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Genetic markers of predisposition to athletic performance in Kazakhstani combat athletes: Results of a GWAS study

Madina B. Baurzhan[1,2*], Aibol T. Kulbayev[1], Alina V. Ten[1], Leyla B. Djansugurova[3], Nazym K. Altynova[4]

[1]*Academy of Physical Education and Mass Sports, Astana, Kazakhstan.*
[2]*Research Institute of Balneology and Medical Rehabilitation, Ministry of Health, Astana, Kazakhstan.*
[3]*Institute of Genetics and Physiology, Al-Farabi Kazakh National University, Almaty, Kazakhstan.*
[4]*Institute of Genetics and Physiology, Almaty, Kazakhstan.*
Corresponding author: Madina B. Baurzhan (*Email: madina_baurzhan@mail.ru*)

Abstract

This study investigated genetic markers associated with athletic performance in Kazakhstani combat sport athletes and assessed their potential role in developing a genetic passport for personalized training. A total of 235 elite wrestlers and boxers and 172 controls were enrolled. DNA was extracted from blood and genotyped using the Illumina iScan platform (654,027 SNPs). Genome-wide association analysis was performed in PLINK, and significant variants were annotated using genomic databases. No genome-wide significant associations ($p < 5 \times 10^{-8}$) were observed, but several SNPs reached suggestive significance ($p < 5 \times 10^{-6}$). Variants in CDH13, LIMD1, and ZNF215 were most notable. The CDH13 rs79764367 G allele was enriched in wrestlers, while the A allele was associated with boxing. LIMD1 rs77194734 showed allele-specific differences between wrestling and boxing. ZNF215 rs76704889 was linked to elite-level traits in both groups. This pilot GWAS provides the first evidence of candidate genetic markers in Kazakhstani combat athletes, highlighting allele-specific differences across sports disciplines.

Keywords: CDH13, Combat sports, GWAS, LIMD1, SNP, ZNF215.

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Institutional Review Board Statement: The study was approved by the Local Ethics Committee of the Institute of Genetics and Physiology (Protocol No. 5, July 25, 2022) and the Local Ethics Committee of the Academy of Physical Education and Mass Sports (Protocol No. 1, November 8, 2022). All participants provided written informed consent.
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1. Introduction

Athletic performance is a complex phenotype determined by the interplay of genetic, environmental, and psychosocial factors. Numerous studies have demonstrated that genetic variation significantly contributes to interindividual differences in physical capabilities, accounting for an estimated 50–80% of variability in traits such as endurance, strength, and power [1-3]. In contrast, the contribution of training regimens, nutrition, and psychological preparation is considered to explain 20–50% of performance outcomes [4].

In the past two decades, the application of molecular genetics has advanced the understanding of sports performance. More than 200 single nucleotide polymorphisms (SNPs) have been reported in genome-wide association studies (GWAS) as being linked to endurance, strength, and exercise recovery [5-7]. At least 20 markers have been proposed to distinguish elite-level athletes from non-athletes, including variants in ACTN3, ACE, PPARGC1A, and IL6 [8, 9]. These discoveries have led to the concept of a "genetic passport" for athletes, which could support talent identification, optimize training load, and guide recovery strategies.

However, despite significant progress, most of the existing data have been derived from European, East Asian, and North American populations [10-12]. Data on Central Asian athletes, including those from Kazakhstan, are virtually absent from the literature. This gap is particularly relevant given that genetic profiles vary considerably between populations, and allele frequencies may influence both the strength and direction of associations with performance traits [13, 14].

Kazakhstan has a strong tradition in combat sports such as Kazakh wrestling, Greco-Roman and freestyle wrestling, judo, sambo, karate, taekwondo, combat jiu-jitsu, and boxing. Athletes from these disciplines consistently achieve success at international competitions, making them an ideal cohort for investigating genetic determinants of performance. Understanding the genetic predisposition of Kazakhstani athletes may not only support personalized sports medicine but also strengthen the country's position in global sports science research.

The present study aimed to perform a GWAS analysis of combat sport athletes from Kazakhstan in order to: (i) identify SNPs associated with athletic qualities; (ii) assess their potential contribution to combat sport-specific traits; and (iii) explore their applicability for the development of a genetic passport.

