNPs in a homozygous state per sample, while non-athletes' exomes (Tatar controls, n = 19) contained 18 nonsense SNPs. Finally, we applied genomic data for the association analysis and used reaction time (RT) as an example. Out of 1884 known genome-wide significant SNPs related to RT, we identified four SNPs (*KIF27* rs10125715, *APC* rs518013, *TMEM229A* rs7783359, *LRRN3* rs80054135) associated with RT in wrestlers. The cumulative number of favourable alleles (*KIF27* A, *APC* A, *TMEM229A* T, *LRRN3* T) was significantly correlated with RT both in wrestlers ($P = 0.0003$) and an independent cohort ($n = 43$) of physically active subjects ($P = 0.029$). Furthermore, we found that the frequencies of the *APC* A (53.3 vs 44.0%, $P = 0.033$) and *LRRN3* T (7.5 vs 2.8%, $P = 0.009$) alleles were significantly higher in elite athletes (n = 107) involved in sports with RT as an essential component of performance (combat sports, table tennis and volleyball) compared to less successful (n = 176) athletes. The *LRRN3* T allele was also over-represented in elite athletes (7.5%) in comparison with 189 controls (2.9%, $P = 0.009$). In conclusion, we present the first WGS study of athletes showing that WGS can be applied in sport and exercise science.

CITATION: Boulygina EA, Borisov OV, Valeeva EV et al. Whole genome sequencing of elite athletes. Biol Sport. 2020;37(3):295–304.

Received: 2020-05-16; Reviewed: 2020-05-26; Re-submitted: 2020-05-29; Accepted: 2020-06-01; Published: 2020-06-10.

INTRODUCTION

Sports genomics is a relatively new scientific discipline focusing on the organization and functioning of the genome of elite athletes. It postulates that genetic and epigenetic factors play a key role in athletic performance and related phenotypes such as power, strength, aerobic capacity, flexibility, height, muscle mass, coordination, and personality traits. Despite a relatively high heritability of athlete status and performance related phenotypes, the search for genetic variants contributing to predisposition to success in certain types of sport has been a challenging task. So far, 185 DNA polymorphisms associated with athlete status have been identified in the last 21 years [1–3].

Among common tools for the detection of performance-associated DNA polymorphisms researchers use case-control or genotype-phenotype studies based on a candidate gene design [4–7]. The limitation of this approach is that one cannot detect the polymorphic variant which lies within a non-coding (possibly, regulatory) genome region. Another approach is a genome-wide association study (GWAS) using micro-array analysis which proved to be extremely successful to uncover genetic association in sport-related phenotypes [8–11]. However, micro-array analysis covers only a limited number (up to 5 M) of DNA polymorphisms (< 0.2% of the genome). Although these polymorphisms are designed to evenly cover most of the genome regions, linkage disequilibrium differences in various populations restrict the generalizability of such an approach. To overcome this, the whole-genome based technique can be effectively used. Whole genome sequencing (WGS) refers to the construction of the complete nucleotide sequence of a genome (~3.2 billion base pairs in humans) and provides a powerful tool to obtain greater insight into the genetic variability that could produce a range of benefits for sport and exercise science.

Here we performed, for the first time, a low coverage whole-genome analysis in a group of athletes which was homogeneous in terms of ethnicity (Tatars), sex (males) and sport discipline (belt wrestling). A number of factors determine athletic performance in wrestling, among them both physiological (strength and endurance, muscle mass, dexterity, displacement speed, flexibility, coordination, balance) and psychological (reaction time, decision-making speed, ingenuity, patience) [12]. For example, most successful wrestlers show a significantly quicker reaction time during fights [13]. The heritability of reaction time has been shown to reach 60% [14]. At least 5 genetic markers (located within *ACE*, *ACTN3*, *FTO*, *HIF1A* and *MCT1* genes; involved in metabolism, skeletal muscle structure and function) have previously been shown to be linked to wrestler status [5, 15–18]. However, genetic association analysis in wrestlers using DNA polymorphisms previously associated with reaction time has not been performed.

The aim of our study was, therefore, to characterize the whole genome sequence of wrestlers and apply this information to establish an association between DNA variants and reaction time.

MATERIALS AND METHODS

Ethical approval
The study was approved by the Ethics Committee of the Federal Research and Clinical Center of Physical-chemical Medicine of the Federal Medical and Biological Agency of Russia. Written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki and ethical standards in sport and exercise science research.

