

Perfil genómico de atletas e
ex-atletas de alto rendimento
desportivo

Hugo Henrique Silva



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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR



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Declaração de Honra

Declaro que a presente tese é de minha autoria e não foi utilizada previamente noutro curso ou unidade curricular desta, ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referência. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

Lista de Publicações

Ao abrigo do disposto do n.º 2, alínea a) do artigo 31.º do Decreto-Lei n.º 115/2013 de 7 de agosto, constam as seguintes publicações, por ordem cronológica, de acordo com a sequência metodológica dos capítulos da tese:

Artigos

- 1 Silva H-H, Silva M-RG, Cerqueira F, Tavares V, Medeiros R. **Genomic profile in association with sport-type, sex, ethnicity, psychological traits and sport injuries of elite athletes: review and future perspectives.** Journal of Sports Medicine and Physical Fitness. 2021, Mar 5, <https://doi.org/10.23736/S0022-4707.21.12020-1>
- 2 Silva H-H, Tavares V, Silva M-RG, Neto BV, Cerqueira F, Medeiros R. 2022. **FAAH rs324420 polymorphism is associated with performance in elite rink-hockey players.** Biology, 11(7), 1076; <https://doi.org/10.3390/biology11071076>
- 3 Silva H-H, Tavares V, Silva M-RG, Neto BV, Cerqueira F, Medeiros R. 2023. **Association of FAAH rs324420 (C385A) Polymorphism with Athletic High-level Performance in the Portuguese Volleyball Players.** Genes 14(6), 1164; <https://doi.org/10.3390/genes14061164>
- 4 Silva H-H, Tavares V, Neto BV, Cerqueira F, Medeiros R, Silva M-RG. **FAAH rs324420 Polymorphism: Biological Pathways, Impact on Elite Athletic Performance and Insights for Sport Medicine.** Genes. 2023; 14(10):1946. <https://doi.org/10.3390/genes14101946>

A presente tese integra resultados, que foram parcialmente publicados na forma de resumo e apresentados na forma de conferência a convite e de poster, em dois congressos internacionais, respetivamente:

Resumos

- 1 Silva H-H, Silva M-RG, Cerqueira F, Medeiros R. 2020. **Genes and elite gymnasts.** In Silva M-RG, Araújo C, Saavedra F, Barata P (Eds.). Livro de Resumos do 8º Congresso Nacional de Ginástica - 6º Congresso Internacional - Ginástica: O Futuro Hoje. Lisboa: Federação de Ginástica de Portugal/Instituto

Português do Desporto e da Juventude I.P. ISBN: 978-989-8650-60-3 (impresso), 978-989-8650-57-3 (eletrónico), p. 54.

Conferência a convite

Silva H-H, Silva M-RG, Cerqueira F, Medeiros R. 2020. Genes and elite gymnasts. 8th Gymnastics National Congress/6th International - Gymnastics: the future now. Lisbon: 01 a 08 December 2020.

- 2 Silva H-H, Tavares V, Silva M-RG, Neto BV, Cerqueira F, Medeiros R. 2023. **Differences in the vitamin D pathway-related genetic variant between elite rink-hockey players' sex, but no association with athletic performance.** Clinical Nutrition ESPEN; 58: 541-542. <https://doi.org/10.1016/j.clnesp.2023.09.344>

Poster

Silva H-H, Tavares V, Silva M-RG, Neto BV, Cerqueira F, Medeiros R. 2023. Differences in the vitamin D pathway-related genetic variant between elite rink-hockey players' sex, but no association with athletic performance. 45th ESPEN Congress on Clinical Nutrition & Metabolism, 11 - 14 September. Lyon, France.

Em cumprimento das disposições do referido Decreto-Lei e das recomendações da comissão de ética da Universidade do Porto, o candidato declara que efetuou trabalho experimental, recolha e tratamento dados, tendo elaborado os referidos artigos com a colaboração ativa dos respetivos coautores.

Índice de Figuras

As Figuras apresentam-se numeradas, de acordo com o capítulo em que estão inseridas.

- Figure 4.1.** Endocannabinoid system as regulator of stress response. Under normal circumstances (A), the endocannabinoid system suppresses the release of the neurotransmitter glutamate via AEA, modulating the synaptic function. However, acute stress (B) can trigger a mechanism mediated by CRH and its receptor CRHR1, which is known to increase FAAH activity in the basolateral amygdala. As a result, there is a decrease in AEA levels, which is no longer able to suppress glutamate release. Consequently, increased neural excitability in the basolateral amygdala promotes anxiety-like behavior (adapted by Lutz et al.) [41]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; AEA: N-arachidonylethanolamine; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; *FAAH*: fatty acid amide hydrolase; NMDAR: NMDA receptor; LMO4: LIM domain only 4; CRH: corticotropin-releasing hormone; CRHR1: corticotropin-releasing hormone receptor 1. 83
- Figure 5.1.** The central role of the endocannabinoid system in regulating the stress response of high-level performance volleyball players. In normal conditions, the endocannabinoid system represses the release of the neurotransmitter glutamate via N-arachidonylethanolamine (AEA), modulating the synaptic function. Nevertheless, under acute stress, the mechanism mediated by corticotropin-releasing hormone (CRH) and its receptor (corticotropin-releasing hormone receptor 1-CRHR1) can be triggered and increase the fatty acid amide hydrolase (FAAH) activity in the basolateral amygdala. Consequently, AEA levels decrease, which are no longer able to restrain glutamate release. As a result, increased neural excitability in the basolateral amygdala enhances anxiety-like behavior (adapted by Silva et al.) [3]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; NMDAR: NMDA receptor; LMO4: LIM domain only 4. 104
- Figure 6.1.** Scientific publications selected in PubMed reporting associated ($n = 54$) and not associated ($n = 6$) traits linked to psychobiological pathways of *fatty acid amide hydrolase* (*FAAH*) rs324420. Genetic variants of this polymorphism have been associated with the increased (↑) or decreased (↓) risk of experiencing various pathologies, disorders and/or behaviours, such as: (1) suffering acute respiratory distress syndrome [15]; (2) consuming alcohol and/or smoking [11,16–21]; (3) experiencing anxiety and/or stress [22–25]; (4) achieving better athletic performances [6,26–28]; (5) suffering substance dependences in adults, including cannabis [12,29–33], cocaine [34], marijuana [35–38] and methamphetamine use [39]; (6) practicing a healthier dietary intake and/or body composition [40–43]; (7) suffering neurological disorders, such as epilepsy [26]; (8) dealing better with fear extinction [44–48]; (9) suffering irritable bowel syndrome [13,49,50]; (10) being obese [51–53]; (11) experiencing opioid-related effects in children [54–56]; (12) suffering myocardial infarction [57]; (13) feeling less pain [58–60]; (14) having personality disorders [10,42]; and (15) feeling satiation more easily [61]. On the other hand, a few studies have not found associations between *FAAH* rs324420 variants and body composition in children and adolescents [62], cannabis dependence [29], functional dyspepsia [63], insulin response [64], irritable bowel syndrome [65] and obesity [66]. 114
- Figure 6.2.** Effects of the endocannabinoid system in central nervous and peripheral systems (adapted from Wang, Dey and Maccarrone) [74]. The activation of several molecular targets by the endocannabinoid anandamide or the 2-arachidonoidglycerol results in a variety of biological actions, affecting practically all central and peripheral systems in animals, as shown. 115
- Figure 6.3.** Geographic distribution of allelic frequencies of the *fatty acid amide hydrolase* (*FAAH*) rs324420 [90]. * Minor allele. 117
- Figure 6.4.** The endocannabinoid system plays a key role in controlling how highly skilled athletes react to stress. Under normal circumstances, the endocannabinoid system modulates synaptic 120

function by inhibiting the release of the neurotransmitter glutamate via *N*-arachidonylethanolamine (AEA). However, under severe stress, the corticotropin-releasing hormone (CRH) and its receptor (corticotropin-releasing hormone receptor 1-CRHR1) can be activated, increasing (↑) the basolateral amygdala's fatty acid amide hydrolase (*FAAH*) activity. As a result, AEA levels drop (↓) and lose their ability to control glutamate release. Consequently, enhanced anxiety-like behaviour is caused by increased neuronal excitability in the basolateral amygdala (adapted by Silva et al.) [6]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; NMDAR: NMDA receptor; LMO4: LIM domain only 4.

Índice de Tabelas

As Tabelas apresentam-se numeradas, de acordo com o capítulo em que estão inseridas.

Table 3.1. Factors influencing the elite athlete potential/performance.	39
Table 3.2. Key projects/studies in genomics, genetics, and exercise biology (adapted from Georgiades et al. ⁹ and Bouchard & Malina ³⁹). ^{29,37,41-48}	43
Table 3.3. Genetic and epigenetic factors for endurance. ^{3,7,13,47,51-59}	44
Table 3.4. Genetic and epigenetic factors for power/strength. ^{7,25,50,55,60-72}	45
Table 3.5. Gene variants for sex and ethnicity and psychological traits. ^{25,80-84}	54
Table 3.6. Gene variants for sports injuries. ⁸⁶⁻⁹⁴	57
Table 4.1. Elite rink-hockey players' characteristics (n = 116, 18 females and 98 males).	75
Table 4.2. Selected polymorphisms' description and TaqMan® assays used.	78
Table 4.3. Genotype frequencies of the selected genetic polymorphisms in elite rink-hockey players (n= 116, 18 females and 98 males).	79
Table 4.4. Multivariate analysis using binomial regression on the athletic performance considering FAAH rs324420 and relevant factors.	80
Table 5.1. Demographic, sports, and training characteristics of high-level performance volleyball players by sex (n = 228; 66 females and 162 males).	97
Table 5.2. Description of the evaluated genetic polymorphisms and the TaqMan® assays used.	98
Table 5.3. Genotype distribution of the evaluated genetic variants among high-level performance volleyball players (n = 219; 60 females and 159 males).	100
Table 5.4. Multivariate analysis on the elite volleyball players' achievements (n = 219, 59 super athletes and 160 others) using binomial regression.	101
Table 6.1. Distribution of <i>FAAH</i> rs324420 genotypes among research studies involving elite athletes.	119

Resumo

O desempenho do atleta de alto rendimento resulta da interação entre variantes genéticas raras e fatores ambientais e comportamentais resultantes da especificidade do desporto praticado. Nos últimos anos, algumas centenas de polimorfismos têm sido estudadas e associadas ao desempenho físico, nomeadamente à capacidade de resistência e de potência dos atletas. Contudo, no que concerne à capacidade mental dos atletas de elite, nomeadamente à disciplina mental e resiliência, que o desporto deste nível competitivo exige, pouco se tem investigado.

O objetivo geral desta tese foi caracterizar o perfil genómico de atletas e ex-atletas de elite de hóquei em patins e de jogadores de voleibol de alto rendimento. Assim, estabeleceram-se os seguintes objetivos específicos: i) analisar a literatura científica que relacionasse as variantes genéticas com o desempenho desportivo de atletas de alto rendimento; ii) estudar o perfil genómico de jogadoras e jogadores de hóquei em patins de elite, as suas características físicas e antropométricas, e determinar eventuais variantes genéticas e outras associadas com o desempenho desportivo desses atletas; iii) identificar o perfil genómico de voleibolistas de alto rendimento, de ambos os sexos, as suas características físicas e antropométricas, e identificar eventuais variantes genéticas e outras associadas com o desempenho desportivo desses atletas; e iv) adicionalmente, e de acordo com os resultados do polimorfismo identificado nos dois tipos de atletas anteriores, caracterizar esse polimorfismo no contexto do desempenho desportivo e refletir sobre os eventuais contributos futuros do seu estudo e implicações no desempenho, na saúde e bem-estar, e na medicina desportiva.

Para responder ao primeiro objetivo específico, procedeu-se à revisão da literatura sobre o tema e construiu-se um primeiro artigo científico, que relaciona os genes associados ao desempenho desportivo nos desportos de resistência, de potência e em ambos, tendo-se ainda identificado diferenças genéticas entre os atletas, de acordo com o seu sexo, etnia, traços psicológicos e lesões desportivas. Para se atingir o segundo objetivo, foram avaliados 116 jogadores de hóquei em patins ($28,2 \pm 8,7$ anos; $24,0 \pm 2,2$ kg/m²; $23,3 \pm 8,8$ anos de experiência desportiva; $13,8 \pm 6,3$ h/semanais de treino; 15,5% de atletas do sexo feminino), representando cumulativamente mais de 85% das quatro melhores seleções mundiais e das cinco melhores equipas portuguesas no Campeonato Nacional de Hóquei em Patins em Portugal. Para se responder ao terceiro objetivo, foram estudados 228 voleibolistas ($26,7 \pm 8,1$ anos; $23,4 \pm 2,1$ kg/m²; $16,3 \pm 8,4$ anos de experiência desportiva; $22,0 \pm 10,5$ h/semanais de treino; 28,9% de atletas do sexo feminino), participantes no campeonato português e com múltiplos medalhados em competições nacionais e internacionais. Nos dois grupos de atletas foram recolhidos os seguintes dados: antropométricos, condições de treino, experiência desportiva e histórico de lesões

desportivas. Foram ainda analisados sete polimorfismos genéticos, tendo a genotipagem do polimorfismo sido efetuada com recurso à Metodologia de Discriminação Alélica TaqMan®. Dado o impacto biológico do polimorfismo encontrado anteriormente nos dois grupos de atletas, redigiu-se um artigo de revisão narrativa acerca desse impacto e refletiu-se sobre as perspetivas futuras na saúde, bem-estar e desempenho desportivo.

Com base nos dois estudos originais, verificou-se que os jogadores de hóquei em patins demonstraram características significativamente diferentes, de acordo com o sexo, ao nível das suas características antropométricas, hábitos de treino, lesões desportivas e variantes genéticas, como o *Receptor de Vitamina D* (VDR) rs731236 ($p < 0,05$). Por seu lado, os voleibolistas apresentaram diferenças significativas, em função do sexo, apenas nos indicadores antropométricos avaliados e nos hábitos de treino ($p < 0,05$). Em resposta ao objetivo principal deste estudo, o perfil genómico dos jogadores de hóquei em patins de elite, caracterizou-se pela presença do alelo rs324420 A da *Hidrólase Amida de Ácidos Gordos* (FAAH) associado à melhoria do desempenho desportivo (AA/AC vs. CC, OR = 2,80; 95% CI, 1,23-6,35; $p = 0,014$; $p = 0,008$ após *Bootstrap*), confirmado na análise multivariada (OR ajustado = 2,88; CI 95%, 1,06-7,80; $p = 0,038$). Também nos jogadores de voleibol de alto rendimento, o alelo A da variante genética *FAAH* rs324420 esteve associado a desempenhos desportivos superiores no modelo genético dominante (AA/AC vs. CC, OR = 1,70; 95% CI, 0,93–3,13; $p = 0,026$; $p < 0,001$ após *Bootstrap*), o que foi corroborado pela análise multivariada (AA/AC vs. CC OR ajustado = 2,00; 95% CI, 1,04-3,82; $p = 0,037$).

Os nossos resultados confirmaram a expressão do *FAAH* no desempenho desportivo de alto rendimento e de elite. Trata-se da primeira descrição deste gene, especificamente do polimorfismo rs324420 em jogadores de elite de hóquei em patins e em voleibolistas de alto rendimento, abrindo caminho para a sua identificação noutros desportos. Por outro lado, o nosso trabalho abre também a porta ao desenvolvimento de estratégias técnicas, táticas e terapêuticas, que considerem a disciplina mental no desporto de alto rendimento e de elite, para contornarem situações de stress, resistência à dor e à inflamação.

Abstract

The performance of a high-performance athlete results from the interaction between rare genetic variants and environmental and behavioral factors, resulting from the specificity of the sport practiced. In recent years, a few hundred polymorphisms have been studied and associated with physical performance, namely the endurance and power capacity of athletes. However, regarding the mental capacity of elite athletes, namely mental discipline and resilience, which sport at this competitive level requires, little has been investigated.

The general objective of this thesis was to characterize the genomic profile of elite rink-hockey players and former athletes and high-performance volleyball players. Therefore, the following specific objectives were established: i) to analyse the scientific literature that relates genetic variants with sport performance of high-performance athletes; ii) to study the genomic profile of elite rink-hockey players, their physical and anthropometric characteristics, and determine possible genetic and other variants associated with these athletes' sport performance; iii) identify the genomic profile of high-performance volleyball players of both sexes, their physical and anthropometric characteristics, and identify possible genetic and other variants associated with these athletes' sport performance; and iv) additionally, and according to results of the polymorphism identified in two previous athletes' groups, characterize this polymorphism in the sports performance context and reflect on future possible contributions of its study and implications for performance, health and well-being, and in sports medicine.

To respond to the first specific objective, the literature on the subject was reviewed and a first scientific article was written, highlighting genes associated with sport performance in endurance and power sports and both, having also identified genetic differences between athletes, according to sex, ethnicity, psychological traits, and sport injuries. To achieve the second objective, 116 roller hockey players were evaluated (28.2 ± 8.7 years; 24.0 ± 2.2 kg/m²; 23.3 ± 8.8 years of sport experience; 13.8 ± 6.3 h/week of training; 15.5% female athletes), representing cumulatively more than 85% of the four best world teams and the five best Portuguese teams in the National Roller Hockey Championship in Portugal. To answer the third objective, 228 volleyball players were studied (26.7 ± 8.1 years; 23.4 ± 2.1 kg/m²; 16.3 ± 8.4 years of sport experience; 22.0 ± 10.5 h/week of training; 28.9% of female athletes), participating in the Portuguese championship and with multiple medals in national and international competitions. The following data were collected from both groups of athletes: anthropometrics, training conditions, sport experience and history of sport injuries. Seven genetic polymorphisms were also analysed, with polymorphism genotyping carried out using the TaqMan® Allelic Discrimination Methodology. Given the biological impact of the polymorphism previously found in the two groups of athletes, a narrative review article

was written about this impact and reflected on future perspectives on health, well-being, and sport performance.

Based on the two original studies, it was found that rink-hockey players demonstrated significantly different characteristics, according to sex, in terms of their anthropometric characteristics, training habits, sport injuries and genetic variants, such as the *Vitamin Receptor D* (*VDR*) rs731236 ($p < 0.05$). On the other hand, volleyball players showed significant differences, according to sex, only in anthropometric indicators and training habits ($p < 0.05$). In elite rink-hockey players, carriers of the rs324420 A allele of *Fatty Acid Amide Hydrolase* (*FAAH*) demonstrated improved sport performance (AA/AC vs. CC, OR = 2.80; 95% CI, 1.23-6.35; $p = 0.014$; $p = 0.008$ after Bootstrap), confirmed by the multivariate analysis (adjusted OR = 2.88; 95% CI, 1.06 -7.80; $p = 0.038$). Also, in high-performance volleyball players, carriers of the A allele of the *FAAH* rs324420 genetic variant demonstrated a superior sport performance in the dominant genetic model (AA/AC vs. CC, OR = 1.70; 95% CI, 0.93–3 .13; $p = 0.026$; $p < 0.001$ after Bootstrap), which was corroborated by multivariate analysis (AA/AC vs. CC adjusted OR = 2.00; 95% CI, 1.04-3.82; $p = 0.037$).

Our results confirmed the expression of *FAAH* in high-performance and elite sport performance. This is the first description of this gene, specifically the rs324420 polymorphism, in elite rink-hockey players and high-performance volleyball players, highlighting the way for its identification in other sports. On the other hand, our work also opens the door to the development of technical, tactical, and therapeutic strategies, which consider mental discipline in high-performance and elite sport, to overcome situations of stress, resistance to pain and inflammation.

Abreviaturas

A	Adenosine
ACE	Angiotensin I converting enzyme
ACSL1	Acyl-CoA synthetase long chain family member 1
ACTN3	Actinin alpha 3
ACVR1B	Activin A receptor type 1B
ADHD	Attention deficit hyperactivity disorder
ADBR2	Adrenoceptor Beta 2
ADN	Adenosina trifosfato
AEA	N-arachidonoyl ethanolamine
AGT	Angiotensinogen
AMPD1	Adenosine monophosphate deaminase 1
aOR	Adjusted odds ratio
AQP1	Aquaporin 1 (Colton blood group)
Arg or R	Arginine
AUD	Alcohol Use Disorder
BDKRB2	Bradykinin receptor B2
BDNF	Brain-derived neurotrophic factor
BMD	Bone mass density
BMI	Body mass index
C	Cytosine
CB1(R)	Cannabinoid type 1 receptor
CB2(R)	Cannabinoid type 2 receptor
CEUFP	Ethical Committee of the University Fernando Pessoa
CHRN3	Cholinergic receptor nicotinic beta 3 subunit
CI	Confidence interval
CKM	Creatine kinase
CKMM	Creatine kinase, M-type
CNDP1	Carnosine dipeptidase 1
CNDP2	Carnosine dipeptidase 2
CNS	Central nervous system
CNTFR	Ciliary neurotrophic factor receptor
COL5A1	Collagen type V alpha 1 chain
COMT	Catechol-O-methyltransferase
COVID-19	Doença causada pelo Coronavírus
CpG	Region citosine-guanine
CREM	cAMP responsive element modulator
CRH	Corticotropin-releasing hormone
CRH(R)1	Corticotropin-releasing hormone receptor
CSA	cross sectional area

CTNP2	Contactin-Associated Protein-Like 2
D	Deletion
DAGL α	Diacylglycerol lipase- α
DNA	Deoxyribonucleic acid
DRD2	D2 dopamine receptor
DZ	Dizygotic twins
eCBs	Endocannabinoids
ECS	Endocannabinoid system
ENCODE	Encyclopedia of DNA Elements Project
ESR1	Estrogen receptor 1
F	Phenylalanine
FAAH	Fatty Acid Amide Hydrolase
FABP3P2	Fatty acid binding protein 3 pseudogene 2
FCRL3	Fc receptor like 3
FEV	FEV transcription factor, ETS family member
FIVB	Fédération Internationale de Volleyball
FOXP3	Forkhead box P3
FTO	Alpha-ketoglutarate dependent dioxygenase
G	Guanine
GABA	Gamma-aminobutyric acid
GABPB1	GA binding protein transcription factor subunit beta 1
GALNTL6	Polypeptide N-acetylgalactosaminyltransferase like 6
GALNT13	Polypeptide N-acetylgalactosaminyltransferase 13
GAMES	International consortium on the endurance athlete
GBF1	Golgi brefeldin A resistant guanine nucleotide exchange factor 1
GENATHLETE	International consortium on the endurance athlete
GJA1	Gap Junction Alpha 1
Gq	Family G protein
GWAS	Genome-Wide Association Studies
HC	Hip Circumference
HERITAGE	HEalth, RIsk factors, exercise Training And GENetics
HFE	Hemochromatosis
HIF1A	Hypoxia inducible factor 1 subunit alpha
HPA	Hypothalamic-pituitary-adrenal
h ²	Heritability
I	Insertion
IGF	Insulin like growth system
IGF1	Insulin like growth factor 1
IGF1R	Insulin like growth factor 1 receptor
IGFBP	Insulin like growth factor binding protein 1

IGFBP-3	Insulin like growth factor binding protein 3
IL6	Interleukin 6
KCNJ11	Potassium inwardly rectifying channel subfamily J member 11
KLF14	Kruppel like factor 14
LINC02341	Long intergenic non-protein coding RNA 2341
LMO4	LIM domain only 4
LRP5	LDL receptor related protein 5
MAF	Minor allele frequency
MAGL	Monoacylglycerol lipase
MAOA	Monoamine Oxidase A
MCT1	Malonyl-CoA-acyl carrier protein transacylase
mGluR5	Metabotropic glutamate receptor 5
MLN	Motilin
mRNA	messenger ribonucleic acid
MSTN	Myostatin
mtDNA	Mitochondrial DNA
MTHFR	Methylenetetrahydrofolate reductase
MZ	Monozygotic twins
Na ⁺	Sodium gradient
NFATC2	Nuclear factor of activated T cells 2
NFIA-AS2	Nuclear factor I A antisense RNA 2
NMDAR	N-methyl-D-aspartate receptor
NOS3	Nitric Oxide Synthase 3
NRF-1	Nuclear respiratory T factor-1
NRF-2	Nuclear respiratory T factor-2
OPG	Osteoprotegerin
OR	Odds ratio
p	Probability
PPARA	Peroxisome proliferator activated receptor alpha
PPARD	Peroxisome proliferator activated receptor delta
PPARG	Peroxisome Proliferator Activated Receptor Gamma
PPARGC1A	Peroxisome Proliferator Activated Receptor Gamma coactivator 1 alpha
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTPRK	Protein tyrosine phosphatase receptor type K
PTP1B	Protein tyrosine phosphatase 1B
PubMed	US National Library of the Medicine National Institutes of Health
P2RX7	Purinergic receptor P2X 7
RANK	Receptor activator of NF-κB
RANKL	Receptor activator of NF-κB ligand
RNA	Ribonucleic acid

RT-PCR	Real-Time Polymerase Chain Reaction
SD	Standard deviation
SEMA4A	Semaphorin 4A
SFEA	Stress Fracture Elite Athlete cohort
SLC6A4	Solute Carrier Family 6 Member 4
SNP(s)	Single nucleotide polymorphism(s)
SOD2	Superoxide dismutase 2
SOST	Sclerostin
SPSS	Statistical Package for the Social Sciences
T	Tyrosine
TERT	Telomerase reverse transcriptase
TFAM	Transcription factor A, mitochondrial
TRIM59	Tripartite motif containing 59
UCP3	Uncoupling protein 3
VAT1L	Vesicle Amine Transport 1-Like
VDR	Vitamin D receptor
VO ₂ max	Maximum oxygen consumption
WC	Waist circumference
WHR	Waist circumference/hip circumference ratio
ZNF608	Zinc finger protein 608
χ ²	Chi-square test
2-AG	2-arachidonoyl glycerol

Índice

Agradecimentos	iii
Declaração de Honra	v
Lista de publicações	vii
Índice de Figuras	ix
Índice de Tabelas	xi
Resumo	xiii
<i>Abstract</i>	xv
Abreviaturas	xvii
1. Introdução geral	25
1.1. Objetivos	29
1.2. Organização da tese	30
2. Metodologia	31
2.1. Desenho do estudo	33
2.2. Participantes	33
2.3. Instrumentos e métodos	33
2.3.1. Confidencialidade e tratamento dos dados	35
3. Genomic profile in association with sport-type, sex, ethnicity, psychological traits and sport injuries of elite athletes: review and future perspectives	37
3.1. Introduction	39
3.2. Genomic profile	42
3.2.1. Genetic polymorphisms associated with endurance-based sports	46
3.2.2. Genes associated with power-based sports	47
3.2.3. Genes and polymorphisms associated with both endurance- and power-based sports	50
3.2.4. Genes associated with sex and ethnicity differences	54
3.2.5. Genes associated with psychological traits	56
3.2.6. Genes associated with sport injuries	57
3.3. Conclusion and future perspectives	60
3.4. References	61
4. FAAH rs324420 polymorphism is associated with performance in elite rink-hockey players	71
4.1. Introduction	73
4.2. Materials and Methods	74
4.2.1. Participants	74
4.2.2. Procedures	76

4.2.2.1. Anthropometric profile	76
4.2.2.2. Training data, sport experience and sport injuries history	76
4.2.2.3. Sample collection and DNA extraction	77
4.2.2.4. Polymorphism selection and genotyping	77
4.2.2.5. Statistical analysis	78
4.3. Results	78
4.3.1. Characteristics of elite rink-hockey players	78
4.3.2. Genotype frequencies in elite female and male rink-hockey players	79
4.3.3. Univariate and multivariate analysis of <i>FAAH</i> rs324420 and relevant factors of elite performance in rink-hockey	80
4.4. Discussion	81
4.5. Conclusions	85
4.6. References	86
5. Association of <i>FAAH</i> rs324420 (C385A) polymorphism with high-level performance in volleyball players	93
5.1. Introduction	95
5.2. Materials and methods	96
5.2.1. Enrolled players' description and data collection	96
5.2.1.1. Biological sample processing	98
5.2.1.2. SNP selection and genotyping	98
5.2.2. Statistical analysis	99
5.3. Results	99
5.3.1. High-level performance volleyball players	99
5.3.2. SNP genotype frequencies	100
5.3.3. Univariable and multivariable analyses	100
5.4. Discussion	101
5.5. Conclusions	106
5.6. References	106
6. <i>FAAH</i> rs324420 polymorphism: biological pathways, impact on elite athletic performance and insights for Sport Medicine	111
6.1. Introduction	113
6.2. The endocannabinoid system and the biological pathways of <i>FAAH</i> rs324420	115
6.2.1. The endocannabinoid system signalling	115
6.2.2. <i>FAAH</i> protein functioning and <i>FAAH</i> gene variation	116
6.2.3. <i>FAAH</i> rs324420 polymorphism	116
6.3. Geographic distribution of <i>FAAH</i> rs324420 alleles	117
6.4. <i>FAAH</i> rs324420 polymorphism and elite athletic performance	118

6.4.1. Biological evidence in elite athletic performance	118
6.4.2. Biological pathways, elite athletic performance and Sport Medicine	119
6.5. <i>FAAH</i> rs324420 polymorphism and other psychobiological associations	121
6.5.1. <i>FAAH</i> rs324420 polymorphism, stress, anxiety and fear extinction	121
6.5.2. <i>FAAH</i> rs324420 polymorphism, pain and inflammation	122
6.5.3. <i>FAAH</i> rs324420 polymorphism and neural dysfunctions	123
6.6. Implications for Sport Medicine	124
6.7. Conclusions and future directions	125
6.8. References	126
7. Conclusões e perspectivas futuras	141
8. Referências gerais	145

1 Introdução geral

1.1. Objetivos

1.2. Organização da tese

1. INTRODUÇÃO GERAL

Os seres humanos apresentam uma ampla variação no seu desempenho físico (Bouchard et al., 1998); alguns são naturalmente talentosos, outros não (Careau & Wilson, 2017; Williams, Ford, & Drust, 2020). Importa, por isso, compreender o atleta de elite pelo seu talento e pela sua vertente genética no desenvolvimento do rendimento desportivo.