2. Materials and Methods

The study involved EDTA-treated peripheral blood samples from 235 qualified athletes involved in martial arts (masters of sports of the Republic of Kazakhstan and candidates for master of sports of the Republic of Kazakhstan), who are winners and prize winners of the championships of the Republic of Kazakhstan and Asian championships. The control group consisted of 172 volunteers without any sports training.

All participants provided written informed consent prior to inclusion. The study protocol was approved by the Local Ethics Committee of the Institute of Genetics and Physiology, Ministry of Education and Science of the Republic of Kazakhstan (Protocol No. 5, 25 July 2022).

2.1. DNA Extraction

DNA was extracted from frozen (–20 °C) EDTA-treated peripheral blood samples using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, USA) and the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA), according to the manufacturers' protocols. DNA samples were stored at –20 °C. The quantity and quality of the isolated DNA were assessed spectrophotometrically (NanoDrop 2000 and NanoDrop One, Thermo Scientific, USA), fluorometrically (Quantus, Promega, USA; Qubit Fluorometric Quantification, Thermo Scientific, USA), and by electrophoresis in a 0.8% agarose gel. DNA samples with a purity ratio of 1.75–1.80 were used for further analysis. Samples contaminated with RNA (ratio 1.8–2.0) or proteins (ratio 1.5–1.7) were additionally washed and reprecipitated with ethanol until the desired DNA purity was achieved.

2.2. Genome-Wide SNP Microarray Genotyping

For genome-wide microarray genotyping (654,027 single nucleotide polymorphisms, SNPs, across the whole genome), DNA samples with a concentration of at least 50 ng/μL and a purity ratio of 1.75–1.80 were used. DNA sample preparation was performed using the Infinium Automation Kit – 8 Tip Tecan Non LIMS (Illumina, USA). Genotyping of the study cohorts was conducted on the iScan System platform (Illumina, USA) using the Infinium Global Screening Array-24 Kit (Illumina, USA), according to the Infinium HTS Automated Workflow protocol.

2.3. Processing of Genome-Wide SNP Genotyping Data

Raw microarray genotyping data were processed using Illumina GenomeStudio v.2010.3 (Illumina, USA), PLINK 1.09, and RStudio (R package *minfi*). Samples with a call rate below 98% (percentage of successfully genotyped SNPs) were excluded from further analysis.

The bioinformatics workflow included:

- Evaluation of data reliability ($p < 0.05$),
- Chromosomal (autosomal, sex chromosomes) or mitochondrial SNP localization,
- Determination of genetic status (homozygosity/heterozygosity at polymorphic loci).

Case–control genome-wide association studies (GWAS) were conducted using PLINK v1.9. Quality control filters excluded samples with >10% missing genotypes (--mind 0.1). Mitochondrial and XY chromosome SNPs (--not-chr x y mt) with call rate <95% (--geno 0.05), minor allele frequency <0.05 (--maf 0.05), or deviation from Hardy–Weinberg equilibrium ($P < 10^{-3}$, --hwe 0.001) were removed. After filtering, 296,772 SNPs were available for the "Wrestling" cohort and 295,873 SNPs for the "Boxing" cohort. Association analysis was performed using the --assoc option.

Manhattan and QQ plots were generated in R v4.2.2 using the *qqman* package [15] while annotation of significant SNPs was performed with the *BioMart* package [16, 17].

2.4. Functional Annotation and Gene Interaction Analysis

Annotation and interpretation of genetic variants obtained with the iScan system were performed using the Genome-Wide Association Studies Catalog (GWAS Catalog, https://www.ebi.ac.uk/gwas/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), and the 1000 Genomes Project database (https://www.internationalgenome.org/1000-genomes-browsers).

Potential interactions between SNPs associated with athletic performance and their corresponding genes were explored using GeneMANIA (http://genemania.org). Protein–protein interactions were analyzed using STRING (http://string-db.org).

3. Results

After filtering according to the exclusion criteria (missing genotype rate >10%, call rate <95%, minor allele frequency <0.05%, and deviation from Hardy–Weinberg equilibrium, $P < 10^{-3}$), 296,772 SNPs were retained for the *Wrestling* cohort. The Manhattan plot for the *Wrestling–Control* analysis is presented in Figure 1.