Participants
Twenty professional male belt (kurash) wrestlers (age 20 ± 4.4 years; height 173.0 ± 10.0 cm, weight 73.6 ± 10.6 kg) volunteered for the WGS study. The athletes were all Caucasian Tatars from the Republic of Tatarstan (Russian Federation). Exomes (protein-coding regions of genes in a genome) of wrestlers were compared with exomes of ethnicity-matched controls (Tatars, n = 19) from a previous study [19]. The second cohort consisted of 43 physically active participants (27 males, age 35.8 ± 7.9 years, height 178.4 ± 6.2 cm, weight 77.1 ± 11.0 kg; 16 females, age 29.4 ± 8.7 years, height 168.8 ± 6.4 cm, weight 57.3 ± 5.2 kg) and was used for validation of findings from the association study (reaction time). The case-control study involved 283 athletes (110 females, 173 males; age 24.1 ± 3.6 years) from the following sporting disciplines: boxing (n = 101), wrestling (n = 82), karate (n = 21), taekwondo (n = 24), volleyball (n = 45), table tennis (n = 10). All athletes were international-level competitors who represented Russia in international competitions (107 elite (prize winners) and 176 sub-elite) and have been tested negative for doping substances. The control group (n = 189, 38 females, 151 males; age 45 ± 4.3 years) included unrelated citizens, without any competitive sport experience.

Sample collection, DNA sequencing, SNV calling and SNP genotyping
Fasting venous blood samples (a total of 9 ml of blood) of wrestlers were collected in the morning in tubes containing K2-EDTA and stored at −20°C until DNA extraction. DNA extraction was performed using a Wizard Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, USA). DNA libraries were sequenced by Illumina HiSeq 2500 platform using the HiSeq SBS Kit v4 (Illumina, San Diego, USA) according to the manufacturer's recommendations, with pair-end 125-bp read length at an average sequencing depth of 9.9x (ranging from 2.6x to 16.8x). Raw reads were mapped to the human reference genome hg19 using BWA [20]. The low coverage whole genome variant calling was performed using Strelka v. 2 [21]. Hard filtering was applied to the detected raw single nucleotide variants with parameters as follows: MQ < 40, LowDepth > 3, HighSNVSB < 10. Variants were annotated using Annovar [22] equipped with additional databases (ClinVar, COSMIC, dbSNP, ESP6500, ExAC). The whole genome variants were validated by the microarray technology with HumanOmniExpress Bead-Chips (Illumina Inc, USA) for genotyping of ~900,000 SNPs.

SNP genotyping (micro-array analysis) of physically active participants, athletes and controls was performed with DNA samples obtained from leukocytes (venous blood). Four ml of venous blood were collected in tubes containing EDTA (Vacuette EDTA tubes, Greiner Bio-One, Austria). Blood samples were transported to the laboratory at 4°C and DNA was extracted on the same day. DNA extraction and purification were performed using a commercial kit according to the manufacturer's instructions (Technoclon, Russia) and included chemical lysis, selective DNA binding on silica spin columns and ethanol washing. Extracted DNA quality was assessed by agarose gel electrophoresis at this step. HumanOmni1-Quad Bead-Chips (Illumina Inc, USA) were used for genotyping of 1,140,419 SNPs

in 283 athletes and 189 controls, while HumanOmniExpress Bead-Chips (Illumina Inc, USA) were used for genotyping of ~900,000 SNPs in 43 physically active participants. Reaction time related DNA variants (n = 1884; including leading and tag SNPs) for validation in wrestlers and physically active participants were selected from published studies [23, 24].

Reaction time measurement
Visual reaction time was evaluated using the computer test 'Traffic light'. Laboratory-based testing was carried out under same conditions for participants (i.e. in morning, in the resting state, using the same computer, under supervision of the same test administrator). Subjects sat in front of a table with the palm of the dominant hand supported and their index finger on a computer mouse. The participants were consistently presented with light signals in the centre of the monitor screen, and were asked to press the button when the green signal appeared. The duration of the intervals between the red and green signals ranged from 0.5 to 5 s. The first 5 signals were trial and were not recorded. The best three attempts from the following 5 signals were recorded and the average reaction time was calculated. All attempts were observed by the test administrator.

Statistical analysis
Statistical analyses were conducted using PLINK v1.90, R (version 3.4.3), and GraphPad InStat (GraphPad Software, Inc., USA) software. The chi-square test (χ^2) was used to test for the presence of the Hardy-Weinberg equilibrium in the genotype distribution, to compare the proportions of subjects with a high number of reaction time improving alleles or allelic frequencies between groups. To evaluate the associations between polygenic profiles and reaction time, the Spearman rank correlation coefficient was calculated. *P* values < 0.05 were considered statistically significant.