O talento refere-se ao potencial ou à capacidade individual do atleta em elevar um domínio específico, que solicita habilidades especiais e treino (Den Hartigh, Hill & Van Geert, 2018), sendo que a qualidade dessas habilidades e desse treino se traduz no desempenho de alto rendimento (Teunissen et al., 2023). Parece que os atletas com desempenho superior são então, dotados de um elevado potencial genético, concretizado através de um esforço árduo (Georgiades et al., 2017), quer da componente física, quer mental.

Pela experiência dos autores na área do treino de alto rendimento, as características genéticas para o sucesso têm um papel fundamental e se o treino desse atleta for intenso, ele terá um melhor desempenho do que outros menos treinados. Assim, em atletas semelhantes, o treino faz a diferença. Nos super-atletas, os resultados mais elevados são alcançados de forma mais fácil, rápida e natural (ou seja, com menor esforço ou sacrifício) do que nos outros atletas.

Observou-se que os atletas talentosos são muitas vezes oriundos de famílias talentosas (De Moor et al., 2007) e, apesar das famílias partilharem vários aspetos do seu ambiente, elas também partilham o seu ADN, dependendo da ligação genética entre eles (Moran & Pitsiladis, 2017). Antero et al. (2018) avaliaram dados sociodemográficos de 125051 atletas participantes nos Jogos Olímpicos entre 1896 e 2012, tendo verificado que a probabilidade de um atleta olímpico, com um parente medalhado, ser medalhado foi significativamente superior à de um atleta olímpico, sem qualquer parente nos ciclos olímpicos estudados.

Desta forma, apenas um número reduzido de atletas consegue atingir um nível de desempenho desportivo excecional, porque o desenvolvimento do atleta não é linear. Este é influenciado por fatores previsíveis (e.g., transição da adolescência para a idade adulta com padrões de desenvolvimento próprios) e por fatores imprevisíveis (e.g., lesões, traumas) (Hardy et al., 2017).

Desta forma, o desempenho do atleta de alto rendimento depende da interação complexa de diversos fatores, incluindo o treino, as capacidades físicas e volitivas, a nutrição, a genética e o meio sociocultural em que está inserido (Thomas et al., 2016; Silva & Paiva, 2015, 2016). Para além destes, as exigências específicas do calendário competitivo podem igualmente influenciar esse desempenho (Fietze et al., 2009; Silva & Paiva, 2019).

Assim, é consensual que a compreensão do desempenho do atleta de alto rendimento exige a integração de duas principais fontes de especificidade individual, nomeadamente

a ligação entre as suas características genéticas e o ambiente envolvente (Georgiades et al., 2017).

A análise do perfil genómico de atletas tem vindo a despertar interesse na comunidade científica, de forma a apoiar a melhoria do treino do atleta talentoso, e otimizar a sua prestação desportiva, tornando-o num atleta medalhado.

Contudo, esta área é a que mais carece de investigação, pelo facto de outras áreas do conhecimento, tais como, a Fisiologia, a Biomecânica, a Traumatologia e a Nutrição terem conseguido primeiramente responder a problemas ligados à saúde, ao rendimento e à segurança dos atletas. Também as questões éticas e morais na condução prática das investigações científicas na área da Genética podem ter contribuído para essa menor abrangência científica. Neste contexto, indica-se o caso da Liga norte-americana de beisebol, que tem vindo a aplicar testes genéticos de parentalidade para confirmar a idade dos atletas e evitar falsificações (Zitelli, 2011).

Sabe-se que há atletas com bons desempenhos em desportos de resistência ou em desportos de potência, mas raramente em ambos. Estas diferenças individuais podem ser potencialmente explicadas não apenas pelo impacto da variação da sequência de ADN na biologia e no comportamento, mas também pelos efeitos das alterações epigenéticas, que afetam o fenótipo, modificando a expressão genética, como por exemplo, o tipo de treino (de resistência ou de potência) (Amato et al., 2018) e as oportunidades de participação no próprio desporto (Moran & Pitsiladis, 2017; Careau & Wilson, 2017).

De Moor et al. (2007) propuseram que os fatores genéticos podem explicar, em cerca de 66%, a variância observada no desempenho desportivo, estando identificadas mais de 200 variantes genéticas associadas ao desempenho físico (Ahmetov, Egorova, Gabdrakhmanova, & Fedotovskaya, 2016; Semenova, Hall, & Ahmetov, 2023).

Por seu lado, as lesões são um dos principais constrangimentos à otimização da forma desportiva do atleta, sendo que a investigação também se tem debruçado sobre as variantes genéticas, que se podem relacionar com o maior risco de lesão, conforme demonstrado por Salles et al. (2018), que concluíram que o polimorfismo do *recetor Fc 3 - Fc receptor like 3 (FCRL3)* –169C esteve associado a um maior risco de tendinopatia em voleibolistas.

Estima-se que no desporto de elite britânico, os testes genéticos sejam usados em cerca de 17% dos atletas com o objetivo de se avaliar o desempenho e a ocorrência de lesões desportivas, e que 66,7% dos atletas consideram o teste genético, como um instrumento valioso no processo de identificação de talentos no desporto (Varley et al., 2018).

Apesar de 70% a 85% do sucesso desportivo se dever aos traços da personalidade (Raglin, 2001) e à capacidade mental do atleta no contexto competitivo (Peplonska et al., 2017), a investigação tem-se centrado maioritariamente na avaliação do desempenho físico de

atletas de desportos de resistência e de potência, e a identificação de variantes genéticas, ligadas às estruturas emocionais com efeito no desempenho de alto rendimento, tem sido pouco investigada (Peplonska et al., 2019; Semenova, Hall, & Ahmetov, 2023).

Desta forma, parece haver uma lacuna de conhecimento entre a componente genética e o desempenho desportivo de alto rendimento, ao nível das qualidades mentais, isto é, da disciplina mental desses atletas (controlo emocional, capacidade de tomada de decisões em situações de stress competitivo, resiliência, paciência, liderança).

Neste sentido, propusemo-nos a atingir os objetivos do estudo indicados a seguir.

1.1. Objetivos

O objetivo geral desta tese foi caracterizar o perfil genómico de atletas e ex-atletas de hóquei em patins de elite e de jogadores de voleibol de alto rendimento.

A escolha destes dois desportos prendeu-se com o facto de termos acesso facilitado a estes atletas, aumentando a possibilidade de participação representativa nestes desportos, e sermos capazes de responder aos objetivos propostos. Mesmo assim, e devido às limitações decorrentes da pandemia, o trabalho de campo esteve temporariamente suspenso (explicado no capítulo seguinte - Metodologia).

Importa desde já esclarecer ainda a definição de atleta de elite, como o indivíduo que atinge objetivos elevados a nível nacional e/ou internacional, e que apresenta capacidades desportivas excecionais (Den Hartigh, Hill & Van Geert, 2018).

Neste contexto, os jogadores de hóquei em patins, que fizeram parte do nosso estudo, foram considerados de elite, dado que integravam a modalidade, em que Portugal era campeã do mundo e cujo campeonato nacional envolvia os melhores jogadores mundiais (nacionais e internacionais). No caso dos voleibolistas, dada a universalidade da modalidade e a crescente competitividade dos campeonatos nacionais (masculino e feminino), os atletas foram categorizados como atletas de alto rendimento, de acordo com o menor número de vitórias (essencialmente títulos europeus e mundiais) relativamente aos hoquistas (explicado no capítulo seguinte – Metodologia).

Assim, estabeleceram-se os seguintes objetivos específicos:

- 1) analisar a literatura científica que relacionasse as variantes genéticas com o desempenho desportivo de atletas de alto rendimento;
- 2) estudar o perfil genómico de jogadoras e jogadores de hóquei em patins de elite, as suas características físicas e antropométricas, e determinar eventuais variantes genéticas e outras associadas com o desempenho desportivo desses atletas;
- 3) identificar o perfil genómico de voleibolistas de alto rendimento, de ambos os sexos, as suas características físicas e antropométricas, e identificar eventuais variantes genéticas e outras associadas com o desempenho desportivo desses atletas;

4) adicionalmente, e de acordo com os resultados do polimorfismo identificado nos dois tipos de atletas anteriores, surgiu o último objetivo, nomeadamente o de caracterizar esse polimorfismo no desempenho desportivo e refletir sobre os eventuais contributos futuros do seu estudo e implicações no desempenho, na saúde e bem-estar, e na medicina desportiva.

1.2. Organização da tese

A seguir ao capítulo da Introdução, apresenta-se o segundo capítulo referente à Metodologia do trabalho desenvolvido, iniciando-se com a descrição do desenho do estudo, a identificação dos participantes e a caracterização dos métodos e instrumentos aplicados durante o trabalho de campo para a colheita e análise dos dados.

Posteriormente, são apresentados os capítulos três a seis, que correspondem aos artigos científicos publicados em revistas indexadas com fator de impacto, que resultaram do trabalho desenvolvido, respetivamente:

- > o capítulo três, que se refere ao artigo de revisão narrativa - *Genomic profile in association with sport-type, sex, ethnicity, psychological traits and sport injuries of elite athletes: review and future perspectives*. Journal of Sports Medicine and Physical Fitness. 2021, Mar 5. DOI:10.23736/S0022-4707.21.12020-1;
- > o capítulo quatro, que compreende o primeiro artigo original - *FAAH rs324420 polymorphism is associated with performance in elite rink-hockey players*. Biology. 2022; 11(7), 1076. DOI:10.3390/biology11071076;
- > o capítulo cinco, que concerne o segundo artigo original - *FAAH rs324420 (C385A) Polymorphism with Athletic High-level Performance in the Portuguese Volleyball Players*. Genes. 2023; 14(6), 1164. DOI:10.3390/genes14061164 e;
- > o capítulo seis, que diz respeito ao segundo artigo de revisão narrativa - *FAAH rs324420 Polymorphism: Biological Pathways, Impact on Elite Athletic Performance and Insights for Sport Medicine*. Genes. 2023; 14(10):1946. DOI:10.3390/genes14101946.

Por fim, abordam-se as conclusões, refletindo-se sobre os resultados encontrados e as diretivas futuras sobre o perfil genómico dos atletas, sendo a seguir incluídas, as respetivas referências bibliográficas, constantes nos capítulos um e dois (Introdução e Metodologia) da tese.

2 Metodologia

2.1. Desenho do estudo

2.2. Participantes

2.3. Instrumentos e métodos

2.3.1. Confidencialidade e tratamento dos dados

2. Metodologia

2.1. Desenho do estudo

Realizou-se um estudo transversal, que decorreu de acordo com as seguintes fases:

1. Apresentação do projeto junto dos clubes, através de uma reunião informativa com os atletas, que voluntariamente decidiram participar no estudo, e treinadores. Nessa reunião foram explicados os objetivos e a metodologia do trabalho. Posteriormente, procedeu-se ao esclarecimento de eventuais dúvidas, informou-se sobre o anonimato e a confidencialidade dos dados, bem como da possibilidade de, em qualquer momento, os eventuais participantes poderem abandonar o estudo, sem com isso serem alvo de qualquer penalização. De seguida, obteve-se o respetivo consentimento informado, através da assinatura, que tinha por base a Declaração de Helsínquia.
2. Recolha da informação seguinte por cada atleta: dados sociodemográficos, treino desportivo e competição, história clínica e dados genéticos.

Este estudo obteve a aprovação da Comissão de Ética da Universidade Fernando Pessoa, conforme apresentado inicialmente no projeto de candidatura ao Doutoramento no Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

O estudo contou a colaboração do Instituto Português de Oncologia do Porto para a análise laboratorial das amostras de material genético dos participantes, e da Faculdade de Ciências da Saúde da Universidade Fernando Pessoa para o armazenamento dessas amostras.

2.2. Participantes

Foram avaliados todos os atletas e ex-atletas de elite, de ambos os sexos, com idade igual ou superior a 18 anos, praticantes de desportos individuais e coletivos, que aceitaram participar voluntariamente neste estudo e que forneceram consentimento informado.

Os critérios de inclusão foram: situar-se na faixa etária indicada anteriormente, e; enquadrar-se dentro do perfil de atleta de elite (conceito definido anteriormente nos objetivos do estudo).

Os critérios de exclusão foram: sofrer de alguma incapacidade física ou outra, que impeça a recolha de saliva, e; não ter obtido qualquer internacionalização como atleta até ao momento do estudo.

2.3. Instrumentos e métodos

Depois da obtenção do consentimento informado, os dados foram recolhidos sempre pelo mesmo investigador, previamente treinado, através da aplicação de procedimentos padronizados e de um questionário específico para atletas e para ex-atletas, através do qual serão recolhidos os dados seguintes:

> Dados sociodemográficos (idade, sexo, profissão, estado civil, número de filhos e de filhas).

> Dados antropométricos (peso, estatura, perímetro da cinta, perímetro da anca, comprimento da mão, número que calça).

> Treino desportivo (desporto praticado, idade de início no alto rendimento, experiência desportiva em anos, frequência diária e semanal de treinos, número de participações em competições nacionais e internacionais, idade na primeira lesão desportiva, mês da primeira lesão desportiva, e motivos para a prática desportiva).

> História clínica; no caso de atletas e ex-atletas do sexo feminino, recolheram-se dados relativos à sua história ginecológica (idade na menarca, regularidade dos ciclos menstruais e uso de contraceptivos orais).

> Perfil genómico foi avaliado através de *swab* bucal (foram recolhidas duas amostras por atleta: uma do lado direito e uma do lado esquerdo). Cada amostra recolhida foi guardada dentro de um frasco esterilizado fechado e identificado com o mesmo código atribuído ao questionário, e transportada numa mala térmica e armazenadas no frio, até à realização da respetiva análise laboratorial do perfil genómico ligado ao desempenho desportivo.

Antes da análise do perfil genómico, procedeu-se primeiramente à revisão da literatura sobre todos os fatores genéticos associados ao desempenho desportivo em diferentes modalidades desportivas, a qual se encontra publicada no primeiro artigo de revisão (capítulo três desta tese). Da lista destes fatores (88 variantes genéticas), foram selecionados sete polimorfismos genéticos: i) *FAAH* - Fatty Acid Amide Hydrolase/Hidrólase Amida de Ácidos Gordos, ii) *PPARGC1A* - *PPARG Coactivator 1 Alpha/Coativador I-Alfa do Receptor Gama Ativado por Proliferador de Peroxissoma*, iii) *PPARD* – *Peroxisome Proliferator Activated Receptor Delta/Recetor-Delta Ativado pelo Proliferador de Peroxissoma*, iv) *PPARG* – *Peroxisome Proliferator Activated Receptor Gamma/Recetor Gama Ativado pelo Proliferador de Peroxissoma*, v) *VDR* – *Vitamin D Receptor/Recetor de Vitamina D*, vi) *ADRB2* - *Adrenoceptor Beta 2/Adrenocetor Beta 2* e vii) *NOS3* – *Nitric Oxide Synthase 3/Óxido Nítrico Síntase-3*). Esta seleção teve por base os seguintes critérios (publicados nos dois artigos originais – capítulos quatro e cinco desta tese): a sua associação prévia com o desempenho desportivo numa população caucasiana (considerando desportos de resistência, de potência e/ou lesões desportivas), o polimorfismo de menor frequência alélica na população ibérica ser superior a 10% para garantir a representação do genótipo, a sua consequência funcional, a existência de estudos de validação e a disponibilidade de ensaios *TaqMan®* (*Applied Biosystems*).

Por forma a uniformizar-se a avaliação dos participantes, produziu-se um manual de procedimentos da investigação com a descrição do trabalho e com todas as indicações pormenorizadas para a sua execução.

Criou-se um livro de códigos e a respetiva base de dados no *Statistical Package for the Social Sciences* (SPSS), versão 25.0 para *Windows*.

Por fim, aplicou-se o questionário a uma amostra de conveniência para deteção de eventuais correções e padronização das técnicas de aplicação do questionário e da recolha de dados genéticos, tendo-se efetuado a análise desses dados (resultados não incluídos nesta tese) com vista a obtenção das respetivas alterações no manual de procedimentos e no livro de códigos.

O trabalho de campo iniciou-se em outubro de 2019 e terminou em julho de 2022.

Apesar de este trabalho ter estado suspenso entre os meses de março 2020 a junho 2021, devido à pandemia por COVID-19, foram realizados esforços no sentido do desenvolvimento do projeto, de acordo com o proposto no plano de intenções do doutoramento.

Assim, procedeu-se à análise preliminar dos resultados sociodemográficos, do treino desportivo e da história clínica dos participantes, tendo-se elaborado e submetido um artigo de revisão narrativa para publicação numa revista científica indexada com fator de impacto. Posteriormente, e com os resultados laboratoriais disponíveis dos jogadores de hóquei em patins, avançou-se para a redação e submissão do primeiro artigo original referente ao perfil genómico dos hoquistas, dado que foram os primeiros atletas a ser avaliados.

Em seguida, e com os resultados da análise laboratorial, submeteu-se o segundo artigo original, que caracteriza o perfil genómico dos voleibolistas.

E dada a escassez de estudos nas variantes genéticas ligadas ao desempenho físico e mental do atleta de elite, a importância do polimorfismo *FAAH rs324420* encontrado nos dois grupos de atletas, e o facto do atual ciclo olímpico estar a terminar, submeteu-se um artigo de revisão narrativa, que refletisse a evolução deste polimorfismo e despertasse interesse no contexto do desporto e da saúde no alto rendimento.

2.3.1. Confidencialidade e tratamento dos dados

De forma a assegurar a confidencialidade dos dados, todos os questionários e declarações de consentimento informado foram separados e guardados em local seguro, de forma a garantir a impossibilidade de os emparelhar e aos quais só tiveram acesso, os investigadores responsáveis pelo estudo.

Os questionários foram codificados e tratados na forma de análise estatística descritiva e inferencial, de forma a comparar-se os grupos amostrais nas variáveis avaliadas com recurso ao programa informático SPSS, versão 25.0 para *Windows*.

3

Genomic profile in association with sport-type, sex, ethnicity, psychological traits and sport injuries of elite athletes: review and future perspectives

3.1. Introduction

3.2. Genomic profile

3.2.1. Genetic polymorphisms associated with endurance-based sports

3.2.2. Genes associated with power-based sports

3.2.3. Genes and polymorphisms associated with both endurance- and power-based sports

3.2.4. Genes associated with sex and ethnicity differences

3.2.5. Genes associated with psychological traits

3.2.6. Genes associated with sport injuries

3.3. Conclusion and future perspectives

3.4. References

3.1. Introduction

In the broad sense, “elite” is defined as “belonging to a group of people in society that is small in number, but powerful and with a lot of influence” or “(especially in sport) having or showing the greatest ability at the highest level of competition”.¹ In the athletic population, however, a consensus definition is not clear yet. For some, an elite athlete is a person who is a member of any national team or another high-level representative team in any sport organised by a National Sports Federation,² or the athlete achieving selection for international teams.^{3,4} Regardless of a core definition, it is consensual that an elite athlete is a person who demonstrates high athletic achievements, either national and/or internationally, and presents particular performance abilities, i.e., is a talent.⁵ Therefore, elite athletes are thought to have exceptional genetic potential⁶ with an incredible inter-individual variability of physical performance traits, including power, strength, endurance, muscle fibre composition and size, neuromuscular coordination, flexibility, personality profile,⁷ VO₂ max (meaning maximum oxygen consumption) and injury susceptibility⁸ (Table 3.1).

Table 3.1. Factors influencing the elite athlete potential/performance.

The **elite athlete** is determined by **exceptional characteristics influenced** by (not necessarily by this order):

- ⌘ Genetic potential
 - ⌘ Physical performance traits
 - ⌘ Adequate nutrition
 - ⌘ Dedicated training
 - ⌘ Personality profile
 - ⌘ Motivation
 - ⌘ Family support
 - ⌘ Auxiliary training/scientific approaches
-

Ahmetov et al.⁷ have reported a variance of 66% in the athlete status attributed exclusively to genetics factors, whereas other researchers reinforced that environmental factors are likely to give a contribution of at least 50%, but less than 100%.⁹ In fact, gifted athletes are the result of the combination of favourable genetic and environmental factors,^{9,10} i.e., the adult athletic phenotype irreversibly results from the individual’s growth and development trajectory influenced by both genetic background and environmental effects.

From the authors’ experience in the field of high-performance training, genetic traits for athletic success have a key role and if that athlete’s training is intense, he/she will have better and higher achievements than others less trained. Hence, in similar athletes, the training makes the difference. In gifted-athletes, higher results are easier, faster and more naturally achieved (i.e., without great effort or sacrifice) than others.

A recent twin-family study¹¹ of a retrospective cohort (125,051 worldwide athletes that have participated in the Olympic Games between 1896 and 2012, including 90 dizygotic (DZ) and monozygotic (MZ) twins) found that the medal probability was significantly greater in Olympians carrying a kinship with a former Olympic Medallist: 44.4% for niece/nephew (33.7-54.2), 43.4% for offspring (37.4-48.6), 64.8% for siblings (61.2-68.8), 75.5% for DZ twins (63.3-86.6), and 85.7% for MZ twins (63.6-96.9); a significantly greater concordance was found in MZ than DZ ($P=0.01$) and heritability (h^2) estimated at 20.5%. Thus, the closer kinship with a former Olympic medallist, the greater probability for an Olympic athlete to become a medallist.

On the other hand, it would be inconceivable to win the Tour de France without a year-long program of physical training.¹⁰ Therefore, associated to genetics and epigenetics, several other factors determine the elite athletic success, such as adequate sport-specific practice^{12,13} and dedicated training, nutrition, motivation, equipment improvements and other environmental factors^{3,6,7,12,14} (Table 3.1).

The rapid increase and improvement of tools for DNA analysis has allowed the identification of several genes and genetic markers correlated with specific phenotypes.¹²

In the early 2000s, the first genetic markers associated to athletic performance were identified, including gene variations of the *Angiotensin I converting enzyme (ACE)* and the *Actinin alpha 3 (ACTN3)*, which have provided new insights regarding the elite athletic performance and genomic profile.⁷

However, athletic performance and trainability are complex traits, being influenced by a plethora of genes.¹⁵ In the last few years, some genetic variants have been associated with elite athletic performance, but its polygenic trait character has limited the progress for a consistent definition of the genomic profile of elite athletes, especially due to minor contributions of each genetic variant.^{16,17} In addition, small and mostly heterogeneous cohorts resulting in conflicting findings,¹⁵ and the complexity of genetic influences underlying the adaptations of various traits in performances of high-level¹⁸ have not been favourable for robust and conclusive associations. It is well-known that the elite-level performance is not for all;¹⁹ exactly, it is rare,²⁰ i.e., only few athletes can achieve and develop particular performance abilities at a high-level making elite performance rare. The question is no longer whether or not there is a genetic component to elite athletic potential and performance, but exactly which genes are involved in and by which mechanisms and pathways they exert their effect.⁷

The lack of studies confirming candidate genes variants with this regard may be due to the fact that complex traits are mostly polygenic, i.e., numerous genes can demonstrate small effects as each DNA locus can possibly show a phenotype variance between ~0.1 to ~1.0%.^{7,21} This occurs because of genetic alterations across the genome consisting of the

replacement of a single nucleotide by another, named single nucleotide polymorphisms (SNPs).¹⁵ A polymorphism is a DNA variant that is common in a population and typically observed in more than 1% of individuals.¹² In the human genome, there are from 3 to 4 million common variants and from 200,000 to 500,000 rare variants.²² From these latter, some genetic markers are associated with athletic performance and trainability,¹⁵ namely with endurance, strength and power, or combined power with strength.⁷ From over than 200 polymorphisms associated with elite athletic status, the *ACTN3* rs1815739 variant has presented the most consistent results²³⁻²⁵ and was even recently considered as a powerful influence for becoming an elite sprint or power athlete.²⁶ The aforementioned genetic variant in the *ACTN3* gene (11q13.1) results in the replacement of an arginine (Arg or R) with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X). The 577X allele (T allele) contains a sequence change that prevents the production of functional α -actinin-3 protein, which is almost exclusively restricted to fast, type 2X muscle fibres, responsible for producing force at high velocity or powerful contraction, typically observed in sprint/power athletes.⁷

Herein, this review summarises the current understanding about genetic and epigenetic factors in elite athletes, as well as their genomic profile in association with sport-type, sex, ethnicity, psychological traits and sport injuries.

A search of the published literature that investigated the association between genetics and the performance of elite athletes was conducted through the PubMed (US National Library of the Medicine National Institutes of Health) platform and obtained according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Data collection included: scientific articles, books and book chapters published between 2015 and 2020. The descriptors that were used to search for the articles were: “elite athlete”, “performance”, “genetics”, “genes” and “genome” in the title. The title and the corresponding abstract were judged according to relevance. If promising, they were selected for more detailed reading and included in this review. Both review and original articles were considered for this study. Additional publications were also considered by cross-referencing. A hand search of the reference lists of articles included in the final analysis that were identified via the database search was conducted, as were the first 20 “related articles” of those included database search articles on PubMed. The inclusion criteria were: to be published in the last five years, to be done only in human beings and to be written in four languages (Portuguese, English, Spanish and French). After the data were collected, the results were analysed through careful reading and organized according to the topics presented in this review. The articles suggested during the peer review process were included later. A total of 121 publications were included in this review.