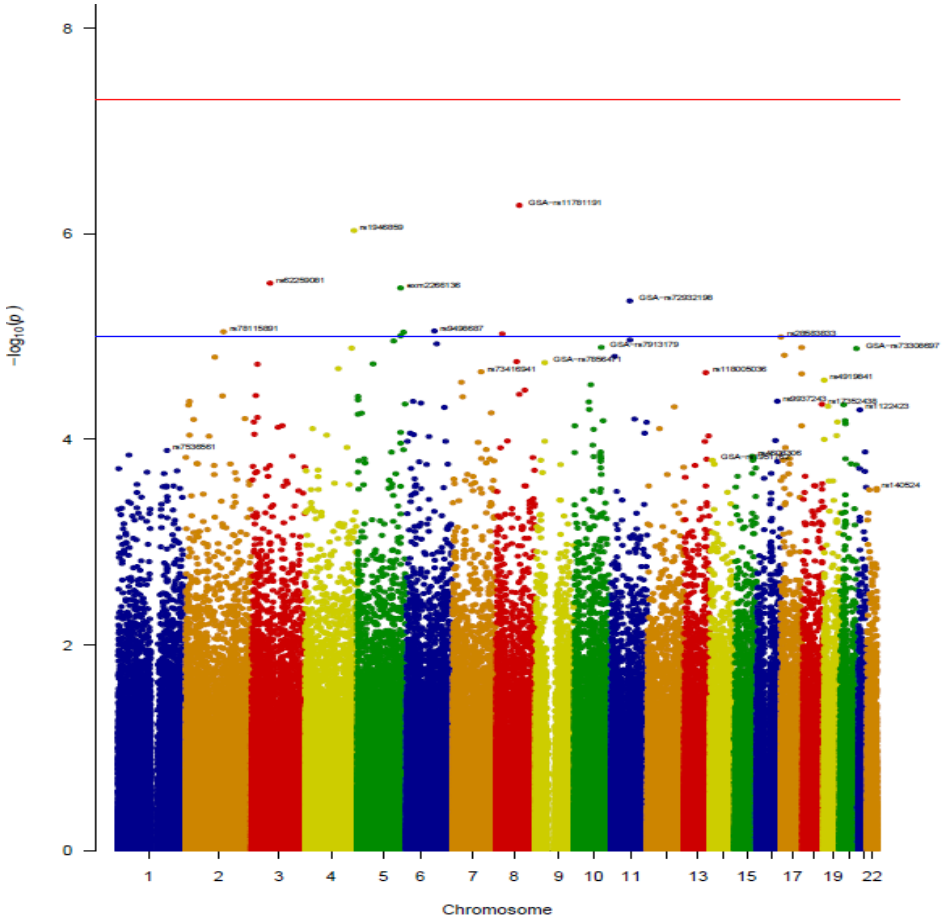

Figure 1.
Manhattan plot of SNP associations with athletic performance in the Fight–Control cohort.

Table 1 summarizes the annotation of SNPs showing highly significant associations (–log10P < 10^{-5}) with athletic performance traits in wrestlers.

Table 1.
Annotation of SNPs with significant associations with athletic performance traits in wrestlers.

SNP	Chromosome	Alleles	Associated Gene	Gene Localization	Variant Type
rs11781191	8	C/A	-	-	-
rs12546622	8	A/G/T	POLR3D	upstream	upstream transcript variant (2kb)
rs1473247	5	T/C	RNF145, IL12B	RNF145 (intron)	intron variant
rs1946859	4	T/A/C/G	LOC105377557	intron	intron and upstream transcript variant
rs62259081	3	G/A	-	-	-
rs72932198	11	G/A	LOC105369370	intron	intron variant
rs7297848	12	C/T	FBXW8	intron	intron variant
rs78115891	2	C/T	GRIK2	intron	intron variant
rs9498687	6	G/A/T	GRIK2	intron	intron variant
rs978914	5	G/A/C/T	DOCK2, INSYN2B	3′ UTR, intron	3′ UTR/intron variant, downstream transcript variant

As shown in Table 1, all associated SNPs represent intronic and transcript variants, potentially exerting regulatory effects on gene expression. Subsequently, potential gene–gene interactions and protein–protein interactions were analyzed (Figure 2).