RESULTS
The metrics of genomic variants
As a genomic data quality metric, we used the transition/transversion (Ts/Tv) ratio, which was detected to be consistent with previous studies ($\approx$2) [25]. For 20 wrestlers' genomes, using a low coverage protocol we detected over 11.5 million raw genomic variants in total. Taking into account only sufficiently covered genomes ($\geq$ 12x), for the 12 most deeply covered samples, average numbers of SNVs and indels were 3.8 million and 0.64 million per sample, correspondingly. About 11 million raw variants passed the quality filters (3.6 mil-

TABLE 1. Basic statistics of raw WGS data and genomic variants in wrestlers (n = 20)

Sample #	Raw reads number	Mapped reads number	Mapped reads, %	Mean coverage	Ts/Tv ratio	Raw SNPs number	Raw indels number	Filtered SNPs number	Filtered indels number
1	135419040	134973712	99.67	2.65	2.01	1170490	94591	1132716	92585
2	152651625	152180976	99.69	3.00	2	1338431	115651	1296133	113359
3	135166473	134674889	99.64	2.64	1.98	1156025	100851	1119445	98894
4	138380470	137897091	99.65	2.71	2.01	1187357	97416	1149187	95394
5	138247447	137870773	99.73	2.72	1.98	1197711	105756	1160354	103601
6	170747873	170301203	99.74	3.36	1.98	1504990	139290	1455811	136558
7	156619056	156211771	99.74	3.08	1.98	1389524	126783	1345519	124277
8	167468957	167046801	99.75	3.29	1.99	1468924	133239	1422379	130714
9	349634959	349479692	99.96	14.13	1.98	3845412	638443	3646325	619174
10	354831314	354646812	99.95	14.27	1.98	3864805	644140	3662651	624830
11	419085260	418962576	99.97	16.81	1.98	3381380	559592	3212089	539752
12	370391660	370236765	99.96	14.93	1.99	3899130	644534	3703033	624312
13	305447291	305279572	99.95	12.27	1.99	3785574	617903	3587268	599639
14	318191054	318037831	99.95	12.85	1.98	3789480	628848	3586741	609087
15	362707535	362532795	99.95	14.62	1.98	3879120	651822	3666744	631928
16	384884409	384695366	99.95	15.52	1.98	3887156	660029	3676958	639700
17	330816726	330647548	99.95	13.31	1.98	3809749	634330	3603943	615521
18	366875825	366706680	99.95	14.78	1.98	3852608	645399	3649519	625418
19	366788900	366617355	99.95	14.78	1.98	3893521	654992	3691772	635218
20	412081693	411940287	99.97	16.62	1.98	3933194	659427	3727201	636988

TABLE 2. Association between DNA polymorphisms and reaction time in wrestlers (n = 20)

Closest gene	Favourable allele	Reaction time, s			r	P
		Genotype 1	Genotype 2	Genotype 3		
APC	rs518013 A	GG (n = 4) 0.287 ± 0.023	GA (n = 10) 0.286 ± 0.017	AA (n = 6) 0.283 ± 0.007	-0.52	0.028*
KIF27	rs10125715 A	TT (n = 1) 0.301	AT (n = 12) 0.289 ± 0.016	AA (n = 7) 0.277 ± 0.011	-0.49	0.034*
TMEM229A	rs7783359 T	–	AT (n = 10) 0.292 ± 0.016	TT (n = 10) 0.279 ± 0.012	-0.44	0.048*
LRRN3	rs80054135 T	AA (n = 17) 0.289 ± 0.013	AT (n = 2) 0.277 ± 0.004	TT (n = 1) 0.255	-0.56	0.012*

*$P < 0.05$, significant correlation. Values are mean ± SD.

TABLE 3. Frequencies of the favourable alleles in athletes and controls

Groups	n	Frequencies of favourable alleles,%			
		APC rs518013 A	*KIF27* rs10125715 A	*TMEM229A* rs7783359 T	*LRRN3* rs80054135 T
Elite athletes	107	53.3*	71.5	65.4	7.5**
Sub-elite athletes	176	44.0	70.2	63.6	2.8
Russian controls	189	46.6	69.6	66.7	2.9

*$P = 0.033$, statistically significant differences between elite and sub-elite athletes.
** $P = 0.009$, statistically significant differences between elite and sub-elite athletes or controls.

lion SNVs and 0.62 million indels on average per mostly covered samples). 47.8% of variants were annotated as synonymous SNV and 46.2% as nonsynonymous SNV; about 1.2% were frameshift and non-frameshift indels. The average numbers of stop-gain and stop-loss mutations were 120.8 and 14.8, respectively, for deeply covered samples; the maximum number of these nonsense mutations was 148 SNVs per individual. As expected, the vast majority of variants localized in intergenic and intronic regions (≈56% and ≈34%, respectively). About 2.7% of variation lay within exons, upstream and downstream, and in 3' and 5' UTRs. Basic statistics of raw WGS data and genomic variants in wrestlers are given in Table 1 and Supplementary Tables 1–7.