3.2. Genomic profile

Performance is a complex phenomenon as it is not a trait *per se*, but the combination of many traits working together and in mutual influence.²⁰

Heritability studies in exercise performance firstly started with twin and family cohorts. It is important to note that talented athletes frequently have talented ancestries/family members²⁷ and share environmental conditions, as well as, DNA characteristics, depending on their genetic relationship.²⁸

Klissouras²⁹ primarily observed a 93% variation in VO₂max on 25 pairs of male twins (15 MZ and 10 DZ), 96.5% for skeletal muscle fibre composition and that such variations were due to genetic factors; no correlation was identified on genotype-environment interaction, which may have overestimated the h^2 .

Other following studies also conducted in twins and families also demonstrated a high h^2 estimation for VO₂max in the order of 77%,³⁰ 69-87%³¹ and 71%,³² for maximal power ($h^2=97\%$) and maximal isometric force ($h^2=83\%$).³³

In addition, a h^2 estimate of 72% for VO₂max was also reported in a recent meta-analysis of eight twin studies.³⁴

Since, heritability depends on the studied population and time, the responses' heritability to exercise training (i.e. trainability)¹³ through highly controlled studies using resistance strength, aerobic and intermittent training protocols have been performed.³⁵ For instance, a large-scale family study named HHealth, RIsk factors, exercise Training And GENetics (HERITAGE)³⁶ has reported a variance improvement in VO₂max from 47 to 50% after exercise training due to genetics.

The HERITAGE study³⁷ among two others Genome-Wide Association Studies (GWAS)^{27,38} have boosted an increasing interest in the scientific community for athletic performance and genes expression by identifying markers associated to athletic status; however, only few were common. Yan et al.¹⁷ even considered that twin and family studies did not identify individual genes influencing performance.

In 2001, the Human Genome Project³⁹ used the so-called "candidate gene approach" for identifying genes with a specific function for exercise.¹²

Other initiatives and projects were conducted as it is illustrated in Table 3.2, which will be latter discussed.

Table 3.2. Key projects/studies in genomics, genetics, and exercise biology (adapted from Georgiades et al.⁹ and Bouchard & Malina³⁹).^{29,37,41-48}

Year	Key events
1971	Twin Studies of VO2 max. ²⁹
1984	Twin Studies of trainability of VO2 max. ⁴¹
1999	Heritage Family Study. ³⁷
2000	<i>ACE</i> , Candidate Gene Approach. ⁴²
2001	The Human Genome Project aimed at sequencing and analysing of the human genome. ³⁹
2003	The Encyclopedia of DNA Elements Project (ENCODE) aimed at studying functional elements in the human genome sequence. ⁴³
2003	<i>ACTN3</i> . ⁴⁴
2007	Genetics of East African runners. ⁴⁵
2008	The 1000 Genomes Project, a large public catalogue about the human variation and genotype data. ⁴⁶
2016	GAMES, the first GWAS related to athletic performance. ⁴⁷
2016	The Athlome Project aimed at calling for international collaborative efforts to discover the elite human performance's genetics, adaptive exercise training and muscle injury prevention. ⁴⁸

Although over 200 SNPs have been associated to physical performance,⁴⁹ only 25 SNPs have been related to athletic status⁵⁰ due to their very small effect size in performance traits and status.¹⁵ In fact, only two SNPs demonstrated consistent replication among different studied populations, with these variants being located at *ACE* gene²⁶ and *ACTN3* genes.⁴⁴ The *ACTN3* rs1815739 and *ACE* I/D (I= insertion, D= deletion) polymorphisms appear to be associated to specific sprint phenotypes and influence the athletic status, as the *ACTN3* rs1815739 variant is more influential to 200-m performance and the *ACE* I/D polymorphism is more involved in the longer, 400-m sprint performance.²⁵

Indeed, it was found that the *ACE* DD (−0.60, 95 % CI −1.09 to −0.11) and *ACE* ID (−0.68, 95 % CI −1.18 to −0.18) individuals had significantly faster sprint time than *ACE* II individuals ($P < 0.05$).²⁵

Normally, athletes show associations between endurance-based sports characteristics (Table 3.3) or power-based sports characteristics (Table 3.4) and a particular genetic or epigenetic factor²⁸, however, some studies demonstrated some genetic and epigenetic associations to both based-characteristics.

Table 3.3. Genetic and epigenetic factors for endurance.^{3,7,13,47,51-59}

Gene	Genetic or epigenetic factors	Athletes/reference
<i>ACE</i>	I/D ^a	196 elite triathletes ¹³ 3,889 elite endurance athletes (2,984 marathon runners) and 6,109 controls/systematic review ⁵¹ 30 sub-elite Australian Football players ⁵² 107 Caucasian male elite athletes (17 sprint/power from short-distance runners, swimmers <200m, 36 endurance athletes from rowers, football players, middle distance swimmers and 54 athletes from mixed sports - water polo, handball and volleyball) ⁵³
	rs4341	413 Polish Caucasian elite athletes (225 endurance and 188 power) ⁵⁴
<i>ACTN3</i>	rs1815739 ^a	196 elite triathletes ¹³ 107 Caucasian male elite athletes (17 sprint/power from short-distance runners, swimmers <200m, 36 endurance athletes from rowers, football players, middle distance swimmers and 54 athletes from mixed sports - water polo, handball and volleyball) ⁵³
<i>ADRB2</i>	rs1042713 A	Endurance athletes ⁷
<i>AQP1</i>	rs1049305 C	Endurance athletes ⁷
<i>AMPD1</i>	rs17602729 C	Endurance athletes ⁷
	rs17602729 A	196 elite triathletes ¹³
<i>BDKRB2</i>	–9	Endurance athletes ⁷
<i>CHRNA3</i>	rs4950 G	413 Polish Caucasian elite athletes (225 endurance and 188 power) ⁵⁴
<i>CNDP2</i>	rs6566810 AA	908 Brazilian elite athletes (328 endurance, 415 power and 165 combat) and 967 non-athletes ⁵⁵
<i>CKMM</i>	rs1803285 G ^a	196 elite triathletes ¹³
<i>COL5A1</i>	rs12722 T	Endurance athletes ⁷
	rs12722 T	460 elite rugby union, 88 elite rugby league athletes and 565 controls ⁵⁶
	rs3196378 A	
<i>FAAH</i>	rs324420 AA	413 Polish Caucasian elite athletes (225 endurance and 188 power) ⁵⁴
<i>GABPB1</i>	rs12594956 A	Endurance athletes ⁷
	rs7181866 G	
<i>GALNTL6</i>	rs558129	1520 elite endurance athletes and 2760 controls ⁴⁷
<i>MSTN</i>	rs1805086 ^a	196 elite triathletes ¹³
<i>HFE</i>	rs1799945 G	196 elite triathletes ¹³ Endurance athletes ⁷
<i>IGFIR</i>	rs1464430	111 Israeli track and field athletes, 80 swimmers and 111 controls ³ 159 elite athletes of track and field and/or triathlon and 68 controls ⁵⁷
<i>IGF1</i>	rs35767	111 Israeli track and field athletes, 80 swimmers and 111 controls ³
<i>KCNJ11</i>	rs5219 C	Endurance athletes ⁷
<i>mtDNA</i>	H haplogroup	Endurance athletes ⁷
	K haplogroup	
<i>MCT1</i>	rs1049434 AA	128 Italian elite male soccer players ⁵⁸
<i>MSTN</i>	rs1805086	Israeli track and field athletes, swimmers and controls ^{3,59}
<i>NRF-2</i>	rs12594956	413 Polish Caucasian elite athletes (225 endurance and 188 power) ⁵⁴

^a Tested, but not associated to elite performance.

Table 3.3. Cont.

Gene	Genetic or epigenetic factors	Athletes/reference
<i>NOS3</i>	rs1799983	Endurance athletes ⁷
<i>PPARA</i>	rs4253778 G	Endurance athletes ⁷
<i>PPARD</i>	rs2016520 C	Endurance athletes ⁷
<i>PPARGC1A</i>	rs8192678 T	196 elite triathletes ¹³
<i>TFAM</i>	rs2306604 GG	413 Polish Caucasian elite athletes (225 endurance and 188 power) ⁵⁴
<i>UCP3</i>	rs1800849 T	Endurance athletes ⁷

Table 3.4. Genetic and epigenetic factors for power/strength. ^{7,25,50,55,60-72}

Gene	Genetic or epigenetic factors	Athletes/reference
<i>ACE</i>	I/D	116 elite Jamaican and 114 US sprinters ⁶⁰ 100 Hungarian elite female athletes (30 kayaking/rowing, 29 handball/basketball, 27 water polo and 14 combat sports) ⁶¹ 282 Tunisian athletes (149 endurance and 133 power) and 211 controls ⁶²
<i>ACTN3</i>	rs1815739	59 elite Chinese sprint/power athletes ⁵⁰ 86 male European White male canoe sprint athletes and 354 controls ⁶³ 507 Caucasian men, including 431 rugby union athletes and 710 Caucasian controls ⁶⁴ 346 Caucasian elite sprinters ²⁵
	rs1815739 CC	100 higher elite and elite climbers ⁶⁵
	rs1815739 C	1057 Japanese track and field athletes (627 sprint/power athletes and 430 endurance athletes) and 810 controls ⁶⁶
<i>SEMA4A</i>	rs12401573	1206 football players ⁶⁷
<i>TERT</i>	rs33954691	
<i>NFATC2</i>	rs4811192	
<i>PTPRK</i>	rs55743914	
<i>AGT</i>	rs699 C	Power athletes ⁷
<i>AMPD1</i>	rs17602729 C	Power athletes ⁷
<i>CKM</i>	rs1803285 G	Power athletes ⁷
<i>CNDP1</i>	rs2346061 C	908 Brazilian elite athletes (328 endurance, 415 power and 165 combat) and 967 non-athletes ⁵⁵
	rs2887 A	908 Brazilian elite athletes (328 endurance, 415 power and 165 combat) and 967 non-athletes ⁵⁵
<i>CNTFR</i>	rs41274853 AA	211 Japanese sprint/power track and field athletes and 814 Japanese controls ⁶⁸
<i>CREM</i>	rs1531550 A	Power athletes ⁷
<i>FTO</i>	rs9939609 A	1,329 Brazilians (677 sport combat athletes and 652 non-athletes) and 1,674 Russians (920 sport combat athletes and 754 non-athletes) ⁶⁹
<i>GALNT13</i>	rs10196189 G	Power athletes ⁷
<i>GBF1</i>	rs2273555 A	53 Russian and 76 Polish weightlifters ⁷⁰
<i>HIF1A</i>	rs11549465 T	Power athletes ⁷
<i>IL6</i>	rs1800795 G	Power athletes ⁷

Table 3.4. Cont.

Gene	Genetic or epigenetic factors	Athletes/reference
<i>KLF14</i>		176 Polish top-level power and endurance athletes (top-level athletes, including medallists of Olympic Games and World championships practicing power and endurance sports: 100 athletics, 21 speed skaters, 28 swimmers and 27 rowers) and 128 controls ⁷¹
<i>MLN</i>	rs12055409 G	53 Russian and 76 Polish weightlifters ⁷⁰
<i>MTHFR</i>	rs1801131 C	Power athletes ⁷
<i>NOS3</i>	rs2070744 T	Power athletes ⁷
	rs1799983	Elite athletes (48 water polo players, 22 rowers, 21 kayakers, 19 canoeists and 16 swimmers) ⁷²
<i>PPARA</i>	rs4253778 C	Power athletes ⁷
<i>PPARG</i>	rs1801282 G	Power athletes ⁷
<i>SOD2</i>	rs4880 G	Power athletes ⁷
<i>TRIM59</i>	DNA hypermethylation	176 Polish top-level power and endurance athletes (top-level athletes, including medallists of Olympic Games and World championships practicing power and endurance sports: 100 athletics, 21 speed skaters, 28 swimmers and 27 rowers) and 128 controls ⁷¹

3.2.1. Genetic polymorphisms associated with endurance-based sports

Seven polymorphisms previously associated with an endurance polygenic profile (*ACE* I/D, *ACTN3* rs1815739, *Adenosine monophosphate deaminase 1 (AMPD1)* rs17602729, *Creatine kinase, M-type (CKM)* rs1803285, *Hemochromatosis (HFE)* rs1799945, *Myostatin (MSTN)* rs1805086 and *PPARG coactivator 1 alpha (PPARGC1A)* rs8192678) were genotyped in 196 elite triathletes.¹³ Only the *AMPD1* endurance-optimal C allele was found to be significantly associated with an improvement in performance time ($P= 0.01$).¹³

An international consortium (GAMES)⁴⁷ was established to analyse the cardiorespiratory fitness and its response to exercise training, and to compare elite endurance athletes and ethnicity-matched controls. This resulted from a GWAS undertaken on two cohorts of elite endurance athletes and controls (GENATHLETE and Japanese endurance runners), where 45 promising markers were identified and tested for replication in seven additional cohorts of endurance athletes ($n=1520$, from which 835 took part in World Championships and/or Olympic Games) and 2760 controls, from Australia, Ethiopia, Japan, Kenya, Poland, Russia and Spain. This meta-analysis has demonstrated that only the rs558129 polymorphism at *Polypeptide N-acetylgalactosaminyltransferase like 6 (GALNTL6)* was a significant marker in all available studies ($P= 0.0002$).⁴⁷

Both *Insulin like growth factor 1 (IGF1)* rs35767 and *MSTN* rs1805086 polymorphisms have been associated with increased muscle mass, which are important for sprint and power events. The combined frequency of these genetic determinants was tested in 111 Israeli

track and field athletes (78 males and 33 females, age 17–50 years; 63 long distance runners and 48 power/sprint and long jump athletes) and 80 swimmers (49 males and 31 females, age 16–49 years; 38 long-distance and 42 short-distance) and 111 controls.³ It was observed that exhibiting both mutations was significantly higher ($P<0.05$) among long distance runners (17%) than in short distance runners (10%), long distance swimmers (8%), short distance swimmers (2%) and controls (7%). Presenting both mutations was significantly higher ($P<0.05$) among long-distance runners compared to short-distance swimmers (8% versus 2%, $P<0.05$); 40% of long-distance runners and 25% of long-distance swimmers carriers of both mutations were of elite level.³

A pilot study⁵² recruited 30 sub-elite Australian Football players aged from 17 to 19 years old that completed tests of endurance, power and technical skills. The *ACE* gene was a significant factor in predicting 'all round' athletic performance and skills; the DD genotype was as a predictor for endurance ($P= 0.001$), power ($P\leq 0.008$) and technical skills ($P\leq 0.003$). Others polymorphisms, in the *Brain-derived neurotrophic factor (BDNF)*, *D2 dopamine receptor (DRD2)* and *Catechol-O-methyltransferase (COMT)* genes were also identified as contributors to enhance skill outcomes ($P\leq 0.044$).⁵²

In a recent systematic review⁵¹ including case-controls, cohorts and genome-wide association designs with 3,889 elite endurance athletes (2,984 were marathon runners) and 6,109 controls of both genders and different ethnicities, 16 SNPs were studied in 14 genes (e.g., *NFIA antisense RNA 2 (NFIA-AS2)*, *ACE*, *Nuclear respiratory factor 1 (NRF1)*, among others). Endurance-based sports rely on several factors, mostly associated with cardiorespiratory function (the maximal rate of oxygen consumption, $\text{VO}_{2\text{max}}$), the proportion of slow-twitch fibres in skeletal muscle, aerobic power and technical skills. However, it is still unknown which genes are specifically associated with elite marathon performance due to small effects sizes and limited replication of genotype-phenotype associations.

3.2.2. Genes associated with power-based sports

The *ACE* encoded protein converts the angiotensin I hormone to angiotensin II,⁷¹ which regulates blood pressure and influences skeletal muscle function in a not completely understood form.^{74,76} The *ACE* I/D polymorphism is known to modify the circulating enzyme levels. There can be identified three genotypes at the *ACE* I/D polymorphism, namely DD, ID or II, with the DD being the most associated with the enzyme highest proportion of fast-twitch muscle fibres and greater speed and power/strength.⁶⁰

The *ACTN3* gene is almost exclusively presented in fast, glycolytic type II muscle fibres responsible for explosive contractions.⁵⁰ The *ACTN3* rs1815739 variant is the most studied in sports performance with the CC genotype (RR genotype) being associated with muscle

power and strength and the TT genotype (XX genotype) tending to be associated with endurance,⁷⁴ but less pronounced than the first.^{23,24,77,78}

A case-control study with 53 Russian and 76 Polish weightlifters of both genders demonstrated *Motilin* (*MLN*) rs12055409 G-, *Zinc finger protein 608* (*ZNF608*) rs4626333 G- and *Golgi brefeldin A resistant guanine nucleotide exchange factor 1* (*GBF1*) rs2273555 A alleles are associated with higher levels of strength, muscle mass and muscle fibre size.⁷⁰ In a study with 211 Japanese sprint/power track and field athletes (62 international, 72 national and 77 regional athletes) and 814 Japanese controls⁶⁸, the *Ciliary neurotrophic factor receptor* gene (*CNTFR*), one of the candidate genes contributing to sprint/power performance, was analysed. The rs41274853 AA genotype was significantly more frequent in international sprint/power athletes (19.4%) than in controls (7.9%, $P = 0.036$, OR=2.81 [95% CI: 1.43-5.55]). Reporter assays revealed that the miR-675-5p binds to this polymorphic region within the *CNTFR* mRNA, irrespective of the rs41274853 allele present.⁶⁸

The carnosinase genes (*Carnosine dipeptidase 1* (*CNDP1*) and *Carnosine dipeptidase 2* – (*CNDP2*)) play a key role in the homeostasis of carnosine (found in excitable tissues, such as skeletal muscle, heart and some brain regions) during high-intensity muscles contractions, in which rapid changes in the intramuscular metabolic profile can lead to muscular fatigue. They were investigated in 908 Brazilian elite athletes and in 967 non-athletes; athletes were stratified into endurance (n= 328), power (n= 415), and combat (n= 165).⁵⁵ The *CNDP2* rs6566810 (AA genotype) was overrepresented in international-level endurance athletes. The minor alleles of *CNDP2* rs3764509 (G allele), *CNDP1* rs2346061 (C allele) and *CNDP1* rs2887 (A allele) were more frequent in power-oriented athletes than in endurance-oriented athletes and non-athletes. These differences were higher between power and endurance athletes, thus, these polymorphisms may be favourable for power and strength type exercise. For *CNDP1* rs2346061 (C allele) no significant difference was observed in genotype frequencies between power and combat sports athletes, but the *CNDP1* rs2887 (A allele) showed an inverse distribution between these groups.⁵⁵ Therefore, the minor alleles of *CNDP2* rs3764509, *CNDP1* rs2346061, and *CNDP1* rs2887 are more likely to be novel genetic markers for power athletes.

A study comparing *ACTN3* rs1815739 genotypes and allele frequencies of 103 Chinese athletes (24.3±3.2 years old; 59 elite sprint/power athletes and 44 elite endurance athletes) and 50 controls (25.5±3.0 years old) has shown significant differences in the genotype distribution of the sprint/power athletes compared to endurance athletes ($P = 0.001$) and controls ($P < 0.001$).⁵⁰ Although there were no international-level sprint/power athletes with TT genotype, the CC genotype was significantly higher in international-level than in national-level sprint/power athletes ($P = 0.004$); sprint/power athletes carrying the CC

presented better performances than those with the T allele genotypes ($P= 0.004$ and $P= 0.001$, respectively).⁵⁰

A study of Orysiak and co-workers⁷⁹ involving 398 Caucasian male ($n= 266$, age 16.7 ± 2.1 years, training experience 7.7 ± 2.9 years) and female ($n= 132$, age 15.8 ± 2.0 years, training experience 6.6 ± 2.1 years) athletes from various sports (ice hockey, canoeing, swimming, and volleyball) examined the association between *ACE* and *ACTN3* polymorphisms, independently or in combination, and muscle strength and power components. Physical tests, such as countermovement jump, spike jump and muscle strength, were made. The genotype distribution of the *ACE* and *ACTN3* genetic determinants did not differ significantly between groups of athletes for either sex and there was no association between the polymorphisms' genotypes (alone or in combination) and sum of muscle strength, height of jump or mechanical power in both jump tests for male and female athletes.⁷⁹ Therefore, an influential role of these polymorphisms in determining power/strength performance of elite athletes was not observed.

Concerning the *ACTN3* gene, 100 higher elite and elite climbers (82 Polish, 12 Russian and 6 Austrian): 50 boulderers and 50 lead climbers of both genders (45 male and 5 female professional boulderers and 39 male and 11 female lead climbers) took part in a case-control study.⁶⁵ It was demonstrated that the rs1815739 CC genotype in the boulderers was significantly higher than in lead climbers and controls (62 vs. 26%; 33%, respectively; $\chi^2 = 17.230$, $P= 0.0017$) and frequencies of the C allele in boulderers differed significantly from lead climbers and controls (77 vs. 51%; 58%, respectively; $\chi^2 = 15.721$, $P = 0.0004$). In addition, the frequency of this allele in high elite boulderers was significantly higher than in elite ones (88% vs. 68%, $P = 0.025$).⁶⁵

The *Protein tyrosine phosphatase receptor type K* gene (*PTPRK*), which has been related to cell growth, differentiation, mitotic cycle, and oncogenic transformation, was included in a study with 1206 football players of both genders and unknown ethnicity through a micro-array data.⁶⁷ It was found that the *PTPRK* rs55743914 was significantly associated ($P= 7.7\times10^{-7}$) with the 5-m sprint test and all other three genes (namely *Semaphorin 4A* (*SEMA4A*), *Nuclear factor of activated T cells 2* (*NFATC2*) and *Telomerase reverse transcriptase* (*TERT*)) were significantly associated in the 20-m sprint test (rs12401573, $P= 7.8\times10^{-5}$; rs4811192, $P= 3.7\times10^{-5}$ and rs33954691, $P=6.6\times10^{-5}$, respectively).⁶⁷

A case-control conducted by Guilherme et al.⁶⁹ in 1,329 Brazilians (677 athletes and 652 non-athletes) and 1,674 Russians (920 athletes and 754 non-athletes) found that *FTO alpha-ketoglutarate dependent dioxygenase* (*FTO*) rs9939609 A allele was increased in Russian power athletes (i.e., the A allele presence was associated with an increased OR of being a Russian power athlete compared with non-athletes, Odds ratio (OR): 1.45, $P= 0.002$) and was also over-represented in combat sports athletes of heavier weight

categories (i.e., the allele was associated with an increased OR of being a combat sports athlete of heavier weight categories compared with non-athletes, OR: 1.79, $P=0.018$). The rs9939609 AA genotype was over-represented in strength and heavier athletes. Regarding the muscle fibres, the AA genotype (associated with an enhanced risk of excessive body mass and related phenotypes) had less slow-twitch muscle fibres than T allele genotypes carriers ($P=0.029$).⁶⁹

Power-based sports are influenced by muscle strength associated with fast, glycolytic type II muscle fibres responsible for explosive contraction, mechanical power and blood pressure regulation.

3.2.3. Genes and polymorphisms associated with both endurance- and power-based sports

A female-only study investigated the importance of *ACTN3* and *ACE* polymorphisms among 100 Hungarian elite female athletes from different sports: kayaking/rowing ($n=30$), handball/basketball ($n=29$), water polo ($n=27$) and combat sports (judo, wrestling, $n=14$).⁶¹ Significant differences were only found in *ACE* I/D polymorphism between water polo athletes (I= 61.11%; D=38.89%) and combat athletes (I= 35.71%, D=64.29%, $P<0.03$). Similar frequencies in the I allele were observed for water polo (61.11%) and kayaking/rowing (56.67%) athletes, but not significant, as well as, the *ACTN3* rs1815739 polymorphism showed no differences between sport groups.⁶¹

The association of the endothelial *Nitric oxide synthase 3* (*NOS3*) rs1799983 polymorphism with right ventricular structure in elite athletes highlights the role of this polymorphism and nitric oxide bioavailability in athletes' cardiac adaptation. The influence of this SNP in the right ventricular structure was studied in elite athletes, namely water polo players ($n=48$), rowers ($n=22$), kayakers ($n=21$), canoeists ($n=19$) and swimmers ($n=16$).⁷² Although the genotype distribution of the *NOS3* rs1799983 polymorphism was not significantly associated with sex or physical performance, athletes carrying the polymorphism (T allele) demonstrated increased right ventricular mass index (32 ± 6 g versus 27 ± 6 g, $P<0.01$) and larger right ventricular stroke volume index (71 ± 10 ml versus 64 ± 10 ml, $P<0.01$) than athletes with a GG genotype.⁷²

Ben-Zaken et al.⁵⁹ assessed the prevalence of the *MSTN* rs1805086 polymorphism across 185 Israeli track and field athletes (138 males and 47 females, age 17-50), 80 swimmers (49 males and 31 females, age 16-49) and 118 non-athletes (81 males and 37 females, age 19-29). From track and field athletes, 113 were long distance runners/5000m-marathon and 72 were power-event athletes/100-200m sprints and long-jumpers and; from swimmers, 38 were long-distance swimmers/800-1500m and 42 were short-distance swimmers/50-100 m. The *MSTN* rs1805086 CC genotype was not found in short distance-swimmers or

associated with top level swimming performance in long distance-swimmers.⁵⁹ Nevertheless, it was significantly higher in top-compared to national-level long-distance runners (26% versus 8%, $P < 0.05$), short distance runners (16% versus 9%, $P < 0.05$), and all runners combined (20% versus 8%, $P < 0.05$), but not in top- compared to national-level swimmers. Only 14.6% of track and field athletes ($n = 27$) and 8.8% of swimmers ($n = 7$) carried the rare rs1805086 C allele, and only 0.8% ($n = 2$) carried the CC genotype.⁵⁹

Orysiak et al.⁶³ tested the prevalence of *ACTN3* rs1815739 polymorphism in canoe sprint athletes (canoe and kayak paddlers) and performance results at 200- or 1000-m distance in 86 European white male canoe sprint athletes (32 canoe paddlers and 54 kayak paddlers, age = 15-34 years); 354 non-athletic controls were involved. Canoe and kayak paddlers carrying the rs1815739 TT genotype might provide some competitive advantage in performance at 1000m, since their OR was 2.95 (95% confidence interval [CI]: 1.37-6.35) compared to controls. However, it seems limited at 200m, because in non-successful 200-m athletes compared with controls, it was 2.64 (95% CI: 1.30-5.36).⁶³

A study⁵⁷ conducted with 159 elite athletes of track and field and/or triathlon (118 men and 41 women, mean age of 35.9 ± 12.2 years, 77 endurance and 82 power athletes) and 68 non-physically active controls evaluated the polymorphism *Insulin like growth factor 1 receptor (IGF1R)* rs1464430, which has been associated to exercise-related cardiac hypertrophy. The results have shown that the AA genotype was significantly higher ($P < 0.05$) in endurance athletes (49%) than in power athletes (33%). No significant differences were observed in the AA genotype among elite and national level endurance athletes (44% versus 52%, respectively). On the other hand, this genotype of power athletes was significantly lower in elite athletes than in national ones (17% versus 42%, respectively; $P < 0.05$).⁵⁷ These findings suggested that rs1464430 AA genotype may be beneficial for endurance sports, but not associated with elite endurance performance and that the genotype presence may be a disadvantage in power sports.

Heffernan et al.^{8,64} observed an association of the *ACTN3* rs1815739 polymorphism with playing position in elite rugby union athletes and concluded that inherited fatigue resistance was more prevalent in forwards and inherited sprint ability was overrepresented in backs, especially wings and full backs.