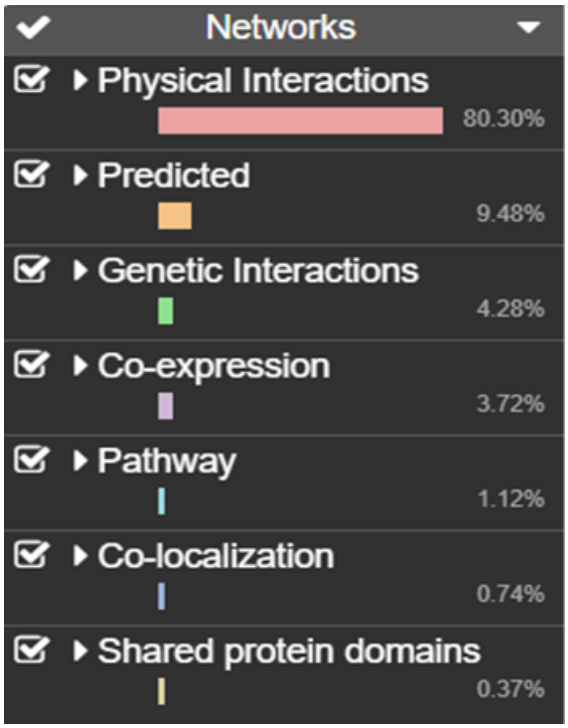

Figure 2.
Gene interaction network for wrestling-associated SNPs.

As shown in Figure 2, genes harboring SNPs associated with wrestling displayed mainly potential physical interactions (80.3%), while the levels of genetic interactions (4.3%), co-expression (3.7%), and shared biochemical pathways (1.1%) were negligible. Protein interaction analysis further confirmed the absence of direct protein–protein interactions (Figure 3).

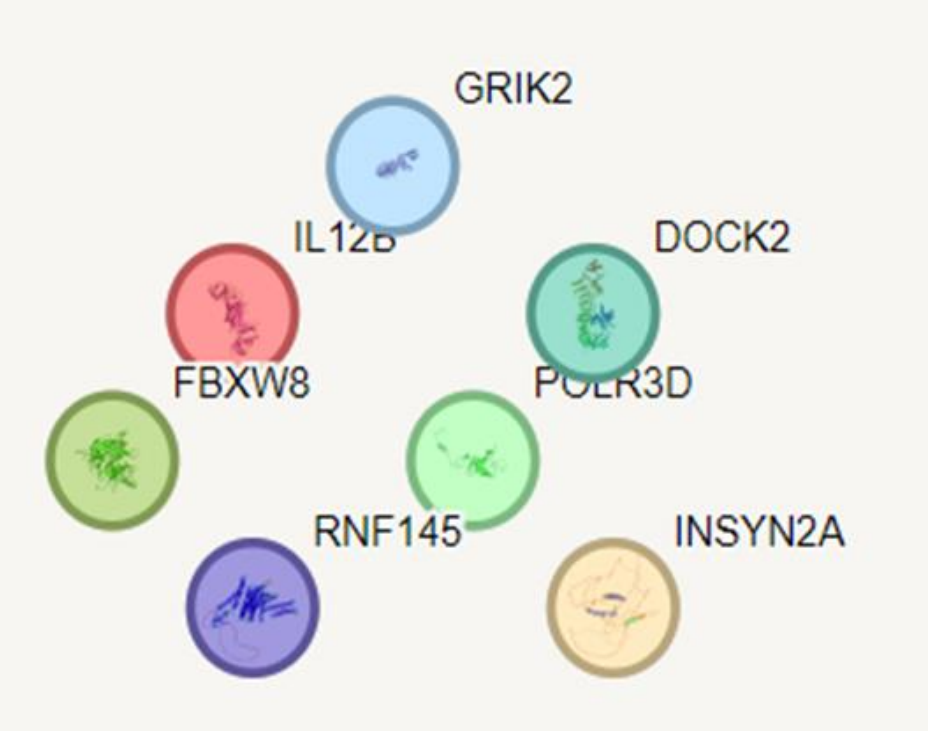

Figure 3.
Absence of protein–protein interactions among proteins encoded by wrestling-associated gene polymorphisms.

Thus, no genetic associations with physical performance traits were identified in the *Wrestling–Control* cohorts.