The fraction of variants that were predicted to be 'benign' and 'likely benign' was the highest (about 91.6%), followed by the fraction that had 'uncertain significance' (2.2%). Variants annotated as 'pathogenic' and 'likely pathogenic' represented 0.66% of total variation. We next compared the number of homozygous stop-gain mutations between wrestlers and 19 Tatar controls from our previous study [19]. Athletes' genomes on average contained 18.9 nonsense SNPs in a homozygous state per sample, while non-athletes' exomes contained 18 nonsense SNPs ($P>0.05$).

Genetic association analysis

Reaction times (RT) did not differ between 20 wrestlers and 43 physically active subjects (0.286 (0.015) s vs 0.274 (0.059) s; $P = 0.372$). RT between males and females in the group of physically active subjects was not significantly different ($P = 0.891$); therefore in the association analysis we used the combined group. In the discovery phase, out of 1884 known genome-wide significant SNPs (including leading and tag SNPs) related to RT, 24 SNPs (four leading and 20 tag SNPs) were associated with RT in wrestlers. Of those, four alleles (*KIF27* rs10125715 A, *APC* rs518013 A, *TMEM229A* rs7783359 T, *LRRN3* rs80054135 T) were found to be independently associated with the best RT in wrestlers (Table 2).

To assess the combined impact of all 4 gene polymorphisms, we classified wrestlers and physically active subjects according to the number of 'short reaction time' alleles they possessed (e.g. carriers of genotype *KIF27* rs10125715 TT, *APC* rs518013 GG, *TMEM229A* rs7783359 AA, *LRRN3* rs80054135 AA had 0 'short reaction time' alleles, and subjects with *KIF27* rs10125715 AA, *APC* rs518013 AA, *TMEM229A* rs7783359 TT, *LRRN3* rs80054135 TT genotype had 8 'short reaction time' alleles). The cumulative number of favourable (i.e. leading to a short reaction time) alleles was significantly cor-

related with RT in wrestlers ($r = 0.73$, $P = 0.0003$). This finding was also validated in the independent cohort of physically active subjects ($r = 0.33$, $P = 0.029$).

Next, we compared allelic frequencies of four SNPs between elite athletes (n = 107) involved in sports with RT as an essential component of performance (combat sports, table tennis and volleyball), sub-elite athletes (n = 176) and 189 controls (Table 3 and Supplementary Table 8). The genotypes distributions of four SNPs met the Hardy-Weinberg equilibrium expectations in athletes and controls. We found that the frequencies of the *APC* rs518013 A (53.3 vs 44.0%, $P = 0.033$) and *LRRN3* rs80054135 T (7.5 vs 2.8%, $P = 0.009$) alleles were significantly higher in elite compared to non-elite athletes. The *LRRN3* rs80054135 T allele was also over-represented in elite athletes (7.5%) in comparison with controls (2.9%, $P = 0.009$). Using the 1000 Genomes database (http://www.ensembl.org), we identified that East Asian populations for most SNPs have the highest frequency of favourable alleles compared to other populations (Supplementary Table 9).

DISCUSSION

To our knowledge, this is the first paper on whole genome sequencing of athletes. We found that the mutational load per Tatar athlete (the number of stop-loss and stop-gained mutations), SNV localization and clinical relevance statistics were comparable with those in Eurasian populations [26–28]. We also found that athletes' genomes on average contained 18.9 nonsense SNPs in a homozygous state per sample, while Tatar non-athletes' exomes contained almost the same (18) number of nonsense SNPs. These observations suggest that the obtained sequencing data have an adequate quality and may serve as a good starting point for further research in sports genomics. Tatars are one of the major Turkic-speaking groups in the Volga-Ural region of the Russian Federation. It was believed that, due to the geographic position of the region and the complex ethnic history of the population, Tatars have an extremely high genetic diversity in which the Asian (Mongolian) component had a significant contribution. The latest studies showed that the trace of East Asian or Central Siberian ancestry in the genomes of Volga Tatars is less than expected (approximately 5%) [29], but nevertheless, it still allows us to evaluate the genome data quality using European and Asian genomic data as a reference.

We also applied genomic information to establish an association between DNA variants and reaction time. The high quality of WGS was confirmed by micro-array analysis of 1884 SNPs previously reported to be associated with RT in the UK Biobank cohorts [23, 24]. As a result, we confirmed the association between four independent SNPs (*APC* rs518013, *KIF27* rs10125715, *TMEM229A* rs7783359, *LRRN3* rs80054135) and RT in two cohorts (wrestlers and physically active subjects). We also found that the frequencies of *APC* rs518013 A and *LRRN3* rs80054135 T alleles were significantly higher in elite athletes involved in sports with RT as an essential component of performance (combat sports, table tennis and vol-

leyball) compared to non-elite athletes and/or controls, indicating that these DNA polymorphisms may play a role in the natural selection process of athletes. Interestingly, for most of the SNPs the highest frequency of favourable alleles compared to other populations was found in East Asians – populations with a long history of cultivation of martial arts and dominance in judo, karate, taekwondo and table tennis. Our approach thus provided for the first time sufficient power of WGS to detect a wide range of candidate alleles that may lead to athletic success.