A literature search⁷ has shown that at least 155 genetic markers are linked to elite athlete status, from which 93 were related to endurance and 62 were associated to power/strength. In fact, 41 markers were found in GWASs with Russian, Japanese, Jamaican and African-American athletes⁷ and 17 genetic markers positive associations were found for endurance and athletic status, such as: *ACE* I-, *ACTN3* rs1815739 T-, *Adrenoceptor beta 2 (ADRB2)* rs1042713 A-, *Aquaporin 1 (Colton blood group) (AQP1)* rs1049305 C-, *AMPD1* rs17602729 A-, *Bradykinin receptor B2 (BDKRB2)* -9-, *Collagen type V alpha 1 chain*

(*COL5A1*) rs12722 T-, *GA binding protein transcription factor subunit beta 1* (*GABPB1*) rs12594956 A- and rs7181866 G-, *Homeostatic iron regulator* (*HFE*) rs1799945 G-, *Potassium inwardly rectifying channel subfamily J member 11* (*KCNJ11*) rs5219 C-, mtDNA H haplogroup, mtDNA K haplogroup (unfavourable), *Peroxisome proliferator activated receptor alpha* (*PPARA*) rs4253778 G-, *Peroxisome proliferator activated receptor delta* (*PPARD*) rs2016520 C-, *PPARGC1A* rs8192678 T- and *Uncoupling protein 3* (*UCP3*) rs1800849 T alleles. On the other hand, 14 genetic markers were associated to power/strength,⁷ namely *ACE* D-, *ACTN3* rs1815739 C-, *Angiotensinogen* (*AGT*) rs699 C-, *AMPD1* rs17602729 C-, *CKMM* rs1803285 G-, *cAMP responsive element modulator* (*CREM*) rs1531550 A-, *Polypeptide N-acetylgalactosaminyltransferase 13* (*GALNT13*) rs10196189 G-, *Hypoxia inducible factor 1 subunit alpha* (*HIF1A*) rs11549465 T-, *Interleukin 6* (*IL6*) rs1800795 G-, *Methylenetetrahydrofolate reductase* (*MTHFR*) rs1801131 C-, *NOS3* rs2070744 T-, *PPARA* rs4253778 C-, *Peroxisome proliferator activated receptor gamma* (*PPARG*) rs1801282 G- and *Superoxide dismutase 2* (*SOD2*) rs4880 G alleles. Also, in three or more studies, five genetic markers were associated to endurance (*ACE* I-, *ACTN3* rs1815739 T-, *HFE* rs1799945 G-, *PPARA* rs4253778 G- and *PPARG coactivator 1 alpha* (*PPARGC1A*) rs8192678 T alleles) and 7 were associated to power/strength (*ACE* D-, *ACTN3* rs1815739 C-, *AMPD1* rs17602729 A-, *HIF1A* rs11549465 T-, *MTHFR* rs1801131 C-, *NOS3* rs2070744 T- and *PPARG* rs1801282 G alleles).⁷

In a study conducted by Kikuchi et al.,⁶⁶ 1057 Japanese track and field athletes (627 sprint/power athletes and 430 endurance athletes) and 810 Japanese controls were studied and a linear and significant correlation was observed between the CC and CT genotypes of the *ACTN3* rs1815739 polymorphism in elite sprint/power athletes (regional: 71%, national: 81%, international: 84%; $P= 0.001$ for trend), and between the same genotypes and long-distance runners (regional: 65%, national: 72%, international: 82%; $P= 0.030$ for trend). Furthermore, elite sprint/power athletes demonstrated a higher frequency of the CC and CT genotypes of the *ACTN3* rs1815739 variant than controls (OR: 1.59, 95% CI: 1.16–2.18; $P= 0.003$).⁶⁶

Znazen et al.⁶² concluded that Tunisian athletes the *ACE* I/D polymorphism were associated with a high level of endurance and power performance, respectively, in 282 Tunisian athletes (149 endurance and 133 power) and 211 sedentary controls. They found significant differences in genotype and alleles between endurance and power athletes ($\chi^2= 10.32$, $P= 0.0057$; $\chi^2= 4,752$, $P= 0.029$, respectively); *ACE* I alleles higher predisposition for in 72 elite endurance-athletes than in controls ($\chi^2= 3.51$, $P= 0.06$); *ACE* genotype in endurance trained athletes and controls ($\chi^2= 6.05$, $P= 0.04$) and; *ACE* D and I alleles between elite power-athletes (n= 59) and controls ($\chi^2= 3.79$, $P= 0.05$).

Massidda et al.⁵⁸ tested the association between the *Malonyl-CoA-acyl carrier protein transacylase (MCT1)* rs1049434 polymorphism and fat-free mass in 128 Italian elite male soccer players and concluded that the rs1049434 T allele was associated with the percentage of fat-free mass. In addition, the percentage of fat-free mass was significantly higher in players with the TT genotype and in the T allele dominant model group (TT/AT) than in players with the AA genotype.⁵⁸

In a study about the association between the *ACE* I/D and *ACTN3* rs1815739 polymorphisms and blood pressure parameters modifications in 107 Caucasian male elite athletes (17 sprint/power from short-distance runners, swimmers < 200m, 36 endurance athletes from rowers, football players, middle distance swimmers and 54 athletes from mixed sports - water polo, handball and volleyball), Durmic et al.⁵³ found that athletes with the *ACE* DD genotype showed higher percentage of the systolic blood pressure decline after maximal incremental stress test, while those with the *ACTN3* rs1815739 CC genotype showed the lowest maximal systolic blood pressure values. Power athletes had a lower blood pressure recovery pattern compared to other participants, with the highest values of resting and maximal systolic blood pressure.⁵³

Heffernan et al.⁵⁶ tested 460 elite rugby union, 88 elite rugby league athletes and 565 non-athlete controls for 2 SNPs of the *COL5A1* gene (rs12722 and rs3196378) and concluded that C alleles, CC genotypes and resulting combinations of both rs12722 and rs3196378 C allele genotypes were important in achieving elite rugby performances and preventing sport injuries. In both SNPs, the injury-protective CC genotype and C allele were significantly higher in all athletes than in controls ($P \leq 0.01$ for rs12722 and $P \leq 0.02$ for rs3196378).⁵⁶

A case-control study with 413 Polish Caucasian elite athletes (age 23.5 ± 4.7 years, 36.3% females) and 451 Caucasian sedentary controls (age 23.0 ± 3.1 years, 51.9% females)⁵³ showed that the elite athletic performance was associated with 4 polymorphisms: *ACE* rs4341 ($P = 0.0095$), *Nuclear respiratory T factor-2 (NRF-2)* rs12594956 ($P = 0.011$), *Transcription factor A, mitochondrial (TFAM)* rs2306604 ($P = 0.049$) and *Fatty acid amide hydrolase (FAAH)* rs324420 ($P = 0.0041$). In addition, *ACE* D alleles ($P = 0.0021$) and *NRF-2* rs12594956 A allele ($P = 0.0067$) were positive predictors, whereas *TFAM* rs2306604 GG genotype ($P = 0.031$) and *FAAH* rs324420 AA genotype ($P = 0.0084$) negatively affect the elite athletic performance.⁵⁴ The *Cholinergic receptor nicotinic beta 3 subunit (CHRN3)* rs4950 G allele involved in coding proteins modulating activity of brain's emotion centres was significantly more frequent in endurance athletes than in power ones ($P = 0.025$); it was highlighted that this gene even contributed to endurance performance ($P = 0.0047$).⁵⁴

Another case-control study in 176 Polish athletes (top-level athletes, including medallists of Olympic Games and World championships practicing power and endurance sports: 100 athletics, 21 speed skaters, 28 swimmers and 27 rowers) and 128 healthy controls of both

genders, developed a 5-CpG model, which is an epigenetic analysis investigating the DNA methylation status. This prediction model revealed an age acceleration of elite athletes ($P=1.503 \times 10^{-7}$) and that was more significant amongst power athletes ($P=1.051 \times 10^{-9}$),⁷¹ demonstrating a higher epigenetic age in athletes, which is contrary to epidemiological data showing their longer life duration. In fact, there was an accelerated DNA hypermethylation for two CpG sites in *Tripartite motif containing 59 (TRIM59)* and *Kruppel like factor 14 (KLF14)* genes. Both markers predicted elite athletes to be several years older than controls and this effect was more substantial in subjects involved in power (*KLF14*, $P=2.116 \times 10^{-8}$ and *TRIM59*, $P=1.239 \times 10^{-10}$) than in endurance sports (*TRIM59*, $P=0.003$ and *KLF14*, $P=3.345 \times 10^{-7}$).⁷¹ However, the two aforementioned genes are involved in anti-tumor and anti-inflammatory activities, respectively, which in turn could affect better life expectancy. Although these studies were both conducted in Polish elite athletes, their main purposes were different and consequently, genes sport-related investigated were distinct.

3.2.4. Genes associated with sex and ethnicity differences

Sex (as a biological genetic difference between women and men) is one major genetic factor that dictates athletic performance, especially in sports objectively measured by weight, distance or time.²⁰ Nowadays, only few sports admit women and men directly competing against each other in the same championship (sailing, equestrian). Men and women's sports performances are considered different in many other sports and sex distinction is understood as necessary.²⁰ However, the basis for those underlying variations is not yet well discussed. Table 3.5 presents gene variants associated to sex and ethnicity-related to athletic performance.

Table 3.5. Gene variants for sex and ethnicity and psychological traits.^{25,80-84}

Sex and ethnicity-related markers		
<i>Gene</i>	<i>Genetic factors</i>	<i>Athletes/reference</i>
<i>ACTN3</i>	rs1815739	346 Caucasian female and male and 91 African lineage male elite sprinters ²⁵
<i>ACVR1B</i>	rs2854464 A	Meta-analysis ⁸⁰ 1672 athletes (482 endurance, 578 sprint/power, 498 mixed) and 1089 controls from both Europe (Caucasian from Italy, Poland and Russia) and Brazil ⁸¹
<i>ACE</i>	DD	346 Caucasian female and male and 91 African lineage male elite sprinters ²⁵
Psychological traits-related markers		
<i>Gene</i>	<i>Genetic factors</i>	<i>Athletes/reference</i>
<i>FEV</i>	rs860573	621 elite athletes (212 endurance, 183 power and 226 combat) and 672 controls ⁸²

Table 3.5. Cont.

Psychological traits-related markers		
<i>Gene</i>	<i>Genetic factors</i>	<i>Athletes/reference</i>
<i>MAOA</i>	9/9	50 power athletes and 100 controls ⁸³
<i>MSTN</i>		50 power athletes and 100 controls ⁸³
<i>SLC6A3</i>		50 power athletes and 100 controls ⁸³
<i>SLC6A4</i>		50 power athletes and 100 controls ⁸³ 133 Italian male elite athletes ⁸⁴

A meta-analysis studied the role of the *ACTN3* rs1815739 polymorphism in elite power athletes and yielded 44 studies from 38 articles observed that the R allele was associated with Western and female athletes, whereas the RX genotype was associated with Asian and male athletes.⁸⁰

Furthermore, it appears that phenotype-genotype associations may be ethnicity-dependent as shown in a cohort of 1672 athletes (482 endurance, 578 sprint/power, 498 mixed) and 1089 controls from both Europe (Caucasian from Italy, Poland and Russia) and Brazil, where the *Activin A receptor type 1B (ACVR1B)* rs2854464 A allele was associated with sprint/power performance in Caucasians, but not in Brazilian athletes.⁸¹ This allele was overrepresented in sprint/power athletes compared with controls in Caucasian sample (adjusted $P=0.048$), but no differences were observed in the genotype distribution between endurance athletes and controls, in neither the Brazilian nor the Caucasian samples (adjusted $P>0.05$).⁸¹

In a case-control conducted in 346 elite sprinters: 255 Caucasian (66 female and 189 male) from Australia, Greece, Poland, Lithuania and Russia and 91 African lineage male from Brazil, Jamaica, US, Italy and Spain, the athletes' genotypes of *ACTN3* rs1815739 and *ACE* ID polymorphisms were investigated.²⁵ The *ACTN3* rs1815739 CC or the *ACE* DD genotype were better associated with faster best 200-m sprint times than rs1815739 TT (21.19 ± 0.53 s vs. 21.86 ± 0.54 s, $P=0.016$) and *ACE* II (21.33 ± 0.56 s vs. 21.93 ± 0.67 s, $P=0.004$) and Caucasian sprinters with the *ACE* DD genotype had faster best 400-m sprint time than their *ACE* II counterparts (46.94 ± 1.19 s vs. 48.50 ± 1.07 s, $P=0.003$).²⁵ Although there was no statistical significant difference in 100-m sprint performance across the *ACTN3* rs1815739 and *ACE* I/D genotypes in Caucasian and African lineage sprinters, elite male African lineage athletes showed some evidence for the prevalence of the *ACTN3* rs1815739 C allele and 200 m sprint speed (20.53 ± 0.64 s for CC and 20.98 ± 0.72 s for CT genotypes).²⁵ Exactly, current relevant World records were different across athletes' sex and ethnicity with female and male African ancestry athletes presenting better times in 100-m, 200-m and 400-m than their Caucasian counterparts.²⁵

3.2.5. Genes associated with psychological traits

It has been suggested that the dopamine neurotransmitter plays a key role in reaching high-level performances through emotional control and psychological management (Table 3.5). Filonzi et al.⁸³ analysed four genes involved in muscles development (*MSTN*) and behavioural insights (the serotonin transporter gene known as *Solute carrier family 6 member 4* (*SLC6A4*), also called *SERT* or *5HTT*, the dopamine transporter gene known as *Solute carrier family 6 member 3* (*SLC6A3*), also called *DAT*, and the *Monoamine oxidase A gene* (*MAOA*) regarding athletic performance. These latter are considered to be directly involved in the management of aggressiveness, anxiety and fear. Fifty elite athletes (soccer, n= 4; basketball, n= 10; tennis, n= 6; volleyball, n= 6; canoeing, n= 2; rugby, n= 10; baseball, n= 6 and track and field, n= 6 from speed running, javelin and shot put) and 100 control athletes were studied. Athletes and controls belonged to Caucasian, Afro-American, Afro-European and Maori ethnicities. The *DAT* exon 15 variable number of tandem repeats (VNTR) genotype and allele frequencies were determined with the VNTR copy number varying between 3 and 11. However, only the 9 and 10 repeats were the most common forms in elite athletes and controls with the heterozygotes 9/10 (elite athletes 52.0% vs. controls 49.0%) and homozygotes 10/10 (elite athletes 22.0% vs. controls 39.0%) being the most frequent genotypes. Furthermore, a significant correlation between the *DAT* genotype 9/9 and allele 9 and elite sport performances was observed, with elite athletes presenting a genotype 9/9 much higher (24.0%) than controls (5.0%, $\chi^2= 11.22$, $P< 0.001$) and the 9-repeat was almost twofold in elite athletes (51.0%) than in controls (29.5%, $\chi^2= 11.35$, $P< 0.001$).⁸³ However, no association was found between muscle development regulation or serotonin pathway and elite performances.

The *SLC6A4* gene encodes the serotonin transporter protein (5-HTT) which is responsible for removing the serotonin from the synaptic cleft. The promoter region of the *SLC6A4* shows a polymorphism with short (s) and long (l) repeats in a region, namely 5-HTT-linked polymorphic region (5-HTTLPR). In spite of the 5-HTTLPR long allele being related to the higher expression of the gene product, the short allele has a lower expression, but is indeed associated with neuroticism behaviours (such as depression and anxiety). In fact, either one or two copies of the short allele present significantly greater levels of neuroticism than those homozygous for the long allele.⁸⁴ A case-control study with 133 Italian male elite athletes (basketball, soccer and hockey) found that the 5HTTLPR s/s genotype was associated with neuroticism ($P< 0.001$) and adverse sport related stress, namely tension/anxiety symptoms ($P< 0.02$), cognitive anxiety and emotional arousal control ($P<0.01$).⁸⁴

The *FEV transcription factor*, *ETS family member* (*FEV*) rs860573 polymorphism as a transcription factor exclusively expressed in neurons of the central serotonin system was

significantly differentiated ($P= 0.26 \times 10^{-5}$) in all groups of elite athletes (212 endurance, 183 power and 226 combat), regardless of discipline, from 672 sedentary controls.⁸² Therefore, it appears athletic performance may be influenced by the serotonergic pathway.

3.2.6. Genes associated with sport injuries

It is well-known the devastating impact of sports injuries and/or traumas in the elite athletic career.⁸⁵ In addition, very little has been investigated about the interaction of gene-environment in the potential injury risk among the athletic population, in particular, due to the small evidence on proposed candidate genes associated with bone mineral density (BMD) (Table 3.6). The SNPs may be important in the regulation of bone remodelling and adaptation to mechanical loading and for the stress fracture injuries' prevention and treatment,⁹⁴ namely through the adaptation of exercise training and diet.⁹⁵

Table 3.6. Gene variants for sports injuries.⁸⁶⁻⁹⁴

Gene	Genetic factors	Athletes/reference
<i>ACTN3</i>	rs1815739 CC	37 top Israeli long-distance runners of Ethiopian origin, 76 Israeli non-Ethiopian origin runners of Caucasian origin and 55 Israeli nonathletic controls ⁸⁶
<i>ESR1</i>	rs9340799 A	151 elite and pre-elite dancers and controls ⁸⁷
<i>FCRL3</i>	rs7528684	271 Brazilian volleyball players of both genders (146 tendinopathy cases and 125 controls) and of different ethnicities ⁸⁸
<i>VDR</i>	rs2228570 FF rs2228570 F	44 Japanese track and field athletes ⁸⁹ 84 male weight-bearing athletes, 48 male swimmers and 80 male controls ⁹⁰
<i>IGFBP</i>	3C	130 track and field athletes and 93 swimmers ⁹¹
<i>IL6</i>	rs1800795	37 top Israeli long-distance runners of Ethiopian origin, 76 Israeli non-Ethiopian origin runners of Caucasian origin and 55 Israeli nonathletic controls ⁸⁶
<i>LRP5</i>	rs1800795 C rs2508836 C GCGT/GCAG haplotypes	130 track and field athletes and 93 swimmers ⁹¹ 151 elite and pre-elite dancers and controls ⁸⁷ 151 elite and pre-elite dancers and controls ⁸⁷
<i>MCT1</i>	rs1049434 AA	37 top Israeli long-distance runners of Ethiopian origin, 76 Israeli non-Ethiopian origin runners of Caucasian origin and 55 Israeli nonathletic controls ⁸⁶
<i>PPARD</i>	rs2016520 CC	37 top Israeli long-distance runners of Ethiopian origin, 76 Israeli non-Ethiopian origin runners of Caucasian origin and 55 Israeli nonathletic controls ⁸⁶
<i>PARGC1A</i>	rs8192678 TT	37 top Israeli long-distance runners of Ethiopian origin, 76 Israeli non-Ethiopian origin runners of Caucasian origin and 55 Israeli nonathletic controls ⁸⁶
<i>P2RX7</i>	rs1718119 rs3751143	99 British elite academy male footballers ⁹² 210 Israeli Defense Forces military conscripts and 518 elite athletes ⁹³ 99 British elite academy male footballers ⁹²

Table 3.6. Cont.

Gene	Genetic factors	Athletes/reference
<i>RANK/RANKL/OPG</i>		
<i>LINC02341</i>	rs9594738	99 British elite academy male footballers ⁹²
<i>RANKL</i>	rs1021188	
<i>FABP3P2</i>	rs9594759	
<i>SOST</i>	rs1877632	99 British elite academy male footballers ⁹² 518 elite athletes (mainly Caucasians), 449 male and 69 female ⁹⁴
<i>VDR</i>	rs10735810 rs731236	518 elite athletes (mainly Caucasians), 449 male and 69 female ⁹⁴

Given the importance of the *vitamin D receptor* gene (*VDR*) with BMD or bone turnover, the *VDR* rs2228570 polymorphism was analysed using endonuclease FokI among 84 male weight-bearing athletes, 48 male swimmers and 80 male controls. The presence or absence of the FokI site was denoted as f/F, respectively, and participants were categorized as ff or FF homozygotes and Ff heterozygotes. A higher total BMD in weight-bearers than controls was found in the FF (7.7%) and Ff (6.9%), but not ff (1.8%) genotypes; however, lower total BMD was only observed in the FF (-4.5%) genotype when comparing swimmers with controls.⁹⁰ These general findings were also observed in 44 Japanese track and field athletes, who showed a higher bone volume in those with the FF genotype, but not in those with the Ff genotype.⁸⁹ Therefore, it seems that participants with the FF genotype may be more responsive to mechanical loading, which results in greater BMD. Exactly, the F allele of the rs2228570 polymorphism, demonstrates greater transactivation (protein expression) and, thus, increased biological activity, including intestinal absorption of calcium, than the f variant, which could explain higher BMD in individuals with the FF genotype.⁹⁶

It is known that the *IGF-1* has a crucial role in muscle growth and function and one of the most significant IGF binding protein is the IGFBP-3, which binds almost 90% of circulating *IGF-1*.⁹¹ It has been observed that the IGFBP3 promoter region A-202C polymorphism may influence the IGFBP-3 circulating levels and modulate the *IGF-1* activity with the A allele being significantly more promoter of activity than the C allele, which results in higher circulating IGFBP-3 levels (AA > AC > CC).⁹¹ In addition, chronic overproduction of the interleucine-6 (IL-6) is associated with reduced IGFBP-3 levels. A study conducted by Ben-Zaken et al⁹¹ with 130 track and field athletes and 93 swimmers found that the prevalence of carrying both IL-6C and IGFBP3C mutations was significantly higher among long-distance swimmers (44%) than in long distance runners (21%, $P < 0.01$), and in short distance swimmers (49%) in relation to sprinters and jumpers (28%, $P < 0.05$).⁹¹ The authors hypothesise that carrying the IGFBP3C polymorphism is needed to compensate for the

potential genetically non-beneficial effect of the higher IL-6C genotype and attenuated IGF system.

Varley et al.⁹³ have observed an association between independent *Purinergic receptor P2X 7 (P2RX7)* polymorphisms with stress fracture injury in 210 Israeli Defense Forces military conscripts and 518 elite athletes. The minor allele of *P2RX7* rs3751143 (C allele) was associated with stress fracture injury in military conscripts and in elite athletes ($P < 0.05$), while the minor allele of *P2RX7* rs1718119 (A allele) was associated with a reduced occurrence of stress fracture injury in both cohorts ($P < 0.05$).⁹³

In another case-control study with 99 British elite academy male footballers,⁹⁰ seven genes (*TNF receptor superfamily member 11a (TNFRSF11A)*, also known as *RANK*), *TNF superfamily member 11 (TNFSF11)*, also known as *RANKL*, *P2RX7*, *Wnt family member 16 (Wnt16)*, *Sclerostin (SOST)*, *MP3K* and *IL6*) and 10 SNPs were analysed, and it was found that *SOST* rs1877632 (trabecular density), *P2RX7* SNPs rs1718119 (cortical thickness and cross sectional area-CSA) and rs3751143 (less bone strength, CSA, cortical CSA and periosteal circumference) and *RANK/RANKL/ TNF receptor superfamily member 11b (TNFRSF11B)*, also known as *OPG* SNPs rs9594738 (periosteal circumference), rs1021188 (cortical thickness and CSA) and rs9594759 (cortical density) were associated with bone phenotypes ($P < 0.05$).⁹² However, these associations were only demonstrated before and not after a 12-week period of increased football training volume. Therefore, no association between genotype and changes in bone parameters over time was shown.

Furthermore, a sample of 518 elite athletes (mainly Caucasians), 449 males and 69 females (mean age 24.2 ± 5.5 years) from the SFEA (Stress Fracture Elite Athlete) cohort, including football, cricket, track and field, running events, rowing, boxing, tennis and others and controls was evaluated.⁹⁴ Genotype was determined using a proprietary fluorescence-based competitive allele-specific polymerase chain reaction assay. The *SOST* rs1877632 and *VDR* SNPs rs10735810 and rs731236 were associated with stress fracture ($P < 0.05$) and homozygotes of the rare allele of rs10735810 and rs731236 showed an association with stress fracture when compared to those homozygotes for the common allele combined with heterozygotes ($P = 0.03$ and $P = 0.01$, respectively).⁹⁴

In a case-control study conducted in 271 Brazilian volleyball players of both genders (146 tendinopathy cases and 125 controls) and of different ethnicities,⁸⁸ *Fc receptor like 3 (FCRL3)* rs7528684 and *Forkhead box P3 (FOXP3)* rs3761549 were studied for tendinopathy risk. The *FCRL3* rs7528684 polymorphism was associated with increased tendinopathy risk (OR = 1.44; 95% CI = 1.02-2.04) and together with knowledge of potential risk factors (age, gender and years playing) could be used to personalize elite athletes' training or treatment by minimizing pathology development risk.⁸⁸

Some elite athletes, such as gymnasts, dancers, swimmers (among others) are at increased risk for low BMD. If untreated, it can lead to early osteoporosis, normally associated to other risk factors, namely low body weight, circadian rhythm disruption, poor sleep, menstrual disturbances and low energy availability.⁹⁷⁻¹⁰⁰ A genetic association study in 151 elite and pre-elite dancers and controls found a very high prevalence of low BMD ($52.98 \pm 7.96\%$) in elite dancers, which was associated to genetic polymorphisms in the oestrogen receptor and Wnt/ β -catenin pathways, in particular, the *Estrogen receptor 1 (ESR1)* rs9340799 A allele, *LDL receptor related protein 5 (LRP5)* rs2508836 C allele and *LRP5* GCGT/GCAG haplotypes at impact sites (lumbar spine and femoral neck) and non-impact site (forearm).⁸⁷ Israeli runners of Ethiopian origin demonstrated a higher prevalence of the *PPARD* rs2016520 CC+*PARGC1* rs8192678 TT genotypes associated with improved endurance performance than Israeli runners (24%) of non-Ethiopian origins (3%, $P < 0.01$); a lower frequency of the *Acyl-CoA synthetase long chain family member 1 (ACSL1)* rs6552828 AA genotype favouring endurance trainability (8 vs. 20%, respectively, $P < 0.05$); a greater frequency of the *ACTN3* rs1815739 CC genotype associated with sprint performance (35 vs. 20%, respectively, $P < 0.05$); a greater frequency of the *MCT1* rs1049434 AA genotype associated with improved lactate transport (65 vs. 45%, respectively, $P < 0.05$); and a lower frequency of *IL6* rs1800795 C allele, associated with reduced post-exercise muscle damage (27 vs. 40%, respectively, $P < 0.01$).⁸⁶ The genetic dominance of Israeli long-distance runners of Ethiopian origin was associated to both endurance and speed-correlated polymorphisms and greater training recovery ability.

Nevertheless, the stress fracture injury prevalence, the most sport injury studied, might be associated to a genetic predisposition associated to the mechanical loading from the intense physical exercise and the outcome of such an interaction for BMD and the potential injury risk.⁹⁵ Therefore, changes on training volume and intensity, recovery or rehabilitation plans based on genetic characteristics could have substantial outcomes on the athletes' health and performance.

3.3. Conclusion and future perspectives

Despite being poorly understood, athletic performance, as all complex traits, is modulated by the complex interaction between genetic and environmental factors¹⁰¹ with the athletic potential and the response ability to training being predetermined by inherited traits. Many of the identified gene variants may be false positive, since they were reported by studies with small cohorts, being the only established genetic variant, the polymorphism *ACTN3* rs1815739. Nevertheless, the combination of the existent GWAS addressing the genetic architecture of athletes and the subsequent replication and validation studies might be

useful for additional data based on previous studies related to others traits or diseases.¹⁰²⁻¹²¹

The study of gene variants associated to sex and ethnicity-related to athletic performance needs further investigation. Athletic performance may be influenced by the serotonergic pathway and the potential injury risk (namely stress fracture) might be associated to a genetic predisposition associated to the mechanical loading from the intense physical exercise.

In this context, genetic studies with larger cohorts of single-sport athletes would be preferable than studies combining athletes of different sports, given the existence of distinct athlete profiles and sport demands.

Although genetic and epigenetic determinants may improve athlete's performance contributing to elite sport practice and high achievements, the importance of dedicated training and coaching, adequate nutrition as well as appropriate prophylactic measures concerning sports injuries is undisputable. In fact, a strong combination of these factors may be the difference between achieving a world record or just participating in the final.