To minimize random effects related to sex and ethnicity, we constructed a unified male Kazakh cohort (*Combat Sports* group). After filtering genome-wide genotyping data in this updated cohort, 18,527 SNPs with high no-call rates were excluded. Additionally, 231,362 SNPs with MAF <3% and 15,652 SNPs deviating from Hardy–Weinberg equilibrium were removed. In total, 328,551 SNP markers were tested for association using the conventional threshold of $-\log10P < 10^{-5}$.

No SNPs reached the genome-wide significance threshold of $P < 5 \times 10^{-8}$ or the Bonferroni-corrected threshold of $P = 1.5 \times 10^{-7}$. The most significant SNPs reached a threshold of $P = 5 \times 10^{-6}$ ($-\log10P = 5.3$) on chromosomes 2, 3, 9, 11, 15, and 16. On chromosome 11, two SNPs in strong linkage disequilibrium ($R^2 = 1$, $D' = 1$, $p < 0.0001$) were located in distinct intronic regions. Other variants were in LD with neighboring markers and mapped to poorly characterized genomic regions (LOCs) or uncharacterized genes. These data are summarized in Table 2. None of the significant SNPs were listed in the ClinVar database or linked to disease.

Table 2.
Associations of SNP markers with athletic performance traits in male Kazakh combat sport athletes.

SNP	Chr	Position	Gene	Alleles	MAF (database)	MAF Wr/Sport	Effective Allele	OR (95% CI)	P-value
rs60914107	2	214053854	LOC107985981	C/T	0.03 (T)	0.03/0.14	T / C	0.2 (0.09–0.42) / 5.1 (2.37–11.03)	5.6×10^{-6}
rs77194734	3	45669879	LIMD1	C/T	0.10 (T)	0.08/0.23	T / C	0.3 (0.17–0.51) / 3.38 (1.95–5.84)	5.7×10^{-6}
rs2027007	9	82345399	–	G/A	0.20 (G)	0.22/0.43	G / A	0.37 (0.24–0.56) / 2.72 (1.77–4.16)	2.8×10^{-6}
rs78027951	11	6932335	LOC107984019	G/T	0.05 (T)	0.04/0.12	T / G	0.22 (0.11–0.44) / 4.64 (2.28–9.45)	4.9×10^{-6}
rs76704889	11	6950490	ZNF215	T/C	0.05 (C)	0.04/0.15	T / C	0.22 (0.11–0.44) / 4.64 (2.28–9.45)	4.9×10^{-6}
rs75908772	15	46075211	LOC105370802	G/A	0.07 (A)	0.01/0.09	G / A	9.60 (3.00–30.69) / 0.10 (0.03–0.33)	4.7×10^{-6}
rs79764367	16	83061956	CDH13	G/A	0.15 (A)	0.01/0.12	G / A	9.04 (3.42–23.84) / 0.11 (0.04–0.29)	1.5×10^{-6}

Despite the limited sample size, odds ratio (OR) values for several SNPs with $P < 1 \times 10^{-6}$ suggest these polymorphisms may serve as candidate genetic markers for combat sport athletes, encompassing both wrestling and striking abilities. Notably, three SNPs were located within intronic regions of the LIMD1, ZNF215, and CDH13 genes.

Interestingly, the SNP rs79764367 (CDH13) showed opposite allele associations between wrestlers and boxers (Kazakh males). The G allele was strongly associated with wrestling-related traits such as strength and endurance (OR = 9.04; $P = 1.5 \times 10^{-7}$), while the A allele was associated with elite boxing performance, characterized by fast reaction, striking power, and endurance.

CDH13 encodes a cadherin family protein involved in calcium-dependent cell adhesion and has been implicated in neuropsychiatric disorders, cardiac failure, and metabolic syndrome [18-23].

Similarly, the C allele of rs77194734 (LIMD1) was enriched in wrestlers (OR = 3.38; $P = 5.7 \times 10^{-6}$), while the T allele was associated with boxing performance. LIMD1 encodes a scaffold protein regulating multiple cellular pathways, including hypoxia signaling, cytoskeletal organization, Wnt signaling, and tumor suppression.

The SNP rs76704889 (ZNF215) showed significant associations in both wrestlers and boxers (OR = 4.64; $P = 4.9 \times 10^{-6}$). ZNF215 encodes a transcription factor with zinc-finger domains, localized in a chromosomal region (11p15) associated with Beckwith–Wiedemann syndrome.