According to the GTEx Portal [30], three of those SNPs are functional and may influence expression of several genes in the brain and other tissues (*APC* rs518013 influences expression of the *SRP19* gene; *KIF27* rs10125715 influences expression of *GKAP1* and *RMI1* genes; *TMEM229A* rs7783359 influences expression of the *RP5–921G16.1* gene). The *APC* (adenomatous polyposis coli protein) gene encodes a tumour suppressor protein that acts as an antagonist of the Wnt signalling pathway and is involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis. Interestingly, the hypermethylation of the APC gene was reported to be inversely associated with physical activity [31]. The *KIF27* (kinesin family member 27) gene encodes a protein involved in ATPase activity and microtubule motor activity [32]. The *TMEM229A* (transmembrane protein 229A) gene encodes a protein involved in DNA-binding transcription factor activity and developmental processes [33]. The *LRRN3* (leucine rich repeat neuronal 3) gene encodes a protein which plays an important role in cerebellum postnatal development [34].

Among the limitations of the current study are the sample size of the wrestler cohort (n = 20) and the low overall sequencing depth. Despite the fact that the total number of SNVs per genome did not reach the level that was observed before [35, 36] (probably due to insufficient sequencing depth), still, such low-coverage sequencing was shown to allow genotyping variants with confidence [37], and this was also confirmed by micro-array analysis in our study. Other efforts to sequence hundreds of genomes of elite athletes are presently underway [38].

CONCLUSIONS

In conclusion, we present the first WGS study of athletes showing that WGS can be applied in sports genomics. By replicating previous findings from non-athletic populations, we demonstrate that the *APC* rs518013 A and *LRRN3* rs80054135 T alleles are associated with the best reaction time in wrestlers and physically active subjects and over-represented in elite athletes involved in sports with reaction time as an essential component of performance.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

1. Maciejewska-Skrendo A, Sawczuk M, Cięszczyk P, Ahmetov II. Genes and Power Athlete Status. In: Barh D, Ahmetov I, editors. Sports, Exercise, and Nutritional Genomics: Current Status and Future Directions. Academic Press; 2019. p. 41–72.

2. Semenova EA, Fuku N, Ahmetov II. Genetic profile of elite endurance athletes. In: Barh D, Ahmetov I, editors. Sports, Exercise, and Nutritional Genomics: Current Status and Future Directions. Academic Press; 2019. p. 73–104.

3. Valeeva EV, Ahmetov II, Rees T. Psychogenetics and sport. In: Barh D, Ahmetov I, editors. Sports, Exercise, and Nutritional Genomics: Current Status and Future Directions. Academic Press; 2019. p. 147–165.

4. Mustafina LJ, Naumov VA, Cieszczyk P, et al. AGTR2 gene polymorphism is associated with muscle fibre composition, athletic status and aerobic performance. Exp Physiol. 2014; 99(8):1042–52.

5. Guilherme JPLF, Egorova ES, Semenova EA, et al. The A-allele of the FTO Gene rs9939609 Polymorphism Is Associated With Decreased Proportion of Slow Oxidative Muscle Fibers and Over-represented in Heavier Athletes. J Strength Cond Res. 2019; 33(3):691–700.

6. Semenova EA, Miyamoto-Mikami E, Akimov EB, et al. The association of HFE gene H63D polymorphism with endurance athlete status and aerobic capacity: novel findings and a meta-analysis. Eur J Appl Physiol. 2020; 120(3):665–673.

7. Kusić D, Connolly J, Kainulainen H, et al. Striated muscle-specific serine/threonine-protein kinase beta segregates with high versus low responsiveness to endurance exercise training. Physiol Genomics. 2020;52(1):35–46.

8. Ahmetov I, Kulemin N, Popov D, et al. Genome-wide association study identifies three novel genetic markers associated with elite endurance performance. Biol Sport. 2015;32(1):3–9.

9. Rankinen T, Fuku N, Wolfarth B, et al. No Evidence of a Common DNA Variant Profile Specific to World Class Endurance Athletes. PLoS One. 2016; 11(1):e0147330.

10. Pickering C, Suraci B, Semenova EA, et al. A genome-wide association study of sprint performance in elite youth football players. J Strength Cond Res. 2019; 33:2344–2351.

11. Al-Khelaifi F, Yousri NA, Diboun I, et al. Genome-wide association study reveals a novel association between MYBPC3 gene polymorphism, endurance athlete status, aerobic capacity and steroid metabolism. Front Genet. 2020. doi: 10.3389/fgene.2020.00595.