Therefore, future studies in this field are required and important aspects should be considered, namely study designs, sample size and cohort stratification, as well as the quality of genetic tools to be used, particularly in none previously studied physical traits (e.g., coordination, flexibility, eyes and hair colour, hand length and shoe number) or poorly studied ones (e.g., psychological profile - temperament). These studies should aim to support and give athletes, coaches and other sports-related professionals, the possibility to pursue athletic performance improvements based on their unique genetic profile.

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4 *FAAH* rs324420 polymorphism is associated with performance in elite rink-hockey players

4.1. Introduction

4.2. Materials and Methods

4.2.1. Participants

4.2.2. Procedures

4.2.2.1. Anthropometric profile

4.2.2.2. Training data, sport experience and sport injuries history

4.2.2.3. Sample collection and DNA extraction

4.2.2.4. Polymorphism selection and genotyping

4.2.2.5. Statistical analysis

4.3. Results

4.3.1. Characteristics of elite rink-hockey players

4.3.2. Genotype frequencies in elite female and male rink-hockey players

4.3.3. Univariate and multivariate analysis of *FAAH* rs324420 and relevant factors of elite performance in rink-hockey

4.4. Discussion

4.5. Conclusions

4.6. References

4.1. Introduction

Elite athletes are thought to have an exceptional genetic potential [1] with incredible interindividual variability of physical performance traits (i.e., power, strength, endurance, muscle fiber composition and size, neuromuscular coordination and flexibility), personality profile [2], VO_2 max (maximum oxygen consumption) and injury susceptibility [3].

According to Silva and coworkers [4], genetic factors play a key role in athletic success as they optimize gifted athletes' achievements in comparison with less talented athletes. Additionally, the athletes' training regime is also determinant in athletic accomplishments, i.e., the more intense the training, the better the results.

Remarkably, similar elite athletes in terms of physical characteristics can have distinct performance, with only few developing high-level performance abilities, making elite performance rare [4]. In fact, the decisive factor of outstanding performances is based on the athlete's ability to perform not only optimal physical abilities but also emotional and mental competences [5].

The physical performance of athletes has been linked to more than 200 genetic variants, among which 155 correlate with elite athlete status [2]. In opposition, genetic foundations of competition-facilitating behavior and low stress response that influence elite performances are still scarce [4–7]. Additional investigation involving elite athletes is crucial to improve training and competition environments and provide more holistic treatment and recovery methods, especially in sports with a high risk of injury, as is the case of rink-hockey [8].

Rink-hockey is a high-intensity intermittent sport with noncontinuous movements at different speed levels and incomplete recovery periods [9,10]. Its effective playing time is 50 min, divided over two periods of 25 min (in categories of seniors, under 23 and under 19) [11], requiring regular endurance training to build tolerance for strenuous exercise and aerobic and anaerobic metabolisms (high-energy phosphate and glycolysis), as well as improving cardiovascular and musculoskeletal systems [9]. Furthermore, rink-hockey daily practice promotes morphological adaptations in the muscle spindle, which induce plastic changes in the central nervous system, decreasing the latency of the stretch reflex response and increasing its amplitude [12]. In particular, the player's repetitive movements induce repetitive afferent inputs from the mechanoreceptors that over time promote plastic changes in the cortex [13], improving neuro-muscular efficiency, which is positively correlated with the sport competition level achieved [12].

In addition to these physical demands, this invasion game also requires a variety of mental abilities [14], particularly optimal decisions making, especially in the stressful last 15 min, when direct free hits, penalty shots and other one-against-one/two and three situations are decisive for the game final score.

Furthermore, given the high risk of potential injuries, mental abilities in the setting of rink-hockey are also required in order to cope with injuries and build pain tolerance [8,15]. In addition, rink-hockey goalkeepers are being increasingly considered of tremendous importance for teams' decisive points, who should also be insightful in reading the game and resilience [16].

Therefore, psychological qualities such as planning skills in line with the game strategy, stress coping, anxiety management, resilience and avoiding impulsivity are determinant in athletic performance training and competition to achieve success [5]. Unfortunately, despite being a quite popular sport worldwide, there are few published studies on rink-hockey [15,17], among which very few are on genetics [4,18].

Previous studies have shown that genetic determinants among *Angiotensin-I-Converting Enzyme* (ACE), *Actinin Alpha 3* (ACTN3), *Angiotensinogen* (AGT), *NFE2-like BZIP Transcription Factor 2* (NRF-2), *PPARG Coactivator 1 Alpha* (PPARGC1A), *Peroxisome Proliferator-Activated Receptor Gamma* (PPARG) and *Transcription Factor A, Mitochondrial* (TFAM) genes are associated with sport-type (for instance endurance versus power) [2,5]. However, results are conflicted, and few genetic variants are linked to psychological traits and sport injuries [4] in relation to elite sport performance. Therefore, the present study aims to analyze genetic variants in genes coding for proteins potentially modulating the functioning of the brain's center of emotions, located at the hypothalamic–pituitary–adrenal axis, especially those highly associated with the limbic system and their physiological pathways to adequately control a stress response [5]. For instance, the *Fatty Acid Amide Hydrolase* (FAAH) rs324420 is a polymorphism thought to play roles in neural functions and has been previously found to be associated with athletic performance in shaping anxiety-like behavior and affecting leadership and persistence [5,6]. To provide more insights into the topic, this study was designed to examine the impact of some of these genetic markers in a sample of elite rink-hockey players, particularly constituted by athletes competing in the Portuguese national championship (one of the world's most competitive leagues, with the national team being the current world champion) [19], and test for any evidence of association with athletic performance.

4.2. Materials and Methods

4.2.1. Participants

Rink-hockey players who participated at the elite level (i.e., competing at any national team or other high-level representative teams in any sport organized by a National Sports Federation) [20], in the National Rink-Hockey Championship in Portugal during the sport seasons of 2019/2020, 2020/2021 and 2021/2022, were recruited to this study ($n = 116$). These athletes are the world's top best rink-hockey players, as more than 85% of them are

cumulatively from the best four world teams and the best five Portuguese teams, as recently published [21].

The participants were selected considering the following inclusion criteria: (1) being a minimum national-level athlete with an age equal or greater than 18 years old; (2) being an experienced competitor at the national level (i.e., a representative cohort of the world elite athletes competing in Portugal); and (3) having already reached a higher competitive level (i.e., international competitions, such as World, European or Continental Championships). The athletes included in the study had rink-hockey experience for 23.3 ± 8.8 years and at the time of recruitment they were undergoing training programs of 5 to 6 days per week with an average of 2.4 ± 0.9 h per day, with a total of 13.8 ± 6.3 h/week. Additional characteristics of the participants are described in Table 4.1.

Table 4.1. Elite rink-hockey players' characteristics (n = 116, 18 females and 98 males).

Characteristics	Female (n = 18)	Male (n = 98)	Total (n = 116)	p
Age (years) *	25.3 $\pm$ 7.9	28.8 $\pm$ 8.7	28.2 $\pm$ 8.7	0.117
Body mass (kg) *	67.4 $\pm$ 8.7	82.8 $\pm$ 10.5	79.0 $\pm$ 12.0	0.000
Height (m) *	1.73 $\pm$ 0.1	1.85 $\pm$ 0.1	1.82 $\pm$ 0.1	0.000
BMI (kg/m ²) *	22.7 $\pm$ 2.6	24.3 $\pm$ 2.1	24.0 $\pm$ 2.2	0.005
WHR *	0.82 $\pm$ 0.06	0.88 $\pm$ 0.11	0.87 $\pm$ 0.11	0.015
Hand length (cm) *	22.3 $\pm$ 2.6	23.5 $\pm$ 2.0	23.3 $\pm$ 2.1	0.029
Training experience (years) *	19.4 $\pm$ 8.6	24.0 $\pm$ 8.7	23.3 $\pm$ 8.8	0.039
Training frequency *				
days/week	4.6 $\pm$ 0.8	5.9 $\pm$ 0.8	5.7 $\pm$ 0.9	0.000
hours/day	2.4 $\pm$ 0.9	2.4 $\pm$ 0.9	2.4 $\pm$ 0.9	0.041
hours/week	11.4 $\pm$ 6.5	14.3 $\pm$ 6.2	13.8 $\pm$ 6.3	0.000
Participations in regional and national teams *	26.44 $\pm$ 13.16	42.38 $\pm$ 37.78	39.9 $\pm$ 35.5	0.080
	Sport injury, n (%)			
No	17 (94.4)	51 (52.0)	68 (58.6)	0.002
Yes	1 (5.6)	47 (48.0)	48 (41.4)	
	Nationality, n (%)			
Portuguese	18 (100)	78 (79.6)	96 (82.8)	0.113
Others	--	20 (20.4)	20 (17.2)	
	Ethnicity, n (%)			
Caucasian	17 (94.4)	96 (98.0)	113 (97.4)	0.956
Others	1 (5.6)	3 (3.0)	3 (2.6)	

* Data presented as mean $\pm$ standard deviation. BMI: body mass index, WHR: waist circumference/hip circumference ratio. Bold values represent significant results ($p < 0.05$).

Table 4.1. Cont.

Characteristics	Female (n = 18)	Male (n = 98)	Total (n = 116)	p
Profession, n (%)				
Athletes	--	45 (45.9)	45 (38.8)	<0.001
Student	10 (55.6)	25 (25.5)	35 (30.2)	
Teacher	2 (11.1)	11 (11.2)	13 (11.2)	
Physical therapist	2 (11.1)	--	2 (1.7)	
Sport coordinator	--	1 (1.0)	1 (0.9)	
Coach	--	1 (1.0)	1 (0.9)	
Podiatrist	1 (5.6)	--	1 (0.9)	
Other	3 (16.7)	15 (15.3)	18 (15.5)	

Bold values represent significant results ($p < 0.05$).

All athletes gave their informed consent to take part in this study and it was approved by the Ethical Committee of the University Fernando Pessoa (Porto, Portugal, Reference: CEUFP05062017).

4.2.2. Procedures

Data regarding anthropometric indicators/measurements, training conditions, sport experience and sport injuries history was collected using questionnaires personally administered to each participant before the training session by the same trained researcher.

4.2.2.1. Anthropometric profile

Body mass was measured by a digital scale (SECA-872, Hamburg, Germany) to the nearest 0.01 kg wearing T-shirt and gym shorts before training. Height was determined with a portable stadiometer (SECA-213, Hamburg, Germany) to the nearest 0.1 cm. Procedures were conducted as recommended by the International Society for the Advancement of Kinanthropometry [22]. Body mass index (BMI) was calculated as a ratio of weight to the squared height (kg/m^2). Hand length was assessed with a flexible tape from the marked mid-stylian line to the most distal point of the third digit with the hand in a supinated position and fingers extended. The waist circumference (WC) was measured with a flexible tape at the end of a normal expiration at the smallest circumference between the thorax and the hips. The hip circumference (HC) was measured with a flexible tape at the largest circumference on trochanters and waist/hip ratio (WHR) was calculated.

4.2.2.2. Training data, sport experience and sport injuries history

Several parameters were obtained, namely the number of training sessions per week and the number of hours of training sessions per day, which were further used to calculate the

number of hours of training per week. Sport experience included the athlete's participations in regional and national teams, as well as national and international achievements. The elite athletic performance or success was defined according to the total number of national and international titles of the players. Based on tertiles, athletes were categorized as super athletes, i.e., if they had more than 11 titles (the highest tertile). Sport injury history was also collected (including the year of occurrence, anatomical part and time of recovery from first severe sport injury) [15].

4.2.2.3. Sample collection and DNA extraction

Genomic deoxyribonucleic acid (DNA) was extracted from buccal cells samples of both sides (left and right) using sterile swabs (FL Medical, Hamburg, Germany), as previously described [23], through the use of the commercial kit QIAamp DNA Blood Mini Kit, Qiagen. Concentration and purity of DNA samples were assessed spectrophotometrically (NanoDrop Lite spectrophotometer, Thermo Scientific®, Waltham, MA, USA). DNA samples with a concentration equal to or higher than 4 ng/μL and with a purity (A260/A280) ranging between 1.70–2.00 were used for polymorphism genotyping.

4.2.2.4. Polymorphism selection and genotyping

Recently, our research group conducted a review concerning all genetic factors associated with athletic performance across different sport modalities [4]. From the list of these factors (88 genetic variants), based on their previous association with athletic performance in a Caucasian population (endurance, power and/or sport injuries), the polymorphism minor allele frequency (MAF) in Iberian population (MAF should be higher than 10% to ensure genotype representation), their functional consequence, existence of validation studies and availability of TaqMan® assays (Applied Biosystems), seven genetic polymorphisms were selected to be evaluated in our cohort of elite rink-hockey players (Table 4.2). Genotyping was performed in a Real-Time Polymerase Chain Reaction (RT-PCR) system (Applied Biosystems) using the TaqMan® Allelic Discrimination methodology. All RT-PCR reactions were conducted in 6 μL volumes containing 2.5 μL of TaqMan™ Genotyping Master Mix (2×), 2.375 μL of sterile water, 0.125 μL of 40× assay mix and lastly 1 μL of genomic DNA. Amplification conditions were the following: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Data capture and analysis were conducted on a Real-Time PCR System (Applied Biosystems) as described elsewhere [24]. To guarantee genotyping quality, two negative controls were included in each reaction, and double sampling was conducted in at least 10% of the samples randomly chosen, with an accuracy above 99%. The results were individually evaluated by two researchers with no previous knowledge regarding the athletes' performance.

Table 4.2. Selected polymorphisms' description and TaqMan® assays used.

Genetic Variant	Functional Consequence	Gene	Encode Protein and Its Functions	TaqMan® Assays ID
rs324420	Missense	FAAH	<i>Nerve plasticity and other neural functions:</i> Fatty Acid Amide Hydrolase	C___1897306_10
rs8192678	Missense	PPARGC1A	<i>Energy metabolism and circadian rhythm:</i> PPARG Coactivator 1 Alpha	C___1643192_20
rs2016520	5 prime UTR	PPARD	Peroxisome Proliferator Activated Receptor Delta	C___8851952_30
rs1801282	Missense	PPARG	Peroxisome Proliferator Activated Receptor Gamma	C___1129864_10
rs731236	Synonymous	VDR	Vitamin D Receptor	C___2404008_10
rs1042713	Missense	ADRB2	<i>Catecholaminergic system:</i> Adrenoceptor Beta 2	C___2084764_20
rs1799983	Missense	NOS3	<i>Neurotransmission, antimicrobial and antitumoral activities:</i> Nitric Oxide Synthase 3	C___3219460_20

The functional consequence of each polymorphism was defined according to Ensembl database [25]. The functions of encoded proteins were described based on GeneCards database [26] and UniProt database [27].

4.2.2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 26.0 (SPSS, Inc., Chicago, IL, USA). Continuous data were expressed as mean $\pm$ standard deviation (SD), while categorical data were described in terms of frequencies. Some variables categories were defined based on median of variable values, namely: age (≥ 26 yrs. vs. < 26 yrs.), WC/HC (≥ 0.85 vs. < 0.85), hand length (≥ 23 cm vs. < 23 cm) and time of recovery from first severe sport injury (≥ 10 months vs. < 10 months). Associations between the genetic polymorphisms and athlete characteristics were assessed using student's *t*-test for continuous variables, while chi-square test (χ^2) or Fisher's exact test were used for categorical ones. Using a binomial regression model, univariate analyses were conducted to identify predictors of elite athletic performance. Only the polymorphisms and other variables significantly associated with this trait in univariate analyses were further investigated in multivariate analysis to identify independent predictors. Bootstrapping analyses were performed through Monte Carlo simulation (1000 replications). Statistical significance was established at the $p < 0.05$.

4.3. Results

4.3.1. Characteristics of elite rink-hockey players

As expected, male rink-hockey players showed significantly higher anthropometric characteristics (body mass, height, BMI, WHR and hand length) than their female counterparts, who were mostly students (55.6%, Table 4.1). Since 45.9% of male athletes

were professional rink-hockey players, they were also more experienced and submitted to a more intensive training program than their female counterparts ($p < 0.05$, Table 4.1) and suffered more sport injuries ($p = 0.002$, Table 4.1).

4.3.2. Genotype frequencies in elite female and male rink-hockey players

The genotypes' distribution of each polymorphism in female and male rink-hockey players is shown in Table 4.3. To be noted, the distribution of *Vitamin D Receptor* (VDR) rs731236 genotypes differed significantly between female and male athletes ($p = 0.019$) with the AG genotype being more prevalent among females (72.2% vs. 36.7%) and the AA and GG genotypes in males (45.9% vs. 22.2%, and 17.3% vs. 5.6%, respectively). Additionally, *Peroxisome Proliferator Activated Receptor Delta* (PPARD) rs2016520 CC, *Peroxisome Proliferator Activated Receptor Gamma* (PPARG) rs1801282 GG and the *Nitric Oxide Synthase 3* (NOS3) rs1799983 TT genotypes were absent among females. These results are most likely attributed to the reduced number of female athletes.

Table 4.3. Genotype frequencies of the selected genetic polymorphisms in elite rink-hockey players ($n = 116$, 18 females and 98 males).

Genotype Frequencies	Female (<i>n</i> = 18)	Male (<i>n</i> = 98)	Total (<i>n</i> = 116)	<i>p</i>
FAAH rs324420				
AA	1 (5.6)	3 (3.1)	4 (3.4)	0.789
AC	6 (33.3)	28 (28.6)	34 (29.3)	
CC	11 (61.1)	67 (68.4)	78 (67.2)	
PPARGC1A rs8192678				
TT	2 (11.1)	17 (17.3)	19 (16.4)	0.705
CT	9 (50.0)	51 (52.0)	60 (51.7)	
CC	7 (38.9)	30 (30.6)	37 (31.9)	
PPARD rs2016520				
CC	0 (0.0)	5 (5.1)	5 (4.3)	0.395
CT	7 (38.9)	32 (32.7)	39 (33.6)	
TT	11 (61.1))	61 (62.2)	72 (62.1)	
PPARG rs1801282				
GG	0 (0.0)	4 (4.1)	4 (3.4)	0.494
CG	3 (16.7)	14 (14.3)	17 (14.7)	
CC	15 (83.3)	80 (81.6)	95 (81.9)	
VDR rs731236				
GG	1 (5.6)	17 (17.3)	18 (15.5)	0.019
AG	13 (72.2)	36 (36.7)	49 (42.2)	
AA	4 (22.2)	45(45.9)	49 (42.2)	

Bold values represent the significant results ($p < 0.05$).

Table 4.3. Cont.

Genotype Frequencies	Female (<i>n</i> = 18)	Male (<i>n</i> = 98)	Total (<i>n</i> = 116)	<i>p</i>
<i>ADRB2</i> rs1042713				
AA	3 (16.7)	15 (15.3)	18 (15.5)	0.989
AG	8 (44.4)	44 (44.9)	52 (44.8)	
GG	7 (38.9)	39 (39.8)	46 (39.7)	
<i>NOS3</i> rs1799983				
TT	0 (0.0)	14 (14.3)	14 (12.1)	0.212
GT	11 (61.1)	47 (48.0)	58 (50.0)	
GG	7 (38.9)	37 (37.8)	44 (37.9)	

Bold values represent the significant results ($p < 0.05$).

4.3.3. Univariate and multivariate analysis of *FAAH* rs324420 and relevant factors of elite performance in rink-hockey

According to univariate analysis, among the seven genetic polymorphisms, *FAAH* rs324420 was the only one significantly associated with athletic performance (AA/AC vs. CC; OR = 2.80; 95% CI, 1.23–6.35; $p = 0.014$) ($p = 0.008$ after Bootstrapping analyses were performed through Monte Carlo simulation for 1000 replications). This effect was corroborated by multivariate analysis using a model with the factors age, sex, BMI, WC/HC, hand length, existence of severe sport injury and time of recovery from first sport injury (AA/AC vs. CC; adjusted OR = 2.88; 95% CI, 1.06–7.80; $p = 0.038$; Table 4.4).

Table 4.4. Multivariate analysis using binomial regression on the athletic performance considering *FAAH* rs324420 and relevant factors.

Characteristics	OR	95% CI	p
<i>FAAH</i> rs324420			
(AA/AC vs. CC ¹)	2.88	1.06–7.80	0.038
Age *			
(≥26 yrs. vs. <26 yrs. ¹)	9.74	3.09–30.74	0.065
Sex			
(male vs. female ¹)	2.48	0.50–12.30	0.265
BMI			
(≥25kg/m ² vs. <25kg/m ² ¹)	0.58	0.19–1.80	0.349
WHR *			
(≥0.85 vs. <0.85 ¹)	0.83	0.30–2.28	0.721
Hand length *			
(≥23cm vs. <23 cm ¹)	0.35	0.12–1.02	0.054

¹ Reference group. * Variable categories were defined based on median of variable values. BMI: body mass index, CI confidence interval, OR odds ratio, WHR: waist circumference/hip circumference ratio. Bold values represent the significant results ($p < 0.05$).

Table 4.4. Cont.

Characteristics	OR	95% CI	p
Sport injury (Yes vs. no ¹)	3.71	1.21–11.31	0.021
Recovery from first sport injury * (≥10months vs. <10months ¹)	0.64	0.15–2.73	0.541

¹ Reference group. * Variable categories were defined based on median of variable values. CI confidence interval, OR odds ratio, WHR: waist circumference/hip circumference ratio. Bold values represent the significant results ($p < 0.05$).

In this model, *FAAH* rs324420 and the existence of severe sport injury presented as independent predictors of elite athletic performance. According to the results, the rs324420 A allele seems to impose a benefit effect among elite athletes, as the carriers are three times more likely to be super athletes than those with CC genotype. As for the impact of severe sport injury (yes vs. no; adjusted OR = 3.71; 95% CI, 1.21–11.31; $p = 0.021$; Table 4.4), it might be due to the high demands of athletic performance at this elite level.

4.4. Discussion

Most variants in genes coding for components of the cardiovascular, respiratory and musculoskeletal systems are thought to be associated with the elite athlete phenotype [2], but the input of genes coding for biochemical and structural elements of the brain regions associated with psychological traits have been less studied [5]. The aim of the present study was to examine the distribution of variants of genes with a potential role in nerve plasticity, neurotransmission, circadian rhythm, energy metabolism and antimicrobial and antitumoral activities in a sample of elite rink-hockey players and test for any evidence of association with elite athletic performance.

Our study group is unique as it comprises a high proportion of top, elite rink-hockey players, including those who achieved medals in the last World Championship.

In this study, as expected, significant differences between female and male athletes were observed, which can be explained by biological and professional-related factors. Inclusive, almost half of the male players were professional, whereas it was not the case for any female participant. As such, male athletes trained and competed more than their female counterparts, reporting also a higher incidence of sport-related injuries.

To the best of our knowledge, this is the first study evaluating the impact of genetic determinants in the athletic performance of elite rink-hockey players, as previous studies were mostly conducted on ice and field-hockey and at high school and college levels. Briefly, a study conducted with nine Spanish rink-hockey players with tendinopathy and five controls (rink-hockey players without tendinopathy) found that carrying one or more alleles A in *Gap*

Junction Alpha 1 (GJA1) rs11154027 (OR = 2.11; 95% CI, 1.07–4.19, $p = 1.01 \times 10^{-6}$) or G allele in *Vesicle Amine Transport 1-Like (VAT1L)* rs4362400 (OR = 1.98; 95% CI, 1.05–3.73, $p = 9.6 \times 10^{-6}$) was associated with a higher risk of tendinopathy [18]. On the other hand, carrying one or more A alleles in *Contactin-Associated Protein-Like 2 (CTNP2)* rs10263021 had a protective role (OR = 0.42; 95% CI, 0.20–0.91, $p = 4.5 \times 10^{-6}$). Sessa et al. [28] suggested a natural sports selection of an Italian group of athletes practicing intermittent sports (football, basketball and hockey players) whose frequency of Glu298Asp allele in *NOS3* rs1799983 affecting the blood pressure response to endurance training was higher compared with controls. A case-control study with Italian male and female elite athletes of basketball, soccer and hockey found that the polymorphism 5HTTLPR SS genotype of the *Solute Carrier Family 6 Member 4 (SLC6A4)* was associated with neuroticism ($p < 0.001$) and adverse sport-related stress, namely tension/anxiety symptoms ($p < 0.02$), cognitive anxiety and emotional arousal control ($p < 0.01$) [7]. However, these two latter studies do not clearly indicate whether they included rink-hockey players (prevalent by sport and/or sex).

In fact, the little existing genetic research is mostly related to the other two disciplines of hockey (ice and field-hockey) and has been conducted in relation to sport performance (in ice-hockey [29–32] and in field-hockey [33]) and brain trauma in ice-hockey [34,35].

Even though it is consensual, the impact of sports injuries and/or traumas on the athlete's physical and mental health and performance is devastating. The literature concerning the interaction between genetic and environmental factors and sports injuries is limited [36–39]. According to the study results, the incidence of severe sport injuries and *FAAH* rs324420 polymorphism are independent predictors of the athletic performance among our elite rink-hockey players, which may be due to significant differences according to the athletes' sex and training frequency, as recently reported by Silva et al. [4]. As for rs324420, this missense SNP lies within *FAAH*, a gene encoding a protein (fatty acid amide hydrolase) reported as a promising target for the discovery of drugs to treat pain, inflammation and other pathologies [40], which is of great interest for athletic performance, in particular in a high-intensity and invasion sport as rink-hockey. This protein is a serine hydrolase engaged in the endocannabinoid metabolism, which degrades N-Arachidonoyl ethanolamide (Anandamide, AEA), a metabolite responsible for the activation of the cannabinoid type 1 receptor (CB1). Under stress exposure conditions (Figure 1), the protein FAAH is mobilized to degrade the AEA, therefore increasing the neuronal excitability in the amygdala, a key anxiety-mediating region of the brain [5]. In opposition, the inhibition of FAAH decreases anxiety-like behavior [41] and may produce an antidepressant effect mediated by CB1 receptor stimulation [42]. Previously, the rs324420 A allele was associated with a lower expression of FAAH levels [41]; in the population of the Iberian and African peninsula, it has

a percentage of 16% and 37%, respectively [43]. Specifically, although the resultant protein displays normal catalytic properties, it shows an increased sensitivity to proteolytic degradation and shorter half-life, which explains the protein's lower levels [5] (Figure 4.1). The SNP A allele might, therefore, be linked to a quicker habituation of amygdala reactivity to threat, decreased anxiety-like behavior, and increased fear extinction learning. This is of utmost importance for athletes to better cope with the personality trait of stress reactivity, deal more quickly with unpredictable situations and improve their motivation for sport competition [6,41,44]. This evidence is in line with our results, since the carriers of A allele (AA or AC genotype) were shown to be three times more likely to be super athletes than their counterparts (athletes with CC genotype), adjusted for other relevant factors (AA/AC vs. CC; adjusted OR = 2.88; 95% CI, 1.06–7.80; $p = 0.038$).