4. Discussion

In this study, we performed a genome-wide association analysis (GWAS) of Kazakh athletes engaged in combat sports, including wrestling and boxing. Our main aim was to identify genetic variants that may contribute to athletic performance traits relevant to strength, endurance, reaction speed, and striking ability.

After rigorous quality control procedures and statistical filtering, we observed no SNPs that reached the classical GWAS genome-wide significance threshold ($P < 5 \times 10^{-8}$). Nevertheless, several variants reached a suggestive level of association ($P < 5 \times 10^{-6}$), providing potential candidate markers for further investigation.

Among the most notable findings were intronic variants in the CDH13, LIMD1, and ZNF215 genes.

CDH13 (rs79764367) encodes a calcium-dependent cell adhesion molecule and has been implicated in neural regulation, cardiac function, and metabolic processes [18-23]. Our results suggest that different alleles of this SNP may be associated with distinct performance phenotypes: The G allele was strongly associated with wrestling traits (strength and endurance), whereas the A allele showed associations with boxing-related traits (reaction time, striking ability, and stamina). This observation aligns with the functional pleiotropy of CDH13, linking it to both physical endurance and cognitive/neurological processes.

LIMD1 (rs77194734) encodes a scaffold protein involved in cytoskeletal organization, cellular adhesion, Wnt signaling, and tumor suppression. Prior studies have linked LIMD1 to cell migration and stress response pathways. In our study, the C allele was enriched in wrestlers, whereas the T allele was associated with boxing performance. This allele-dependent distribution suggests LIMD1 may influence athletic specialization, particularly between strength/endurance-based versus speed/coordination-based combat sports.

ZNF215 (rs76704889) encodes a zinc-finger transcription factor located within the 11p15 chromosomal region, a locus previously associated with growth regulation and developmental disorders such as Beckwith–Wiedemann syndrome. Our findings suggest potential associations of ZNF215 polymorphisms with both wrestling and boxing performance, although the functional relevance of this gene to athletic traits remains largely unexplored.

It is important to emphasize that all the variants identified in this study were intronic or regulatory in nature, rather than protein-coding. This is consistent with current GWAS literature, where the majority of trait-associated SNPs are located in non-coding regions, likely exerting their effects through modulation of gene expression, transcription factor binding, or epigenetic regulation [24, 25].

Another important observation from our results is the allele-specific divergence between wrestling and boxing athletes. While wrestling performance appears to be more strongly associated with variants conferring endurance and muscle strength, boxing athletes showed enrichment in alleles potentially linked to speed, coordination, and reaction-related traits. These differences may reflect underlying physiological requirements of the two disciplines, suggesting a genetic basis for sport-specific adaptation.

Despite these findings, some limitations must be acknowledged. First, the sample size of our study was relatively modest for GWAS, which may have limited the statistical power to detect associations at genome-wide significance levels. Second, although we focused on a relatively homogeneous Kazakh male athlete cohort, residual confounding due to population stratification cannot be entirely excluded. Finally, functional validation studies are required to confirm the biological roles of the candidate variants identified here.

Future research should aim to replicate these findings in larger, independent athlete cohorts and to integrate genetic data with transcriptomic, proteomic, and physiological profiling. Such multi-omics approaches will be essential to clarify the molecular pathways underlying sport-specific performance traits.

In conclusion, our GWAS of Kazakh combat sports athletes revealed several candidate SNPs within CDH13, LIMD1, and ZNF215 that may contribute to inter-individual differences in performance. While preliminary, these findings provide a basis for further genetic and functional studies in the context of sports genomics and may eventually contribute to the development of personalized training and talent identification strategies in combat sports.

5. Conclusions

At least three polymorphisms can be considered as novel candidate markers of physical traits in combat athletes. CDH13 rs79764367 showed allele-specific effects, with the G allele associated with wrestling and the A allele with boxing. LIMD1 rs77194734 demonstrated a similar pattern, where the C allele was enriched in wrestlers and the T allele in boxers. ZNF215 rs76704889 was linked to elite-level performance across both groups. These associations suggest a genetic contribution to sport-specific traits such as endurance, reaction speed, and muscular strength, providing a foundation for further studies in sports genomics.

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