12. Ackland TR, Elliott BC, Bloomfield J. Applied anatomy and biomechanics in sport. Human Kinetics; 2009.

13. Gierczuk D, Bujak Z, Cieśliński I, et al. Response Time and Effectiveness in Elite Greco-Roman Wrestlers Under Simulated Fight Conditions. J Strength Cond Res. 2018;32(12):3433–3440.

14. Kuntsi J, Rogers H, Swinard G, et al. Reaction time, inhibition, working memory and 'delay aversion' performance: genetic influences and their interpretation. Psychol Med. 2006;36(11):1613–24.

15. Kikuchi N, Min SK, Ueda D, et al. Higher frequency of the ACTN3 R allele + ACE DD genotype in Japanese elite wrestlers. J Strength Cond Res. 2012; 26(12):3275–3280.

16. Gabbasov RT, Arkhipova AA, Borisova AV, et al. The HIF1A gene Pro582Ser polymorphism in Russian strength athletes. J Strength Cond Res. 2013; 27(8):2055–2058.

17. Kikuchi N, Ueda D, Min SK, Nakazato K, Igawa S. The ACTN3 XX genotype's underrepresentation in Japanese elite wrestlers. Int J Sports Physiol Perform. 2013;8(1):57–61.

18. Kikuchi N, Fuku N, Matsumoto R, et al. The Association Between MCT1 T1470A Polymorphism and Power-Oriented Athletic Performance. Int J Sports Med. 2017;38(1):76–80.

19. Boulygina EA, Lukianova E, Grigoryeva TV, et al. Lessons from the Whole Exome Sequencing Effort in Populations of Russia and Tajikistan. BioNanoSci. 2016;6:540–542.

20. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics. 2010;26:589–95.

21. Kim S, Scheffler K, Halpern AL, et al. Strelka2: fast and accurate calling of germline and somatic variants. Nat Methods. 2018;15(8):591–594.

22. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.

23. Davies G, Marioni RE, Liewald DC. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N = 112 151). Mol Psychiatry. 2016; 21(6):758–67.

24. Davies G, Lam M, Harris SE, et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. Nat Commun. 2018;9(1):2098.

25. Wang J, Raskin L, Samuels DC, et al. Genome measures used for quality control are dependent on gene function and ancestry. Bioinformatics. 2015;31(3):318–23.

26. Nagasaki M, Yasuda J, Katsuoka F, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat Commun. 2015;6:8018.

27. Sidore C, Busonero F, Maschio A, et al. Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. Nat Genet. 2015;47(11):1272–1281.

28. Genome of the Netherlands Consortium. Whole-genome sequence variation, population structure and demographic history of the Dutch population. Nat Genet. 2014;46(8):818–25.

29. Triska P, Chekanov N, Stepanov V, et al. Between Lake Baikal and the Baltic Sea: genomic history of the gateway to Europe. BMC Genet. 2017;18(1):110.

30. GTEx Consortium. Genetic effects on gene expression across human tissues. Nature. 2017;550(7675):204–213.

31. Coyle YM, Xie XJ, Lewis CM, et al. Role of physical activity in modulating breast cancer risk as defined by APC and RASSF1A promoter hypermethylation in nonmalignant breast tissue. Cancer Epidemiol Biomarkers Prev. 2007; 16(2):192–6.

32. Katoh Y, Katoh M. Characterization of KIF7 gene in silico. Int J Oncol. 2004; 25(6):1881–6.

33. Kang J, Bai R, Liu K, et al. Identification of significantly different modules between permanent and deciduous teeth by network and pathway analyses. Genet Mol Res. 2016;15(4).

34. Yang J, Li F, Qiu L, et al. Role of LRRN3 in the cerebellum postnatal development in rats. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2011;36(5):424–9.

35. Telenti A, Pierce LC, Biggs WH, et al. Deep sequencing of 10,000 human genomes. Proc Natl Acad Sci U S A. 2016;113(42):11901–11906.

36. Stubbs A, McClellan EA, Horsman S, et al. Huvariome: a web server resource of whole genome next-generation sequencing allelic frequencies to aid in pathological candidate gene selection. J Clin Bioinforma. 2012;2(1):19.

37. Rustagi N, Zhou A, Watkins WS, et al. Extremely low-coverage whole genome sequencing in South Asians captures population genomics information. BMC Genomics. 2017;18(1):396.

38. Tanisawa K, Wang G, Seto J et al. Sport and exercise genomics: the FIMS 2019 consensus statement update. Br J Sports Med. 2020 Mar 22. doi: 10.1136/bjsports-2019–101532.