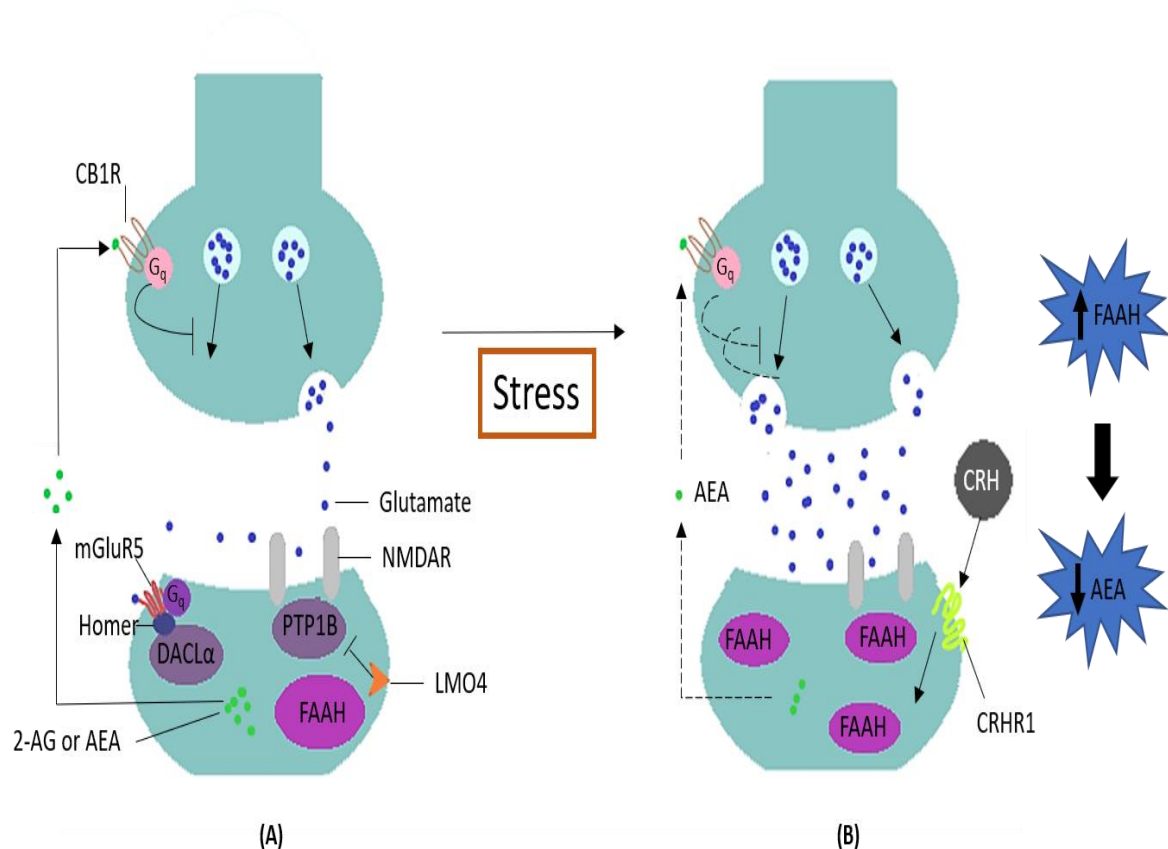


Figure 4.1. Endocannabinoid system as regulator of stress response. Under normal circumstances **(A)**, the endocannabinoid system suppresses the release of the neurotransmitter glutamate via AEA, modulating the synaptic function. However, acute stress **(B)** can trigger a mechanism mediated by CRH and its receptor CRHR1, which is known to increase FAAH activity in the basolateral amygdala. As a result, there is a decrease in AEA levels, which is no longer able to suppress glutamate release. Consequently, increased neural excitability in the basolateral amygdala promotes anxiety-like behavior (adapted by Lutz et al.) [41]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; AEA: N-arachidonylethanolamine; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; FAAH: *fatty acid amide hydrolase*; NMDAR: NMDA receptor; LMO4: LIM domain only 4; CRH: corticotropin-releasing hormone; CRHR1: corticotropin-releasing hormone receptor 1.

In a previous report, Peplonska et al. [6] found that the polymorphism AA genotype is more common among sedentary controls than elite athletes ($p = 0.0084$), which led them to conclude that this genotype could have a negative impact on athletic performance. Similar findings were observed later [5]. In the study, which was conducted with 183 athletes of power, 212 of endurance sports and 451 controls, the authors observed that the SNP AA genotype was underrepresented in both power (in recessive model: OR = 0.36, 95% CI = 0.15–0.86, $p = 0.017$) and endurance athletes (in recessive model: OR = 0.42, 95% CI = 0.20–0.90, $p = 0.022$) compared with controls. In addition, this effect on athletic status was even more evident when the two groups of athletes were analyzed jointly (in recessive model: OR = 0.40, 95% CI = 0.22–0.72, $p = 0.002$), suggesting a negative impact on athletic performance [5].

Here, we hypothesized that such conflict results compared with ours might be attributed to the underrepresentation of AA genotype among elite athletes compared with sedentary controls, as our results suggest a benefit effect of the A allele in elite rink-hockey players when only elite athletes are considered. Additionally, rs324420 might play different roles depending on sport requirements, which needs to be further explored. Furthermore, although significant sex differences among our athletes were not observed for rs324420 genotype frequencies as previously described, it has been shown that estrogens modify emotional behavior through the dysregulation of the FAAH enzyme, thus causing an increase in the signaling of the endocannabinoid system, consequently decreasing anxiety in women [42]. Therefore, further studies would be needed to confirm this hypothesis in female athletes.

Overall, the observed effect of *FAAH* rs324420 is of utmost importance for future research in elite sport, since the mechanism of action of FAAH through the endocannabinoid system [45], applied to pain regulation and inflammation control, might improve mental and physical health and performance among elite athletes who face stressful environments daily [41].

As for the other six polymorphisms, no significant association was detected, despite the existence of some evidence linking these genetic variants to athletic performance [46]. Briefly, *PPARD* (implicated in fatty acid oxidation, cholesterol metabolism and thermogenesis) rs2016520 CC, *PPARGC1A* (involved in mitochondrial biogenesis, fatty acid oxidation, glucose utilization, thermogenesis, angiogenesis and muscle fiber type conversion toward slow-twitch type I fibers) rs8192678 TT genotype and rs8192678 G allele were associated with improved endurance performance ($p < 0.01$) [35,46–49]. The *PPARG* (implicated in adipocyte differentiation, glucose homeostasis and regulation of cardiovascular circadian rhythms) [50] rs1801282 CG genotype has been associated with decreased receptor activity and improved insulin sensitivity [51]. Regarding the *VDR* rs731236 variant, given the importance of vitamins in bone turnover, the polymorphism was

associated with stress fractures ($p < 0.05$), with the GG genotype linked to higher multiple stress fractures when compared with their counterparts ($p = 0.01$) in a sample of 518 elite athletes (mean age 24.2 ± 5.5 years) of football, cricket, track and field, running events, rowing, boxing, tennis and others and controls [52]. The *Adrenoceptor Beta 2 (ADRB2)* (involved in the catecholamine system) rs1042713 A allele was associated with lower protein density (Beta-2 adrenoceptor), resting cardiac output and endurance performance [53]. On the other hand, the rs1042713 G allele was associated with sprint performance in elite youth football players [54]. As for *NOS3* (linked to several biological mechanisms, including angiogenesis) [50] rs1799983 polymorphism, this variant was previously associated with right ventricular structure and nitric oxide bioavailability in athletes' cardiac adaptation. Its influence in the right ventricular structure was studied in elite water polo players, rowers, kayakers, canoeists and swimmers [53]. Although not significantly associated with sport performance, athletes carrying the rs1799983 T allele demonstrated increased right ventricular mass index (32 ± 6 g vs. 27 ± 6 g, $p < 0.01$) and larger right ventricular stroke volume index (71 ± 10 mL vs. 64 ± 10 mL, $p < 0.01$) compared with their counterparts [55].

Nevertheless, our findings concerning these polymorphisms need to be further validated given these previous results.

In terms of study limitations, the sample collection was highly affected by restrictions imposed by the coronavirus pandemic. The same condition also hampered the collection and analysis of some important factors, including for instance the percentage of adipose tissue and/or muscle mass, which can affect athletic performance.

Even so, the recognition of the study relevance by the study participants helped to minimize access constraints. Nevertheless, the participation rate is very representative of the elite in rink-hockey worldwide.

4.5. Conclusions

Elite rink-hockey players carrying the *FAAH* rs324420 A allele were three times more likely to be high-performance/super athletes, which could be attributed to a higher pain tolerance and better stress coping.

This is also of utmost importance for coaches, who can individually plan and adapt the athlete's technical and tactical training and competition, keeping in mind the athlete's specific ability to make decisions and support pain. Moreover, other sport professionals (e.g., medical doctors, physiotherapists, nutritionists and psychologists) should be aware of *FAAH* rs324420 and its associated consequences for the athlete's increased resistance to pain and inflammation and time for injury recovery.

Despite the promising results, as this is a pioneer study, additional studies are needed for further validation of our results in rink-hockey and other sport modalities.

Furthermore, as few studies were conducted to assess the role of *FAAH* rs324420, functional studies are also needed to better characterize this polymorphism and understand its impact on athletic performance. Given its thought biological impact, the role of this polymorphism should be explored in the setting of sport injuries among rink-hockey players, including type and frequency of injuries and recovery time.

4.6. References

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5 Association of *FAAH* rs324420 (C385A) polymorphism with high-level performance in volleyball players

5.1. Introduction

5.2. Materials and methods

5.2.1. Enrolled players' description and data collection

5.2.1.1. Biological sample processing

5.2.1.2. SNP selection and genotyping

5.2.2. Statistical analysis

5.3. Results

5.3.1. High-level performance volleyball players

5.3.2. SNP genotype frequencies

5.3.3. Univariable and multivariable analyses

5.4. Discussion

5.5. Conclusions

5.6. References

5.1. Introduction

High-level performance athletes (i.e., elite athletes) present an extraordinary interindividual variability of physical but also mental traits that greatly influence their sports accomplishments and their career longevity [1–3]. In addition to training regimes [4] and optimal nutrition [5], individual genetic variation is thought to be another preponderant factor in elite athletic performance [1,2].

Over the years, several genes, including, for instance, *Angiotensin-I-Converting Enzyme* (*ACE*) and *Actinin α 3* (*ACTN3*), have been associated with athlete phenotypes and many physiological functions related to muscle-skeletal, respiratory, cardiovascular, nervous, and other systems implicated in sports practice [1,2,6]. However, genetic studies on competition behavior, stress management, and a potential relationship with sports lesions are scarce [2,3,6–8]. Recently, our research team has investigated the implications of the single-nucleotide polymorphism (SNP) *Fatty Acid Amide Hydrolase* (*FAAH*) rs324420 (C385A), among other genetic variants, in elite athletic performance [3]. Our results suggested that the SNP A allele (385A allele) was independently linked to enhanced performance of the world's best rink-hockey players (dominant genetic model, AA/AC vs. CC; adjusted odds ratio (aOR) = 2.88; 95% CI, 1.06–7.80; $p = 0.038$). The gene *FAAH* has been previously associated with pain tolerance, stress, and inflammation, which are features with implications for sports practice [9]. The role of the C385A variant in athletic performance is, however, not consensual, with evidence suggesting a detrimental effect of the 385A allele, as it seems to be more prevalent among inactive individuals than in elite athletes from different sports modalities [7,8]. As the cumulative evidence concerning the C385A variant is inconsistent, validation studies considering other sports modalities in addition to rink-hockey are a must to dissect the role of this SNP in athletic performance.

Volleyball is a high-intensity, intermittent sport modality with repetitive episodes of short durations of effort at diverse speed levels and incomplete recovery periods. Both aerobic and anaerobic metabolisms, as well as cardiovascular and musculoskeletal systems, are crucial for the performance of volleyball players [10]. The game is not time-limited. The team that wins the match is the team that first wins three sets or, in the case of a 2-2 tie, the team that wins the fifth set (deciding set) with at least 15 points and a minimum lead of 2 points (*Fédération Internationale de Volleyball*, FIVB - Volleyball International Federation). Regarding the substitution of players, an athlete may leave and re-enter the game, but only once in a set, occupying only his/her previous position in the line-up [11]. Volleyball is mostly described by its biomechanical component in the vertical plane due to the need for the ball to cross the vertical net to the opponent's court. Although it is a non-invasion game, the frequency of sports injuries is high due to the inherently high training load and mechanical stress during the repetitive technical movements [10]. As such, mental abilities in the context

of high-level performance are needed to manage stress and anxiety, be resilient, and avoid impulsivity to make optimal decisions during training sessions and competition [3,7,12]. This is particularly important in stressful moments of the athletes' sports careers, namely, when they must demonstrate mental discipline and resilience (for instance, in the deciding fifth set of the volleyball match) or cope with sports lesions that may limit their mental and physical readiness to play [13]. In this context, the present study focuses on evaluating the impact of athlete status-related genetic markers with roles in brain function in a cohort of high-level performance volleyball players competing in the Portuguese national championship.

5.2. Materials and Methods

5.2.1. Enrolled players' description and data collection

A cohort of 228 volleyball players of high-level performance (i.e., involved in any national- or high-level team under the responsibility of a National Sports Federation) [3] competing in the Portuguese Volleyball Championship during three consecutive sports seasons (2019/2020, 2020/2021, and 2021/2022) were enrolled in this study. These players are currently the nation's elite volleyball athletes, with many being multiple medalists in national and international competitions.

For participants' selection, the inclusion criteria were the following: (1) be an athlete at least 18 years old at the national minimum level, (2) be an experienced national athlete (i.e., considered a world's elite athlete competing in Portugal), and (3) be a competitor that has reached a higher level (i.e., World, European, and Continental Championships or international competitions such as the Olympic Games) [3].

At the time of recruitment, the athletes' sport experience was 16.3 ± 8.4 years; their training frequency was 6.5 ± 1.5 days per week and 3.3 ± 1.0 h per day, for a total of 22.0 ± 10.5 h per week. The characterization of the enrolled athletes is shown in Table 5.1.

Each participant in the study signed an informed consent prior to their recruitment and this study was approved by the Ethical Committee of the University Fernando Pessoa at Porto (CEUFP05062017).

Data on anthropometrics, training conditions, and sports experience, as well as the history of sports lesions, were assessed by the same trained researcher via questionnaires that were personally distributed to the players prior to training sessions. Determination of height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist/hip ratio (WHR), hand length, and shoe size were described elsewhere [14]. In terms of training regime, training hours per day and training units per week were assessed to determine the number of training hours per week. Athletes' national and international achievements, as well as their participation in national and regional teams were also assessed.

Table 5.1. Demographic, sports, and training characteristics of high-level performance volleyball players by sex ($n = 228$; 66 females and 162 males).

Variables	Total (<i>n</i> = 228)	Females (<i>n</i> = 66)	Males (<i>n</i> = 162)	<i>p</i> -Value
Age (years) *	26.7 ± 8.1	24.9 ± 6.1	27.5 ± 8.7	0.026
Body mass (kg) *	81.4 ± 12.2	69.4 ± 7.5	86.3 ± 10.2	< 0.001
Height (m) *	1.86 ± 0.10	1.77 ±0.07	1.90 ± 0.09	< 0.001
BMI (kg/m ²) *	23.4 ± 2.1	22.2 ± 1.6	23.9 ± 2.0	< 0.001
Waist circumference (cm) *	80.3 ± 6.0	75.4 ± 5.2	82.2 ± 5.1	< 0.001
Hip circumference (cm) *	91.5 ± 10.0	83.4 ± 9.6	94.8 ± 7.5	< 0.001
WHR *	0.88 ± 0.05	0.85 ± 0.04	0.91 ± 0.05	< 0.001
Hand length (cm) *	25.0 ± 1.4	24.2 ± 1.5	25.3 ± 1.2	< 0.001
Shoe size *	44.0 ± 3.1	40.2 ± 1.7	45.6 ± 2.1	< 0.001
Age at first sports lesion (years) *	12.1 ± 9.1	11.8 ± 9.5	12.2 ± 9.0	0.803
Month of the first sports lesion *	5.8 ± 4.2	6.5 ± 4.1	5.5 ± 4.2	0.202
Beginning at high-level performance (years) *	16.9 ± 2.0	16.2 ± 1.4	17.2 ± 2.0	< 0.001
Training experience (years) *	16.3 ± 8.4	14.8 ±6.6	17.0 ± 9.0	0.936
Training frequency *				
Days/week	6.5 ± 1.5	6.8 ± 1.6	6.4 ± 1.5	< 0.001
Hours/day	3.3 ± 1.0	3.2 ± 1.0	3.3 ± 1.0	0.004
Hours/week	22.0 ± 10.5	23.0 ± 12.9	21.6 ± 9.3	< 0.001
Participations in regional and national teams *	27.4 ± 39.2	25.5 ± 37.9	28.2 ± 39.8	0.778
Sport lesion				
No	85 (37.1)	26 (39.4)	59 (36.2)	0.381
Yes	144 (62.9)	40 (60.6)	104 (63.8)	
Nationality				
Portuguese	172 (75.1)	50 (75.8)	122 (74.8)	0.356
Others	57 (24.9)	16 (24.2)	41 (25.2)	
Ethnicity				
Caucasian	208 (90.8)	63 (95.5)	145 (89.0)	0.094
Others	21 (9.2)	3 (4.5)	18 (11.0)	
Other occupation/profession				
Athletes	69 (30.1)	20 (30.3)	49 (30.1)	0.471
Student	99 (43.2)	30 (45.5)	69 (42.3)	
Teacher	22 (9.6)	4 (6.1)	18 (11.0)	
Physical therapist	8 (3.5)	3 (4.5)	5 (3.1)	
Sport coordinator	1 (0.4)	--	1 (0.6)	
Coach	3 (1.3)	--	3 (1.8)	
Podiatrist	3 (1.3)	2 (3.0)	1 (0.6)	
Architect	1 (0.4)	1 (1.5)	--	
Engineer	7 (3.1)	3 (4.5)	4 (2.5)	
Market trader	8 (3.5)	1 (1.5)	7 (4.3)	
Other	8 (3.5)	2 (3.0)	6 (3.7)	

Bold values were regarded as significant ($p < 0.05$). * Variables described as mean $\pm$ standard deviation. BMI: body mass index; WHR: waist circumference/hip circumference ratio.

The history of sports lesions was investigated, including the anatomical part affected, the year of occurrence, and the time of recovery from the first severe injury [3,5].

5.2.1.1. Biological sample processing

Buccal cell samples from each athlete were collected from both right and left using sterile swabs (FL Medical, Hamburg, Germany) [15]. Genomic deoxyribonucleic acid (DNA) was extracted from these samples using QIAamp DNA Blood Mini Kit, Qiagen. Purity (A260/A280) and concentration of the DNA samples were evaluated using NanoDrop Lite spectrophotometer (Thermo Scientific®, Waltham, MA, USA). Only samples with DNA concentration of at least 4 ng/μL and with A260/A280 between 1.70 and 2.00 were used for SNP genotyping.

5.2.1.2. SNP selection and genotyping

In a previous literature review conducted by our research group on genetic factors linked to athletic performance traits (power, endurance, and/or sports lesions) across different sports modalities [2], genetic variants were selected if (1) they were previously reported to influence athletic performance in a Caucasian population; (2) they had roles in brain activity; and (3) their minor allele frequency (MAF) in Iberian population was higher than 10% with the purpose of ensuring genotype representation. Furthermore, the selection was also based on the functional consequence of the genetic variants and the availability of TaqMan® assays (Applied Biosystems). Applying these criteria, three genetic polymorphisms were selected (Table 5.2). SNP genotyping was conducted in a Real-Time Polymerase Chain Reaction (RT-PCR) system (Applied Biosystems) via TaqMan® Allelic Discrimination methodology. All RT-PCR reactions were performed in 6 μL volumes with 2.5 μL of TaqMan™ Genotyping Master Mix (2×), 2.375 μL of nuclease-free water, 0.125 μL of 40× TaqMan® SNP Genotyping Assay (Table 5.2), and 1 μL of genomic DNA. Amplification conditions included the following: 95 °C for 10 min, 45 cycles of 95 °C for 15 s, and 60 °C for 1 min [16]. Two negative controls were included in all PCR runs, and replicates were performed on at least 20% of randomly selected samples (accuracy superior to 99%). Results were assessed independently by two researchers who had no prior knowledge of the athletes' characteristics.

Table 5.2. Description of the evaluated genetic polymorphisms and the TaqMan® assays used.

SNP	Functional Consequence	Biological Functions	TaqMan® SNP Genotyping Assays ID
<i>ADRB2</i> rs1042713	Missense	Catecholaminergic system	C__2084764_20
<i>FAAH</i> rs324420	Missense	Neural functions, including nerve plasticity	C__1897306_10
<i>NOS3</i> rs1799983	Missense	Neurotransmission, antimicrobial, and antitumoral activities	C__3219460_20

SNP functional consequence was characterized based on the Ensembl database [17], and the encoded proteins' biological functions were provided according to the GeneCards database [18] and UniProt database [19].

5.2.2. Statistical analysis

Statistical analyses were performed using SPSS software version 26.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were described as mean $\pm$ standard deviation (SD), while categorical data were presented as frequencies. The categories of the variables age (≥ 26 vs. < 26 years), hand length (≥ 23 vs. < 23 cm), WHR (≥ 0.85 vs. < 0.85) and recovery time from the first severe sports lesion (≥ 10 vs. < 10 months) were defined based on the median value. Associations between the genetic variants and the athlete features were analyzed by the student's *t*-test for continuous variables (assuming normal distribution based on the cohort size), whereas the chi-square test (χ^2) or Fisher's exact test was used for categorical ones. The athletes' accomplishments or success were determined by the total number of national and international player titles. Tertiles were used to classify athletic performance. In the highest tertile (those with more than 11 titles), athletes were regarded as super athletes ($n = 62$), while the two remaining tertiles were combined in one category regarded as others ($n = 166$) for further analyses. Univariate analyses were performed using a binomial regression model to identify SNPs that were predictive of superior athletic achievements. Recessive and dominant genetic models were considered for each genetic polymorphism in the univariate analyses. Only those with statistical significance were presented. Statistical power calculations concerning the univariate binomial regression analyses were performed using the software GPower version 3.1.9.7 (Heinrich Heine University, Düsseldorf, Germany) [20]. Relevant genetic polymorphisms and other relevant variables were further included in a multivariate analysis. Bootstrapping analyses were also conducted using Monte Carlo simulation (1000 replications). All tests, including the statistical power analysis, were two-tailed, and statistical significance was defined as $p < 0.05$.

5.3. Results

5.3.1. High-level performance volleyball players

Female volleyball players were younger ($p = 0.026$) and, as expected, demonstrated significantly lower anthropometric indicators (body mass, height, BMI, waist, hip circumferences, WHR, hand length, and shoe size) than male athletes ($p < 0.01$, Table 4.1). Female players also trained for significantly more days and hours per week and began earlier (16.2 ± 1.4 years old) at the high-level performance level than males (17.2 ± 2.0 years old, $p < 0.01$, Table 5.1), who were more experienced (17.0 ± 9.0 years vs. 14.8 ± 6.6 years) and had a higher number of participations in regional and national teams (28.2 ± 39.8 participations vs. 25.5 ± 37.9 participations), although significant differences were not found ($p < 0.05$). In addition, more than half of the volleyball players reported a high prevalence of sports lesions (63.8% males and 60.6% females), and the first severe sports

lesion occurred at early ages (females, 11.8 ± 9.5 years old and males, 12.2 ± 9.0 years old), mostly in the second trimester of the year (5.5 ± 4.2 months in males and 6.5 ± 4.1 months in females, Table 5.1). Additionally, more than a quarter of the participants were professional volleyball players.

5.3.2. SNP genotype frequencies

The genotypes' distribution of each genetic variant by sex among the players is presented in Table 5.3.

Table 5.3. Genotype distribution of the evaluated genetic variants among high-level performance volleyball players ($n = 219$; 60 females and 159 males).

Genotype Frequencies	Females (<i>n</i> = 66)	Males (<i>n</i> = 162)	Total (<i>n</i> = 219) *	<i>p</i> -Value
<i>ADRB2</i> rs1042713				
AA	13 (21.7)	30 (18.9)	43 (19.6)	0.127
AG	19 (31.7)	74 (46.5)	93 (42.5)	
GG	28 (46.7)	55 (34.6)	83 (37.9)	
<i>FAAH</i> rs324420				
AA	3 (5.0)	8 (5.0)	11 (5.0)	0.996
AC	20 (33.3)	54 (34.0)	74 (33.8)	
CC	37 (61.7)	97 (61.0)	134 (61.2)	
<i>NOS3</i> rs1799983				
TT	10 (16.7)	24 (15.1)	34 (15.5)	0.196
GT	30 (50.0)	61 (38.4)	91 (41.6)	
GG	20 (33.3)	74 (46.5)	94 (42.9)	

* Polymorphism genotyping was not possible in 9 participants (6 females and 3 male athletes, respectively) resulting in a failed genotyping of 4%.

5.3.3. Univariable and multivariable analyses

In the univariable analyses ($n = 219$, 59 super athletes and 160 others), *FAAH* rs324420 under a dominant genetic model (AA/AC ($n = 85$) vs. CC ($n = 134$)) was the only genetic variant significantly associated with sports achievements among high-level performance volleyball players (AA/AC vs. CC; OR = 1.70; 95% CI, 1.13–3.13; $p = 0.026$) ($p < 0.001$ after Bootstrapping analyses were conducted using a Monte Carlo simulation for 1000 replications). It must be noted, however, that the study was only sufficiently powered (power ≥ 0.80) to detect odds ratio (OR) values of at least 1.70, considering the entire cohort with available information for SNP genotypes ($n = 219$).

The effect of *FAAH* rs324420 was confirmed by a multivariate analysis ($n = 219$) adjusted for age, sex, BMI, hand length, and history of severe sports lesions (AA/AC vs. CC; aOR =

2.00; 95% CI, 1.04–3.82; $p = 0.037$; Table 5.4). In the multivariable analysis, *FAAH* rs324420, age, and hand length were independent predictors of high athletic achievements among elite volleyball players. In accordance with previous findings, the 385A allele appears to be beneficial since the AA or AC genotype carriers were two times more prone to be super athletes than players carrying the CC genotype. Additionally, as expected, in the study, older players (≥ 26 vs. < 26 years; aOR = 2.96; 95% CI, 1.55–5.66; $p = 0.001$; Table 5.4) and the ones with bigger hands (≥ 23 vs. < 23 cm; aOR = 5.51; 95% CI, 1.14–26.59; $p = 0.034$; Table 5.4) were found to be more prone to greater achievements.

Table 5.4. Multivariate analysis on the elite volleyball players' achievements ($n = 219$, 59 super athletes and 160 others) using binomial regression.

Factors	aOR	95% CI	p-Value
FAAH rs324420 (AA/AC vs. CC ¹)	2.00	1.04–3.82	0.037
Age (≥ 26 vs. < 26 years ¹)	2.96	1.55–5.66	0.001
Sex (Male vs. female ¹)	0.82	0.37–1.82	0.620
BMI (≥ 25 vs. < 25 kg/m ² ¹)	1.16	0.51–2.64	0.725
Hand length (≥ 23 vs. < 23 cm ¹)	5.51	1.14–26.59	0.034
History of sport lesions (Yes vs. no ¹)	1.34	0.68–2.63	0.400

Bold values were regarded as significant ($p < 0.05$). ¹ Reference group. BMI, body mass index; CI, confidence interval; aOR, adjusted odds ratio.

5.4. Discussion

The influence of genes implicated in the activity of the brain regions associated with psychological traits is poorly explored among elite athletes [3,7]. In addition, the diversity of sports disciplines has made it difficult to detect the potential effect of genetic variants on athletic performance.