SUPPLEMENTARY TABLE 1. Variants localization

Sample #	Exonic	Splicing	ncRNA	UTR5	UTR3	Intronic	Upstream	Downstream	Intergenic
1	7322	46	82943	1512	9541	423608	6817	8045	685167
2	7956	61	93985	1575	10745	485026	7518	8950	793325
3	6251	44	81324	1163	9318	416904	6021	7510	689536
4	7220	51	83069	1487	9786	430918	7000	8218	696527
5	6551	47	82924	1265	9526	433249	6493	7910	715721
6	8466	63	105903	1723	11928	546717	8271	9833	899139
7	7674	49	97758	1471	10934	503454	7439	9229	831484
8	8388	57	102768	1619	11666	532930	7998	9781	877571
9	23368	216	284400	6596	31451	1453515	26561	28486	2408633
10	23550	197	284216	6571	31695	1465213	26999	28594	2418169
11	19231	159	249751	5598	27122	1298338	22436	24082	2103214
12	24072	192	285008	6671	32160	1482433	27346	29089	2438070
13	22444	191	281279	6460	30682	1422247	25857	27787	2368005
14	22666	186	279610	6364	31345	1435265	26285	27783	2364281
15	23366	201	287954	6652	31917	1473141	26928	28946	2417142
16	23674	205	290871	6556	31944	1472548	27057	28951	2432393
17	22957	205	280664	6477	31228	1440480	26498	28157	2380668
18	23328	201	282964	6592	31685	1460742	26779	28494	2411895
19	23345	215	284624	6676	32164	1473218	27066	29128	2448248
20	24045	206	288257	6851	32382	1493547	27650	29073	2459687

SUPPLEMENTARY TABLE 2. Variants effect

Sample #	Frameshift insertion	Frameshift deletion	Stopgain	Stoploss	Non-frameshift insertion	Non-frameshift deletion	Non-synonymous SNV	Synonymous SNV	Unknown
1	30	32	29	7	19	16	3460	3542	195
2	35	35	43	12	20	31	3724	3857	214
3	22	23	35	5	24	18	2943	3009	176
4	30	28	35	3	18	23	3394	3488	221
5	17	24	21	6	16	24	3020	3228	208
6	38	37	38	7	30	30	4008	4049	240
7	21	29	33	4	24	24	3673	3665	213
8	26	41	48	4	32	25	3914	4067	242
9	127	159	133	15	169	176	10780	11137	730
10	128	162	128	14	151	168	10872	11248	725
11	96	150	101	12	112	140	8906	9112	647
12	121	155	129	16	163	185	11110	11519	730
13	127	164	108	19	143	164	10350	10766	662
14	116	153	118	16	138	171	10448	10900	663
15	118	161	126	12	145	167	10856	11135	701
16	127	150	118	14	167	171	10957	11342	685
17	132	152	116	15	132	164	10517	11054	726
18	114	143	127	16	140	182	10780	11150	732
19	114	144	117	16	159	165	10803	11116	766
20	134	196	129	13	145	193	11072	11493	726

SUPPLEMENTARY TABLE 3. Variants clinical relevance

Sample #	Benign	Likely benign	Pathogenic	Likely pathogenic	Uncertain significance	Drug response	Other	Not provided
1	2339	506	17	0	37	28	92	26
2	2463	560	17	3	47	26	117	40
3	1998	490	13	2	48	19	77	28
4	2254	490	15	2	46	18	94	34
5	2179	466	14	2	37	24	104	33
6	2671	633	26	4	61	34	148	44
7	2391	541	16	4	52	22	97	37
8	2591	603	18	5	58	28	119	42
9	7003	1873	57	6	217	73	339	118
10	6453	1736	48	10	212	71	301	125
11	5834	1566	41	6	194	53	311	67
12	6960	1834	55	16	217	69	382	134
13	6541	1664	50	8	240	61	314	125
14	6792	1738	44	10	215	62	350	120
15	6767	1804	54	13	219	63	373	125
16	6768	1760	55	7	255	62	256	119
17	6857	1827	54	14	214	63	318	116
18	6786	1742	45	15	214	59	344	133
19	6915	1812	49	10	242	66	344	150
20	7030	1838	60	11	236	72	373	135

SUPPLEMENTARY TABLE 4. The average of basic statistics

Statistics	20 genomes	12 most covered genomes
Raw reads number	276821878.4	361811385.5
Mapped reads number	276547024.8	361648606.6
Mapped reads,%	99.85	99.95
Mean coverage	9.9	14.6
Ts/Tv ratio	2.0	2.0
Raw SNPs number	2811729.1	3818427.4
Raw indels number	427651.8	636621.6
Filtered SNPs number	2674789.4	3617853.7
Filtered indels number	414847.5	616797.3

SUPPLEMENTARY TABLE 5. Variants with localization (N = 61763469)

Localization	20 genomes average	12 most covered genomes average	% based on 20 genomes
Intergenic	1741943.8	2387533.8	56.4
Intronic	1057174.7	1447557.3	34.2
ncRNA	205513.6	281633.2	6.7
UTR3	22961.0	31314.6	0.7
Downstream	20402.3	28214.2	0.7
Upstream	18751.0	26455.2	0.6
Exonic	16793.7	23003.8	0.5
UTR5	4494.0	6505.3	0.1
Splicing	139.6	197.8	0.005

SUPPLEMENTARY TABLE 6. Variants with predicted effect (n = 336619)

Type of mutation	20 genomes average	12 most covered genomes average	% based on 20 genomes
Synonymous SNV	8043.9	10997.7	47.8
Non-synonymous SNV	7779.4	10620.9	46.2
Unknown	510.1	707.8	3.0
Non-frameshift deletion	111.9	170.5	0.7
Frameshift deletion	106.9	157.4	0.6
Non-frameshift insertion	97.4	147.0	0.6
Stopgain	86.6	120.8	0.5
Frameshift insertion	83.7	121.2	0.5
Stoploss	11.3	14.8	0.1

SUPPLEMENTARY TABLE 7. Variants with clinical significance (n = 136609)

Clinical effect	20 genomes average	12 most covered genomes average	% based on 20 genomes
Benign	4979.6	6725.5	72.9
Likely benign	1274.2	1766.2	18.7
Other	242.7	333.8	3.6
Uncertain significance	153.1	222.9	2.2
Not provided	87.6	122.3	1.3
Drug response	48.7	64.5	0.7
Pathogenic	37.4	51.0	0.5
Likely pathogenic	7.4	10.5	0.1

SUPPLEMENTARY TABLE 8. Frequencies of the favourable alleles in athletes and controls

Groups	n	APC rs518013 A %	Elite vs non-elite	Elite vs controls	KIF27 rs10125715 A %	Elite vs non-elite	Elite vs controls	TMEM229A rs7783359 T %	Elite vs non-elite	Elite vs controls	LRRN3 rs80054135 T %	Elite vs non-elite	Elite vs controls
Elite boxers	41	54.9	0.107	0.172	69.5	0.958	0.991	70.7	0.184	0.477	5.0	0.346	0.340
Sub-elite boxers	60	43.3	-	-	69.2	-	-	61.7	-	-	2.5	-	-
Elite wrestlers	34	55.9	0.205	0.157	69.1	0.813	0.939	57.6	0.718	0.152	5.9	0.202	0.211
Sub-elite wrestlers	48	45.8	-	-	70.8	-	-	60.4	-	-	2.1	-	-
Elite karate athletes	5	40.0	0.834	0.681	80.0	0.899	0.478	60.0	0.359	0.659	20.0	0.071	0.0026*
Sub-elite karate athletes	16	43.8	-	-	78.1	-	-	75.0	-	-	3.1	-	-
Elite taekwondo athletes	5	30.0	0.400	0.299	50.0	0.278	0.186	70.0	0.816	0.825	30.0	0.0053*	0.0039*
Sub-elite taekwondo athletes	19	44.7	-	-	68.4	-	-	73.7	-	-	2.6	-	-
Elite volleyball players	17	50.0	0.667	0.723	82.4	0.315	0.117	64.7	0.478	0.816	6.3	0.862	0.301
Sub-elite volleyball players	28	44.6	-	-	71.4	-	-	57.1	-	-	5.4	-	-
Elite table tennis players	5	70.0	0.074	0.143	80.0	0.159	0.478	90.0	0.531	0.121	10.0	0.305	0.201
Sub-elite table tennis players	5	30.0	-	-	50.0	-	-	80.0	-	-	0	-	-
Elite athletes	107	53.3	0.033*	0.117	71.5	0.737	0.624	65.4	0.564	0.877	7.5	0.009*	0.009*
Sub-elite athletes	176	44.0	-	-	70.2	-	-	63.6	-	-	2.8	-	-
Russian controls	189	46.6	-	-	69.6	-	-	66.7	-	-	2.9	-	-

*P< 0.05, statistically significant differences

SUPPLEMENTARY TABLE 9. Frequencies of the favourable alleles in different populations

Groups	n	APC rs518013 A	KIF27 rs10125715 A	TMEM229A rs7783359 T	LRRN3 rs80054135 T
Tatar wrestlers	20	55.0	65.0	75.0	10.0
Elite Russian athletes	107	53.3	71.5	65.4	7.5
Russian population	189	46.6	69.6	66.7	2.9
African (1000 Genomes)	661	7.7	52.1	63.6	9.8
Admixed American (1000 Genomes)	347	60.7	77.8	59.1	3.5
East Asian (1000 Genomes)	504	68.6	71.2	74.9	25.9
European (1000 Genomes)	503	47.2	71.3	66.4	4.8
South Asian (1000 Genomes)	489	54.2	62.7	72.3	13.1