To the best of our knowledge, this is the first study investigating the role of genetic factors in the high-level performance of volleyball athletes in a homogeneous and unique cohort of elite subjects. Previous studies were mostly performed on diverse sporting disciplines with a reduced number of volleyball athletes, mostly males, and the potential interaction between the athlete's phenotype and biological systems was not consistently observed [3]. Briefly, a cross-sectional study with 131 elite Turkish athletes, including 33 volleyball players ($20.8 \pm$

4.6 years old, 1.97 ± 2.3 m, 91 kg and 23.3 ± 3.1 kg/m²) found a co-beneficial effect of the *ACTN3* R577X (rs1815739) X allele (RX vs. RR, OR = 5.01, 95% CI, 2.45–10.2; $p < 0.0011$; XX vs. RR, OR = 5.06; 95% CI, 2.04–12.59; $p < 0.0013$; RR vs. RX + XX, OR = 0.19; 95% CI, 0.1–0.38; $p < 0.0011$). Although the volleyball players were considered elite athletes by Dogan and co-workers [21], their training regime and sex were not clearly disclosed. In addition, a study concerning the association between *ACTN3* rs1815739 and the *ACE* I/D gene variation with changes in blood pressure of 107 Serbian Caucasian male players (athletes in mixed sports: volleyball, water polo, and handball ($n = 54$); endurance athletes from middle-distance swimmers, football players, and rowers ($n = 36$); sprint/power from swimmers < 200 m, and short-distance runners ($n = 17$) found that the lowest maximal systolic blood pressure was presented by carriers of *ACTN3* rs1815739 CC genotype. In opposition, the highest percentage of systolic blood pressure decline after the maximal incremental stress test was shown by those carrying the *ACE* DD genotype [22]. Although the *ACE* insertion (I allele) has been related to increased endurance performance in elite athletes [23], this Serbian study did not confirm this effect in various sports, including volleyball [20]. More recently, a study conducted by Orysiak and co-workers [24] with Polish Caucasian female (15.8 ± 2.0 years old and 6.6 ± 2.1 years of sport experience; $n = 132$) and male (16.7 ± 2.1 years old and 7.7 ± 2.9 years of sport experience; $n = 266$) athletes from different sports modalities (volleyball, canoeing, swimming, and ice hockey) found that the distribution of genotypes of *ACTN3* and *ACE* genetic factors was not significantly different between athletes' groups of both sexes. Additionally, the polymorphisms' genotypes (in combination or alone) were not associated with the sum of mechanical power, muscle strength, and height of jump for athletes of both sexes [24,25]. Similarly, Ruiz and co-workers [26] did not observe any association between the *ACTN3* rs1815739 and the likelihood of being an elite volleyball player using either a recessive model (RR and RX vs. XX) or a dominant model (RR vs. RX and XX).

The scarce genetic research involving volleyball players has not solidified the role of neurotransmission and nerve plasticity in athletic performance. Although not confirmed, the dopamine neurotransmitter has been suggested to play a key role in high-level performance due to the athlete's ability to develop emotional and psychological control [27]. However, this has not been confirmed. In a study with fifty elite athletes (involving six volleyball players) and one hundred unrelated healthy controls, genes involved in muscle development (*Myostatin (MSTN)*) and the management of aggressiveness, anxiety, and fear (*Solute Carrier Family 6 Member 4 (SLC6A4)*, *Solute Carrier Family 6 Member 3 (SLC6A3)*, and *Monoamine Oxidase A gene (MAOA)*) were evaluated. No association of elite performance with muscle development regulation or the serotonin pathway was found [28].

The literature concerning the gene–environment interaction and sports lesions among volleyball players is also limited [29–32]. In a study involving Brazilian volleyball players (146 and 125 players, with and without tendinopathy, respectively; $n = 271$), the *Forkhead Box P3 (FOXP3)* rs3761549 and the *Fc Receptor Like 3 (FCRL3)* rs7528684 were studied for tendinopathy risk [32], which was more prevalent in males (OR = 2.87; 95% CI = 1.67–4.93) and related to increased age (OR = 8.75; 95% CI, 4.33–17.69) and volleyball experience (OR = 8.38; 95% CI, 3.56–19.73). Salles and co-workers [32] found that athletes carrying the *FCRL3* rs7528684 variant had a higher risk of developing tendinopathy (OR = 1.44; 95% CI = 1.02–2.04). A genotype/phenotype study examined the effect of the VDR-FokI polymorphism in 60 Italian athletes (35 males and 25 females; 11.7% volleyball players) with and without low back pain. Athletes carrying the F allele were two times more likely to develop low back pain (aOR = 2.55, 95% CI 1.02–6.43, $p = 0.046$) [13].

Given the current gaps in our knowledge, the present study was designed to assess the impact of three brain function-related genetic polymorphisms on the sports achievements of elite volleyball players. As expected, significant differences in the anthropometric profile between female and male athletes were shown, which could be due to biological-related factors. More than a quarter of the players were competing at a professional level, and a high incidence of sport-related lesions occurring at very early ages was reported by athletes of both sexes. Age, hand length, and the SNP *FAAH* rs324420 were independent predictors of high sports achievements. Regarding the impact of age, as older players have more sports experience, inevitably they are more prepared for the demands of high-level athletic performance. However, this might also be a mere case of opportunity, meaning older players had more opportunities to win competencies than their counterparts. As for the impact of hand length, this might be due to body composition requirements for technical skills in volleyball (e.g., serving, setting, spiking, and blocking) [32]. Thus, players with bigger hands are more equipped for the demands of volleyball.

The fatty acid amide hydrolase protein, involved in the metabolism of endocannabinoids, is encoded by the *FAAH* gene, which contains the SNP rs324420. The protein is recognized for breaking down the metabolite N-Arachidonoyl ethanolamide (Anandamide, AEA), which activates the cannabinoid type 1 receptor (CB1). The protein FAAH is activated during stress exposure circumstances (Figure 5.1), which raises the neuronal excitability in the amygdala, a critical brain area that mediates anxiety [7]. As opposed to this, FAAH inhibition lessens anxiety-like behavior [33] and may promote an antidepressant impact via stimulation of the CB1 receptor [34].

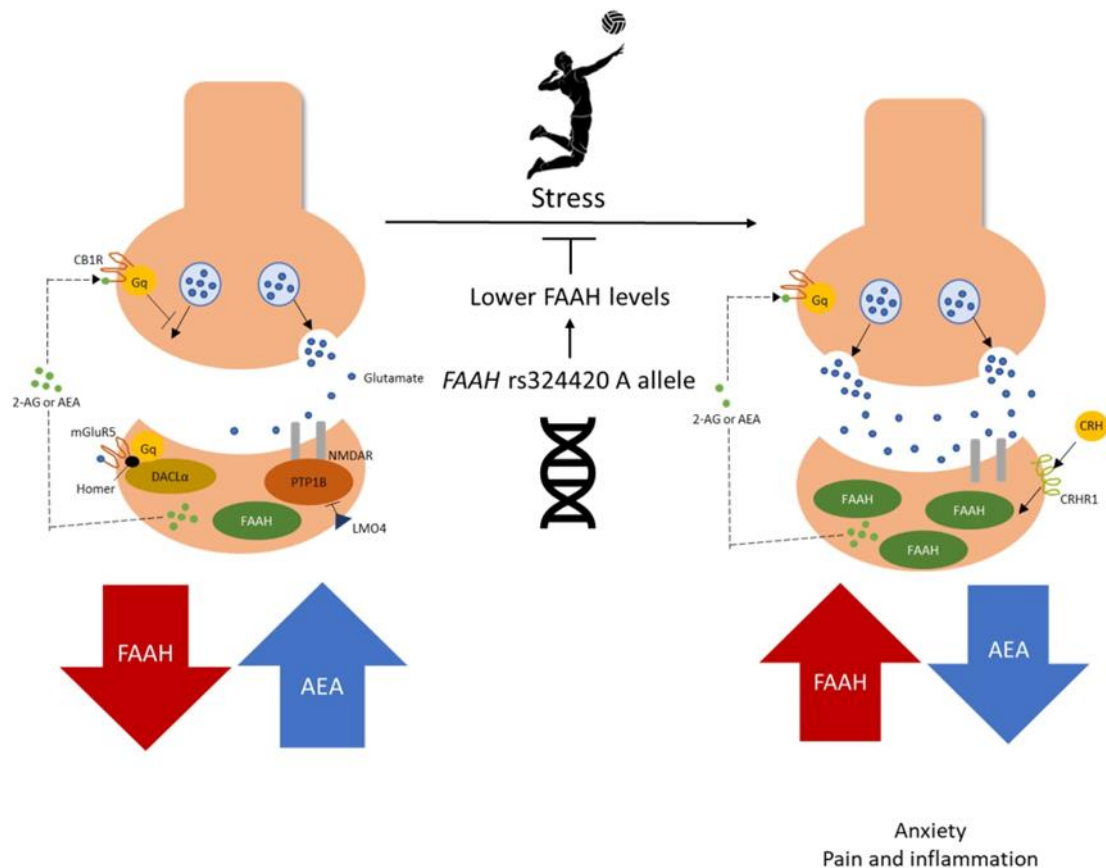


Figure 5.1. The central role of the endocannabinoid system in regulating the stress response of high-level performance volleyball players. In normal conditions, the endocannabinoid system represses the release of the neurotransmitter glutamate via N-arachidonylethanolamine (AEA), modulating the synaptic function. Nevertheless, under acute stress, the mechanism mediated by corticotropin-releasing hormone (CRH) and its receptor (corticotropin-releasing hormone receptor 1-CRHR1) can be triggered and increase the fatty acid amide hydrolase (FAAH) activity in the basolateral amygdala. Consequently, AEA levels decrease, which are no longer able to restrain glutamate release. As a result, increased neural excitability in the basolateral amygdala enhances anxiety-like behavior (adapted by Silva et al.) [3]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; NMDAR: NMDA receptor; LMO4: LIM domain only 4.

According to the literature, FAAH is a good candidate for drug discovery in patients dealing with pain and inflammation [35]. For elite athletes, especially those who play volleyball at a high level of performance with a high incidence of sports lesions, this is of vital importance. Prior research suggested that the 385A allele was associated with reduced FAAH levels [33], with prevalence rates of 16% and 37% in the populations of Africa and the Iberian Peninsula [36]. Although the resulting protein exhibits typical catalytic properties, the 385A allele is associated with a higher sensitivity of FAAH to proteolytic degradation and a shorter half-life, which accounts for the protein's lower amounts [3,7]. The 385A allele may be linked to accelerated amygdala reactivity to threat habituation, enhanced learning of how to

overcome fear, and diminished anxiety-like behavior. This is crucial for athletes to respond and adapt to unpredictably stressful situations, handle stress more effectively, and increase motivation for performing and competing at a high level [8,33,37]. This supports our results that showed that athletes carrying the 385A allele (AA or AC genotype) were twice as likely to be super athletes than those carrying the CC genotype (aOR = 2.00; 95% CI, 1.04–3.82; $p = 0.037$) [3]. Furthermore, the A allele was also previously found to be associated with better athletic achievements among elite rink-hockey players (aOR = 2.88; 95% CI, 1.06–7.80). Therefore, the well-noticed effect of *FAAH* rs324420 in high-level performance sports brings new insights for future research in the *FAAH*'s mechanism of action via the endocannabinoid system [38], applied for inflammation control, and pain regulation. This may also highlight the need to improve not only the athletes' physical health and performance, but also their mental status, especially under stressful conditions [33].

As for the other two SNPs, in opposition to previous studies on athletic performance [13,27,39], no significant association was identified. However, it should be noted that the present study was not sufficiently powered to detect small effects. Hence, future studies with larger cohort sizes are required to better explore their impact. Briefly, the *Adrenoceptor Beta 2* (*ADRB2*) rs1042713 (Arg16Gly) A allele was previously associated with a lower resting cardiac output, protein density, and endurance performance [40], while the G allele was related to the sprint performance of young football players [41]. The *ADRB2* encodes the β 2-adrenergic receptor (a member of the G protein-coupled receptor superfamily), which is primarily responsible for the regulation of many physiological systems with direct implications for athletic performance, including the central nervous, vascular, cardiac, pulmonary, and endocrine systems [41–43]. The protein is involved in the catecholamine system, namely, in the regulation of lipid mobilization from the adipose tissue, glucose uptake, and energy expenditure [40]. As for *Nitric Oxide Synthase 3* (*NOS3*) rs1799983 (G894T), it has been associated with the athletes' susceptibility to sports lesions [44]. The SNP has also been shown to have a potential role in reducing blood pressure in response to physical exercise through the mechanism of endothelium vasodilatation, with implications for athletes' endurance performance [27]. Additionally, athletes carrying the SNP T allele have shown a higher right ventricular mass index (32 ± 6 g vs. 27 ± 6 g, $p < 0.01$) and a larger right ventricular stroke volume index (71 ± 10 mL vs. 64 ± 10 mL, $p < 0.01$) than their counterparts (GG genotype carriers) [45].

Regarding the limitations of our study, although our cohort is highly representative of elite volleyball players competing in Portugal, the small cohort size may have limited the statistical power to detect small effects. Indeed, the sample collection for the present study was extremely affected by the COVID-19 pandemic-imposed restrictions, which also made it difficult to collect data on factors known to affect sports achievements [4]. Beyond

additional studies with larger cohort sizes, further investigation on the effect of *FAAH* rs324420 on athletic performance should also include control groups (sedentary individuals and not elite athletes) to better assess the implications of this SNP in sports practice.

5.5. Conclusions

Elite volleyball players carrying the 385A allele seem to be more prone to be super athletes, which may be attributed to better stress-coping abilities and a higher pain tolerance. This result is in line with our previous findings concerning rink-hockey players of high-level performance. Considering the cumulated data, the SNP *FAAH* rs324420 may help coaches and clinical staff plan the athletes' training, considering their individual mental and physical characteristics that together influence pain tolerance and decision-making in a competition. Thus, sports healthcare professionals, including medical doctors, nutritionists, and physiotherapists, should be aware of the potential of *FAAH* rs324420. However, despite the encouraging results concerning this SNP, additional studies with larger cohort sizes are mandatory to replicate and validate these findings and better characterize the functionality of *FAAH* rs324420. Inclusively, further investigation in other individual sports disciplines is encouraged. Additionally, this genetic polymorphism should also be investigated in the framework of sports lesion prevention, treatment/rehabilitation, and the return of volleyball players to sports activity. As for the other tested SNPs, their implications should be evaluated using larger sample sizes given the lack of study power to discover small effects.

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6 *FAAH* rs324420 polymorphism: biological pathways, impact on elite athletic performance and insights for Sport Medicine

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- 6.1. Introduction
 - 6.2. The endocannabinoid system and the biological pathways of *FAAH* rs324420
 - 6.2.1. The endocannabinoid system signalling
 - 6.2.2. *FAAH* protein functioning and *FAAH* gene variation
 - 6.2.3. *FAAH* rs324420 polymorphism
 - 6.3. Geographic distribution of *FAAH* rs324420 alleles
 - 6.4. *FAAH* rs324420 polymorphism and elite athletic performance
 - 6.4.1. Biological evidence in elite athletic performance
 - 6.4.2. Biological pathways, elite athletic performance and Sport Medicine
 - 6.5. *FAAH* rs324420 polymorphism and other psychobiological associations
 - 6.5.1. *FAAH* rs324420 polymorphism, stress, anxiety and fear extinction
 - 6.5.2. *FAAH* rs324420 polymorphism, pain and inflammation
 - 6.5.3. *FAAH* rs324420 polymorphism and neural dysfunctions
 - 6.6. Implications for Sport Medicine
 - 6.7. Conclusions and future directions
 - 6.8. References

6.1. Introduction

An elite athlete can be defined as a highly specialised person in a given sport discipline, possessing exceptional physiological, psychological, physical and environmental (including family, coach, medical and clinical staff) characteristics, allied with an outstanding sports performance [1]. Strong physical and mental preparation with the combination of an adequate training regime, healthy nutrition and close clinical supervision of the athletes' health are mandatory for elite sport success. In this setting, genetic architecture also plays a major role in athletic performances [2–7].

During the past decade, active research on sports genetics has been engaged with various physiological functions linked to cardiovascular, respiratory, nervous and muscle-skeletal systems and their influence on athlete phenotype [1,8]. However, genetic studies on mental abilities affecting resilience, leadership and anxiety and stress management in training and competitions, as well as pain regulation and sports injuries, are scarce [2–4,9]. In fact, genes encoding proteins that modulate the operating of the brain's emotional centre, located in the hypothalamic-pituitary-adrenal (HPA) axis, particularly those related to the production of a stress response, need to be further investigated in the context of sports performance [1]. One of the genes is *fatty acid amide hydrolase (FAAH)*.

FAAH encodes for a key marker of the amygdala-prefrontal cortex circuit that supports emotion regulation. This protein has been mostly studied in rodent models and more recently in humans. Due to its major catabolic activity for the endocannabinoid anandamide (AEA), testing of *FAAH* inhibitors is important for drug development for diverse diseases, including depression, anxiety, aggressive behaviour, borderline personality disorder, substance use disorders and inflammatory bowel disease [10–13]. Interestingly, in the last five years, the single-nucleotide polymorphism (SNP) *FAAH* rs324420 (also named as c.385C > A or Pro129Thr) has been linked with elite athletic performance in regulating anxiety-like behaviour and influencing persistence and leadership, despite conflicting findings [2,5,6,14].

Given the potential biological impact of this SNP on pain and inflammation regulation, its roles in sports performance and sports medicine should be further dissected. Therefore, this narrative review aims to discuss: (1) the endocannabinoid system (ECS) and the biological pathways of *FAAH* rs324420, (2) the geographic distribution of this SNP, (3) its impact on elite sports performance, (4) its other psychobiological associations and (5) its implications for sports medicine. To do so, a search of the published literature that investigates the association between *FAAH* rs324420 and elite athletes was conducted by screening the PubMed platform. Data collection included scientific articles, books and book chapters published until 23 August 2023. The sole descriptor (i.e., keyword) that was used to search for the articles was “*FAAH* rs324420”. Both review and original articles were

considered, totalling 60 publications. From these, findings of only 54 scientific publications were associated with the *FAAH* rs324420 at different levels. The associations between this polymorphism and different traits were investigated by diverse studies, which are summarised in Figure 6.1.

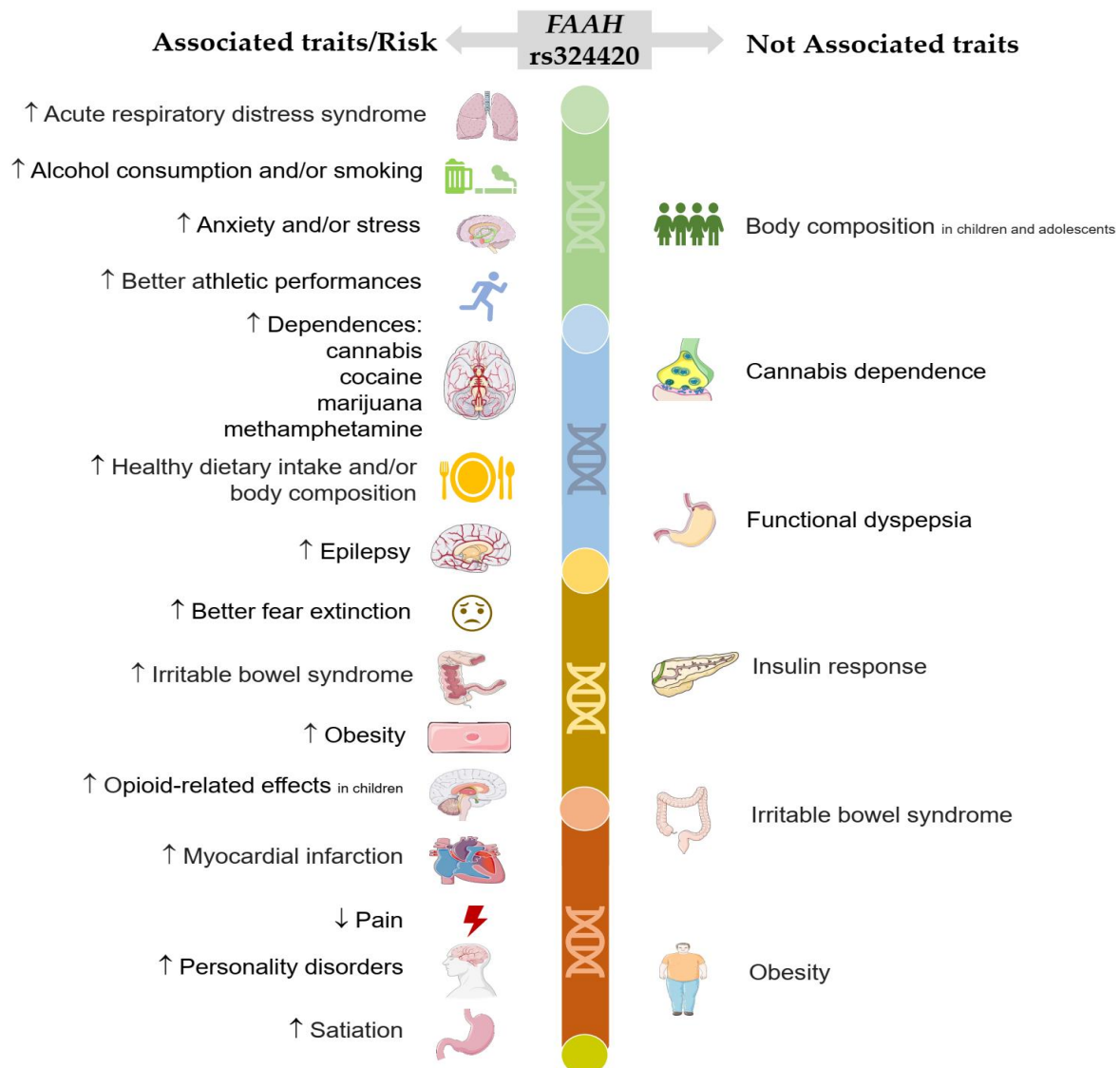


Figure 6.1. Scientific publications selected in PubMed reporting associated ($n = 54$) and not associated ($n = 6$) traits linked to psychobiological pathways of *fatty acid amide hydrolase* (*FAAH*) rs324420. Genetic variants of this polymorphism have been associated with the increased (↑) or decreased (↓) risk of experiencing various pathologies, disorders and/or behaviours, such as: (1) suffering acute respiratory distress syndrome [15]; (2) consuming alcohol and/or smoking [11,16–21]; (3) experiencing anxiety and/or stress [22–25]; (4) achieving better athletic performances [6,26–28]; (5) suffering substance dependences in adults, including cannabis [12,29–33], cocaine [34], marijuana [35–38] and methamphetamine use [39]; (6) practicing a healthier dietary intake and/or body composition [40–43]; (7) suffering neurological disorders, such as epilepsy [26]; (8) dealing better with fear extinction [44–48]; (9) suffering irritable bowel syndrome [13,49,50]; (10) being obese [51–53]; (11) experiencing opioid-related effects in children [54–56]; (12) suffering myocardial infarction [57]; (13) feeling less pain [58–60]; (14) having personality disorders [10,42]; and (15) feeling satiation more easily [61]. On the other hand, a few studies have not found associations between *FAAH* rs324420 variants and body composition in children and adolescents [62], cannabis dependence [29], functional dyspepsia [63], insulin response [64], irritable bowel syndrome [65] and obesity [66].

Supplemental publications were included by cross-referencing the reference lists of the retrieved articles. After data collection, the results were analysed through comprehensive reading and structured in accordance with the themes discussed in this article. A total of 131 publications involving human participants and written in one of five languages (Portuguese, English, Spanish, French or Italian) were included in this review.

6.2. The endocannabinoid system and the biological pathways of *FAAH* rs324420

6.2.1. The endocannabinoid system signalling

One of the most promising biological systems involved in emotion control is the ECS. It is a highly complex signalling system mostly involved in body homeostasis due to its direct action on the central nervous system (CNS) [67]. Specifically, this system acts on immune response modulation [68], motor activity, fear and anxiety regulation [69,70], cardiorespiratory system control [71], stress responses [72], memory process [73] and pain perception [74] by the activation of several molecular targets by the AEA or the 2-AG, resulting in a variety of biological actions, as shown in Figure 6.2.

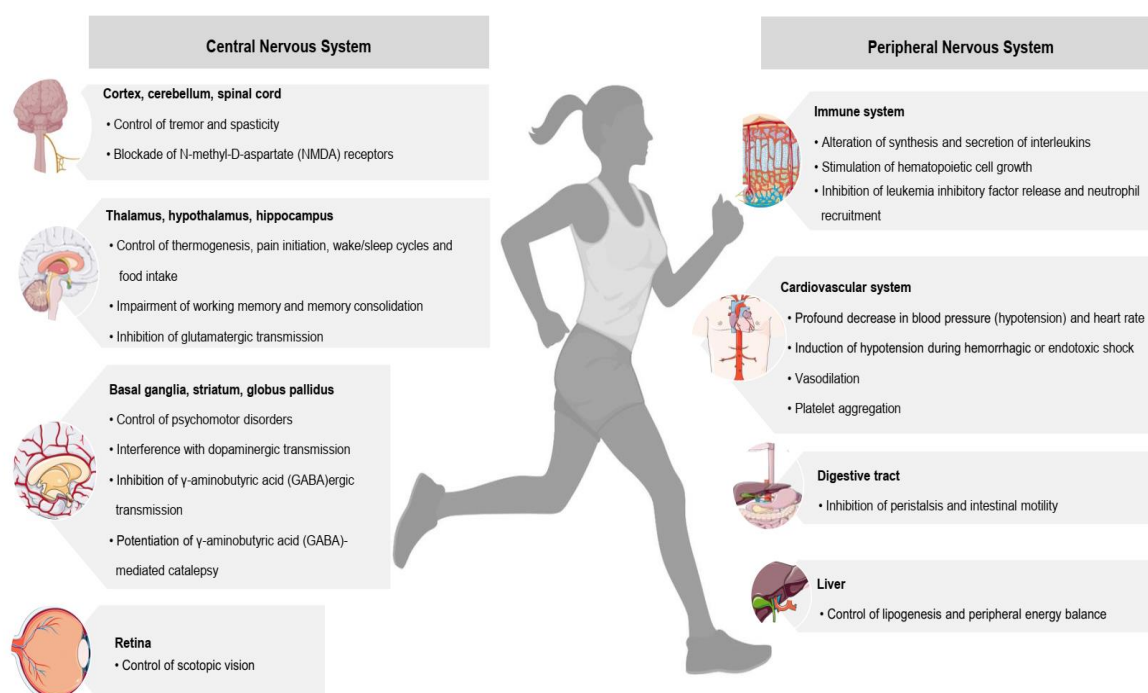


Figure 6.2. Effects of the endocannabinoid system in central nervous and peripheral systems (adapted from Wang, Dey and Maccarrone) [74]. The activation of several molecular targets by the endocannabinoid anandamide or the 2-arachidonoidglycerol results in a variety of biological actions, affecting practically all central and peripheral systems in animals, as shown.

Regarding its composition, ECS encompasses endocannabinoids (eCBs) that are neuromodulators of the CNS, the two most-studied being AEA and 2-arachidonoidglycerol (2-AG). These two act as endogenous ligands for two cannabinoid receptors, namely CB1

and CB2, and as proteins responsible for their biosynthesis, metabolism and release [75]. The eCBs can also function as immunomodulators, such as the AEA, which protects neurons from inflammatory damage during CNS inflammation [67].

The functioning of ECS depends on the interaction between the synthesis, release and inactivation of its endogenous agonists, the eCBs. Both AEA and 2-AG are synthesised via a phospholipid-dependent mechanism, released and taken up by cells via passive diffusion across the plasmatic membrane [67]. After their synthesis, they do not concentrate into synaptic vesicles and are primarily degraded by intracellular enzymatic hydrolysis by the *FAAH*, in the matter of AEA and by the monoacylglycerol lipase (MAGL) and *FAAH*, in the case of 2-AG [67]. In turn, AEA operates on CB1 receptors (R), which are largely found in the CNS, and CB2 receptors, which are predominantly found in the peripheral nervous system and involved in immunological response [66,76]. The CB1R is a G protein-coupled receptor integrated in presynaptic terminals on GABAergic and glutamatergic neurons and is thus integrated in the retrograde signalling of neurotransmission. It is significantly expressed in brain areas involved in emotional behaviour control and memory-related plasticity, namely the prefrontal cortex, amygdala and hippocampal formation [77].

6.2.2. *FAAH* protein functioning and *FAAH* gene variation

FAAH is a serine hydrolase that has been associated with the inactivation of the eCBs, which consists of transporting them back to the cell. This transport is contrary to the normal transport of other neurotransmitters, meaning without a difference in the sodium gradient (Na^+). It is thought that it can occur using lipid transporter proteins by mechanisms of facilitated diffusion and in favour of the concentration gradient [78]. Once inside the cell, the AEA is hydrolysed by the enzyme *FAAH*.

This enzyme is encoded by the *FAAH* gene, which is positioned on the short arm (p) of chromosome 1 at position 33 (1p33). As mentioned, it is considered an intracellular enzyme that can hydrolyse AEA and other bioactive amides. It is also responsible for controlling the brain concentrations of these compounds, having a strategic location in the brain, although its presence has been verified in other tissues, for example, the liver, lungs, kidneys, spleen and testis. In the brain, namely in the hippocampus, cortex and cerebellum, this enzyme is located close to membranes of postsynaptic nerve terminals, where the CB1R are present. Therefore, its location indicates that *FAAH* plays an active role in AEA inactivation, which takes place in postsynaptic neurons [79].

6.2.3. *FAAH* rs324420 polymorphism

There are sequence variations in different regions of *FAAH* [30,35,44,80–83], rs324420 being the most-studied SNP of this gene concerning different phenotypes and functions or

disorders, such as obesity [84], mental diseases [85] or biopsychosocial disorder [86]. This polymorphism consists of the substitution of the nucleotide Cytosine (C) by an Adenine (A) at nucleotide position 385, translating into an amino acid exchange of a Proline (Pro) for a Threonine (Thr) in the codon 129, designated *FAAH* C385A (Pro129Thr; rs324420) (National Center for Biotechnology Information) [34]. This SNP has a minor allele frequency (MAF) of >5% [30]. The A allele, which is the minor, is known to reduce *FAAH* cellular activity and expression due to increased sensitivity to proteolytic degradation. Consequently, compared to C allele individuals, AA genotype carriers have almost 50% decreased *FAAH* activity, which translates into increased signalling and concentrations of AEA [23,87]. The A allele has been related to an increased risk for borderline personality disorder [10], substance use disorders [11,19], cannabis, methamphetamine, and cocaine dependence [27,28,33,34,38] and overweight/obesity [88,89]. However, contradictory results have been observed in cannabis users [12] and regarding athletes' nutritional status [5,6].

6.3. Geographic distribution of *FAAH* rs324420 alleles

As mentioned, the rs324420 A allele has been associated with a lower expression of *FAAH* levels [89]. Its prevalence rates range from 36.8% and 35.2% in the African and the American populations, respectively, to 21.1%, 19.5% and 17.6% in the European, South and East Asian populations, respectively [90,91] (Figure 6.3). Given the recent findings involving Southwestern European athletes [5,6], it is important to note that the frequency of this allele in the Iberic peninsula is even lower (16.4%).

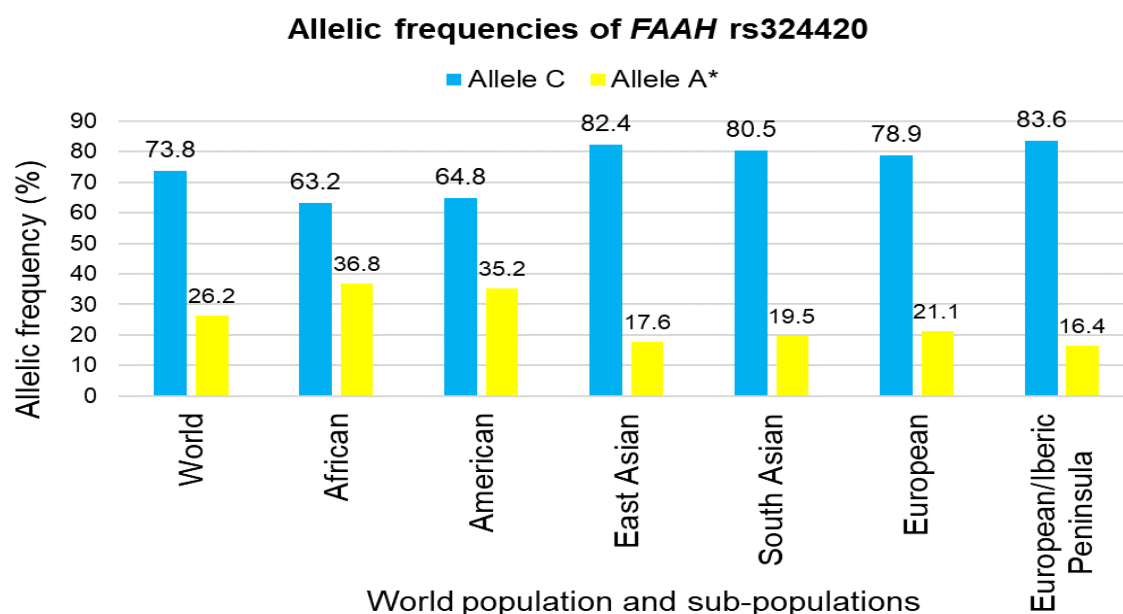


Figure 6.3. Geographic distribution of allelic frequencies of the *fatty acid amide hydrolase* (*FAAH*) rs324420 [90]. * Minor allele.

6.4. *FAAH* rs324420 polymorphism and elite athletic performance

The *FAAH* rs324420 polymorphism has been associated with pain tolerance and inflammation [92], both with strong implications for the athlete's well-being and performance, especially among those competing at high-intensity and/or invasion/contact sports with a high frequency of injuries and/or traumas. However, the biological role of the C385A variant in sports performance is not yet a matter of consensus.

6.4.1. Biological evidence in elite athletic performance

First, evidence suggested a detrimental effect of the 385A allele (A allele), as it seemed to be more prevalent among sedentary people than in elite athletes [2,14]. A case-control study including 413 Polish Caucasian elite power and endurance athletes (aged 23.5 ± 4.7 years, of which 36.3% were females) and 451 Caucasian sedentary controls (aged 23.0 ± 3.1 years with 51.9% females) revealed that the polymorphism AA genotype was more common among controls than athletes and that elite sports performance was negatively affected by the AA genotype (AA versus (vs.) CC + AC; odds ratio (OR) = 0.44; 95% CI, 0.24–0.81; $p = 0.0084$) [14]. Identical results were reported later in 621 elite athletes (183 of power, 212 of endurance and 226 combat sports) and 451 controls [2], where the AA genotype was underrepresented in both power (AA vs. CC + AC; OR = 0.36, 95% CI = 0.15–0.86, $p = 0.017$) and endurance athletes (AA vs. CC + AC; OR = 0.42, 95% CI = 0.20–0.90, $p = 0.022$) in comparison with controls. Furthermore, when the two groups of athletes were examined together, the effect on athletic status was even more pronounced (in the recessive model: OR = 0.40, 95% CI = 0.22–0.72, $p = 0.002$), indicating a negative impact on athletic performance [14].

Recently, a cross-sectional study conducted with 116 of the world's best rink-hockey players (aged 28.2 ± 8.7 years), of which 15.5% were females (25.3 ± 7.9 years old) and 84.4% were males (28.8 ± 8.7 years old), found that the *FAAH* rs324420 and the presence of severe sports injuries were independent predictors of elite sports performance [5]. The A allele appeared to have a positive effect among elite players, as the carriers were three times as likely to be super athletes than those with the CC genotype (AA/AC vs. CC; adjusted OR = 2.88; 95% CI, 1.06–7.80; $p = 0.038$), possibly due to better stress coping and higher pain tolerance. A later cohort study of 228 elite volleyball players (aged 26.7 ± 8.1 years old, 29.0% females) observed that carriers of the 385A allele were two times more likely to be super athletes than athletes demonstrating the CC genotype (adjusted OR = 2.00; 95% CI, 1.04–3.82; $p = 0.037$) [6].

Given the inconsistent results regarding the C385A variant, validation studies considering other homogeneous sports modalities in addition to rink-hockey and volleyball, larger cohort samples and both male and female representation are crucial to investigate the specific role

of this SNP in athletic performance. In addition, the athletes' ethnicity may also influence the prevalence of *FAAH* rs324420 genotypes. Therefore, including homogeneous groups of athletes in this regard may enhance the research impact. For instance, the two first studies were conducted with athletes being exclusively of Polish Caucasian origin, whereas the two more recent studies mostly involved Portuguese people, with almost one-third being of other nationalities (Spanish, Argentinian, Italian, French, Servian, American, Canadian, Brazilian, Chilean, Mozambican, Angolan and Australian) (Table 6.1).

Table 6.1. Distribution of *FAAH* rs324420 genotypes among research studies involving elite athletes.

Authors	Population	Athletes' Profile	Genotypes, <i>n</i> (%)		
			AA	AC	CC
Silva et al. [6]	75.1% Portuguese and 24.9% Others	Elite volleyball players (<i>n</i> =219)	11 (5.0)	74 (33.8)	134 (61.2)
	75.8% Portuguese and 24.2% Others	Female (<i>n</i> = 66)	3 (5.0)	20 (33.3)	37 (61.7)
	74.8% Portuguese and 25.2% Others	Male (<i>n</i> = 162)	8 (5.0)	54 (34.0)	97 (61.0)
Silva et al. [5]	82.8% Portuguese and 17.2% Others	Elite rink-hockey players (<i>n</i> =116)	4 (3.4)	34 (29.3)	78 (67.2)
	All Portuguese	Female (<i>n</i> = 18)	1 (5.6)	6 (33.3)	11 (61.1)
	79.6% Portuguese and 20.4% Others	Male (<i>n</i> = 98)	3 (3.1)	28 (28.6)	67 (68.4)
Peplonska et al. [2]	All Caucasians of Polish origin	Elite athletes (<i>n</i> = 621, 29.3% females)	27 (4.3)	259 (41.7)	335 (54.0)
		Power (<i>n</i> = 183)	6 (3.3)	79 (43.2)	98 (53.5)
		Endurance (<i>n</i> = 212)	8 (3.8)	84 (39.6)	120 (56.6)
		Combat (<i>n</i> = 226)	13 (5.8)	96 (42.4)	117 (51.8)
Peplonska et al. [14]	All Caucasians of Polish origin	Elite athletes (<i>n</i> = 413, 36.3% females)	16 (3.9)	169 (40.9)	228 (55.2)
		Power (<i>n</i> = 188)	6 (3.2)	80 (42.6)	102 (54.2)
		Endurance (<i>n</i> = 225)	10 (4.4)	89 (39.6)	126 (56.0)

6.4.2. Biological pathways, elite athletic performance and Sport Medicine

In addition to their demands regarding training routines and the need for high achievements in international and national competitions, athletes also face very stressful environments daily [89]. Their stress response can be influenced by the mechanism of action of *FAAH* through the ECS, which is corroborated by the recently observed impact of *FAAH* rs324420 on elite athletic performance [93,94].

Stress is described as the body's reaction to an internal or external stimulus to prepare for potential injuries and/or diseases. Physical and psychological stress causes a series of responses that produce immediate threat management followed by a return to homeostasis.

The first brain responses are released in a few seconds of the stimulus [89]. This mechanism involves several neurotransmitters, including serotonin, noradrenaline, the fast-acting stress hormone adrenaline, GABA and glutamate. Endocrine reactions begin minutes to hours after the stressful stimulus, driven by stimulation of the HPA axis and culminating in the production of adrenal glucocorticoids. Preclinical results clearly suggest the concept that stress alters eCB signalling and that this is a fundamental mechanism through which stress alters synaptic plasticity in diverse brain areas [89].

Under stress conditions (Figure 6.4, adapted from Silva et al.) [6], *FAAH* is triggered to breakdown the AEA, raising neuronal excitability in the amygdala, a critical anxiety-mediating part of the brain [2]. In contrast, inhibiting *FAAH* reduces anxiety-like behaviour [89] and may provide an antidepressant effect by stimulating the CB1 receptor [95]. As a result of a higher susceptibility to *FAAH* degradation [2], the SNP A allele may be associated with faster habituation of amygdala responsiveness to danger/threat, lower anxiety-like behaviour and greater fear-extinction learning. This is critical for elite athletes, who need to present personality attributes related to stress response and effective mental discipline to deal with uncertain events more rapidly, boost their motivation for sports competition and decrease the risk of sports injuries [14,78,89].

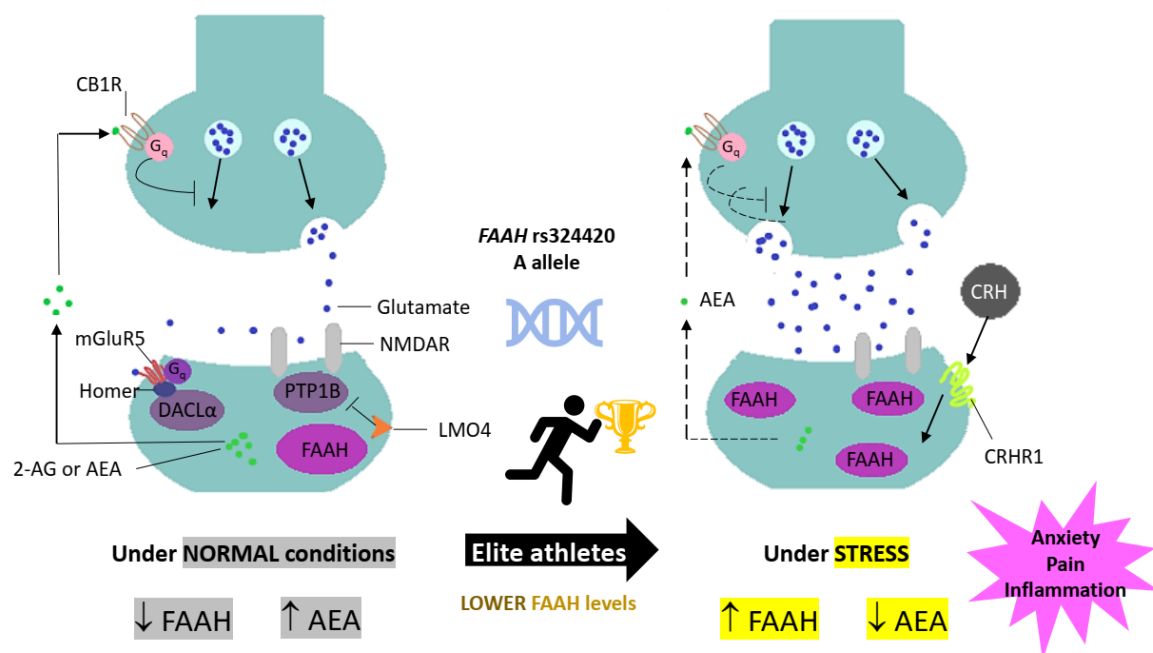


Figure 6.4. The endocannabinoid system plays a key role in controlling how highly skilled athletes react to stress. Under normal circumstances, the endocannabinoid system modulates synaptic function by inhibiting the release of the neurotransmitter glutamate via *N*-arachidonylethanolamine (AEA). However, under severe stress, the corticotropin-releasing hormone (CRH) and its receptor (corticotropin-releasing hormone receptor 1-CRHR1) can be activated, increasing (↑) the basolateral amygdala's fatty acid amide hydrolase (*FAAH*) activity. As a result, AEA levels drop (↓) and lose their ability to control glutamate release. Consequently, enhanced anxiety-like behaviour is caused by increased neuronal excitability in the basolateral amygdala (adapted by Silva et al.) [6]. CB1R: cannabinoid type 1 receptor; Gq: family G protein; mGluR5: metabotropic glutamate receptor 5; 2-AG: 2-arachidonoyl glycerol; DAGLα: diacylglycerol lipase-α; PTP1B: protein tyrosine phosphatase 1B; NMDAR: NMDA receptor; LMO4: LIM domain only 4.

6.5. *FAAH* rs324420 polymorphism and other psychobiological associations

6.5.1. *FAAH* rs324420 polymorphism, stress, anxiety and fear extinction

The exposure to repeated stressful situations results in the habituation of the HPA axis activation and the behavioural stress response. The ability to habituate to repeated exposure to a non-threatening stimulus is protective because it avoids the repercussions of prolonged stress [5,6]. The potential of eCB-mediated synaptic plasticity to facilitate habituation may be one of the most important roles of this mechanism in the setting of human psychopathology [89].

Stress and glucocorticoids both raise 2-AG levels in the hypothalamus, hippocampus, prefrontal cortex and raphe nuclei. When a plasma membrane-associated glucocorticoid receptor in the hypothalamus is activated, 2-AG levels rise rapidly, inhibiting glutamate release [96]. The mechanism by which glucocorticoids increase 2-AG levels in the prefrontal cortex is still unknown, although this increase is known to inhibit GABA release [97]. Activation of CB1R signalling is essential for glucocorticoid-mediated feedback inhibition of the HPA axis [98]. Therefore, the eCB system enhances the activation of resilience elements during and/or after stress exposure [99].

As mentioned before, acute stress modifies the concentrations of the two primary eCBs, AEA and 2-AG, in the brain, altering CB1R signalling [5,6,89]. Acute stress reduces AEA concentrations in the amygdala and prefrontal cortex; these changes are accompanied by an increase in *FAAH* activity and are mediated by CRH effects that modify *FAAH* activity [100]. Reduced AEA concentrations in the amygdala allow activation of the HPA axis, while *FAAH* inhibition lowers the glucocorticoid response [101]. A study conducted with 661 total participants (19.6 ± 1.2 years old), 121 of whom had at least one Diagnostic and Statistical Manual of Mental Disorders-IV diagnosis, concluded that individuals with high AEA inhibitory tone (*FAAH* 385A allele carriers) and high corticotropin-releasing hormone (CRH) signalling (corticotropin-releasing hormone receptor type 1 (CRHR1) rs110402 A allele homozygotes) had the least temporal habituation of the basolateral amygdala, a neuroimaging associated with fear extinction [24]. Stress-induced CRH signalling via CRHR1 in the basolateral amygdala increases *FAAH* activity [102]. In turn, increased activity of this catabolic enzyme leads to the reduction of AEA and a deprivation of inhibitory tone, which is required for lowering anxiety and sustaining fear extinction [78].

When compared to CC homozygotes, healthy individuals carrying the *FAAH* rs324420 A allele demonstrated enhanced fear extinction learning [44] and lower anxiety levels [78,103–105]. This impact was more pronounced in AA homozygotes, who had a simpler degradable *FAAH* enzyme, resulting in higher AEA among carriers [106]. In a sample of 55 healthy male adults, including 17 AC genotype carriers and 34 CC homozygotes, brain activation upon an unextinguished versus extinguished stimulus was greater in AC

genotype carriers than in CC homozygotes in core neural elements related to extinction recall. They also displayed higher AEA levels and lower anxiety levels ($p < 0.05$) [22]. However, controversial results have been found. In a study with 928 Hungarian (all Caucasians) subjects (31.3 ± 10.5 years old; 69.8% females), *FAAH* C385AA allele carriers who experienced childhood adversities demonstrated higher levels of anxiety than CC carriers ($p = 0.0023$) [23]. These findings may be due to a decreased CB1R receptor expression during neurodevelopment in the human brain caused by childhood traumas influencing affective phenotypes, namely the *FAAH* C385A polymorphism [23].

6.5.2. *FAAH* rs324420 polymorphism, pain and inflammation

In response to stress and injuries, ECS has been studied as a key target related to endogenous analgesia [107]. However, very few studies have explained the relationship of *FAAH* rs324420 with pain [60]. The *FAAH* influences eCB concentrations in peripheral and central neurological systems, including immunological cells. It is involved in nociception, inflammatory reactions, and a variety of other processes [108–111]. Inhibiting *FAAH*'s enzymatic activity extends the action of AEA and hence improves eCB-mediated antinociception [60,107].

The amino acid mutation P129T (SNP rs324420) lowers *FAAH* protein expression via a posttranslational system that has not been sufficiently explained. As a result, the SNP rs324420 is the most likely candidate to be the causative variable underlying the connection with sensitivity to cold pain [107]. In a study with women aged 18 to 75 years (900 were tested for cold pain and 1000 for sensitivity to heat pain) and who underwent surgery for breast cancer, patients were divided by the *FAAH* rs324420 genotype (72 for A/A, 380 for A/C and 471 for C/C) [108]. A significant association between the SNP and cold pain sensitivity was found, with greater association in subjects homozygous for the minor allele (AA genotype), who reported less sensitivity to cold pain ($\beta = -1.48$; 95% CI -2.14 to -0.8) than other groups [60].

Given the association of the A allele of the *FAAH* rs324420 with lower *FAAH* activity, 21 highs (with significantly greater pain reduction than lows), 66 low hypnotizable subjects (lows) and 172 controls were genotyped [58]. The A allele frequency increased from lows to controls and from controls to highs (best fitting curve: logarithmic model, $F = 621.93$, $R^2 = 0.998$, $p = 0.026$). Therefore, the role of the *FAAH* polymorphism in high analgesia should not be ruled out, as eCB minor variations can be magnified by eCB interactions with other neurotransmitters [58], as also demonstrated by other researchers [59].

The baseline amount of AEA release in the brain is modest, and neuronal secretion requires a trigger [79]. A powerful stimulus activates the stress response, which can be mediated by the ECS and endogenous opioids and induce stress-analgesia [112]. It seems that the AEA

combined with an *FAAH* inhibitor can generate considerable antinociception. As a result, subjects having a mutation that is expected to reduce *FAAH* function and so prolong the AEA effect reported lower pain intensities and tolerance to cold pain [60].

6.5.3. *FAAH* rs324420 polymorphism and neural dysfunctions

The *FAAH* rs324420 polymorphism has also been related to other neural dysfunctions, such as epilepsy and attention deficit hyperactivity disorder (ADHD). It has been suggested that an altered eCB system can have a neuroprotective effect by activating CB1 receptors by eCBs and selective CB1 agonists, but blocked CB1 receptors by specific antagonists may improve epileptogenesis and lead to diverse neurological conditions such as epilepsy [113] and ADHD [114].

Epilepsy is one of the most prevalent neurological disorders (between 7.60 per 1000) [115] that can be manifested as idiopathic generalised epilepsy and focal epilepsy [116], and it is mainly produced by gene changes and environmental influence. ADHD has a 2–7% global prevalence [117], with candidate gene pathways being influenced by some types of medication administrated for ADHD treatment [118].

As mentioned before, the *FAAH* C385A is responsible for an enlarged vulnerability of the *FAAH* enzyme to proteolytic degradation [76], which increases eCB, preventing the neurotoxicity caused by seizures [119]. Although the literature has investigated the protective effect of *FAAH* inhibitors in the brain area, a recent study conducted with a group of 250 epilepsy individuals, 157 cases with ADHD and 386 healthy controls [26] demonstrated that reduced levels of *FAAH* enzyme produced by this polymorphism increased generalised epilepsy risk by approximately two times (*FAAH* C384A genotype, OR = 1.755, 95% CI 1.124–2.742, $p = 0.013$, and allele, OR 1.462, 95% CI 1.006–2.124, $p = 0.046$). This may be due to potential differences in ligand/receptor ratios of the eCB system [120], as generalised epilepsy affects the broad brain region. In contrast, this SNP was not linked with the risk of ADHD.

The *FAAH* rs324420 variant has also been linked to substance use disorders, specifically cannabis dependence, and that altered *FAAH* activity has been shown to influence alcohol use [20,27], although findings are still complex and controversial [18]. A case-control study with 531 Greek participants (251 alcohol-dependent cases, mean age of 43.5 ± 11.5 yrs., 60 females and 91 males, and 280 controls, mean age of 42.8 ± 14.3 years, 92 females and 188 males) investigated the SNP in patients with Alcohol Use Disorder (AUD) [18]. The authors observed that the A allele was associated with an increased risk of AUD (OR = 0.55, 95% CI 0.41–0.73, $p < 0.0001$). This could serve as a potential biomarker for AUD susceptibility. Another study observed an increased risk of the slow *FAAH* activity group (C/A or A/A) with binge drinking (OR = 2.16, 95% CI 1.36–3.42 at 20 yrs. old, and OR =

1.61, 95% CI 1.10–2.36 at 30 yrs. old), drinking initiation (OR = 1.39, 95% CI 1.09–1.77) and escalation (OR = 2.24, 95% CI 1.05–4.76) and cigarette smoking initiation (OR = 1.20, 95% CI 1.04–1.39), but not with early smoking milestones [17]. A Greek study with 531 participants (251 alcohol-dependent subjects and 280 healthy participants) observed an increased risk of AUD among those carrying the SNP A allele (OR = 0.55, CI 0.41–0.73, $p < 0.0001$) [18]. Regarding alcohol dependence, Sloan et al. [20] observed that, in comparison to people with the CC genotype, American European adults with the A allele exhibited a higher frequency of compulsive drinking behaviours. Also, adolescents carrying AC and AA genotypes showed abnormal drinking attitudes and increased AUD scores [11]. However, due to the complex aetiology of AUD and diversity of genetic and environmental factors [18], further investigation with larger sample sizes and diverse populations are required to examine these findings.

A recent systematic review found that *FAAH* protein contributes to biological and clinical aspects of AUD and that pharmaceutical targeting of this molecule could be useful for alcohol withdrawal by reducing anxiety and resumption of alcohol intake [121]. Since *FAAH* affects brain reward signalling by metabolizing AEA, it might potentially increase addiction vulnerability [122]. As a result, the SNP rs324420 decreases *FAAH* catalytic activity and alters the addictive properties of a variety of substances [20]. The relationship between the SNP and substance use disorders [123] is consistent with previous research that found genetic links between methamphetamine [39], marijuana [36], cannabis [27] and cocaine [34].

6.6. Implications for Sport Medicine

The *FAAH* rs324420 may play diverse functions depending on athletes' age, sex, ethnicity, performance level and type of sport (e.g., athletes submitted to high mechanical impacts may be at greater risk of sport-related injury and medical complications) [5]. Specific attention should be given to the A allele as it was associated with unique athletic achievements [5,6].

Given that the *FAAH* is a good candidate gene for drug discovery in patients dealing with inflammation and pain [124], medical staff working with sports injury prevention and recovery, or athletes' illnesses should advise coaches regarding identification and selection of preventative strategies to be applied in training activities through an individualised training programme [125]. Furthermore, ethical procedures must be respected and followed as the imposition of genetic testing is potentially abusive [125].

While significant sex differences in rs324420 genotype frequencies have not been found in elite athletes [5,6], it seems that oestrogens may modify emotional behaviour by dysregulating the *FAAH* enzyme, increasing the ECS signalling and, as a result, decreasing

women's anxiety [95]. Thus, additional research with female athletes is required to corroborate this hypothesis.

Although scientific studies that genotyped elite athletes for the *FAAH* rs324420 have mostly involved Caucasian players [2,5,6,14], current world records differ by sex and ethnicity, with African ancestry athletes posting faster times in the 100 m, 200 m and 400 m than their Caucasian counterparts [126]. Given the evidence that players carrying the A allele (AA or AC genotype) are two or three times more likely to be super athletes than subjects with the CC genotype [5,6], this might be important for multidisciplinary teams (coaches and clinical staff) responsible for planning athletes' training sessions and preparing them for and helping them recover from competitions, respectively.

The negative impact of sports injuries on athletes' health and performance is undoubtable and can be very devastating, especially if they are recurrent. Therefore, mental qualities are essential to cope with injuries [127–130], acquire pain tolerance [4,131] and be resilient [2,5,6]. The limited scientific literature has shown that the *FAAH* rs324420 input, which codes for physiological aspects of brain regions related to psychobiological qualities, can be a helpful tool for athletic performance [6]. In fact, severe sports injuries and *FAAH* rs324420 were independent predictors of elite athletic performance, probably due to significant differences between athletes' sex and training, as recently reported [5].

Sport medicine specialists and technical staff should be aware that athletes with genetically reduced *FAAH* activity and who are repeatedly submitted to stress (permanently elevated AEA) during their childhood may be vulnerable to anxiety and depression in later life due to long-term effects on stress response possibly by the CB1R downregulation throughout brain neurodevelopment [23].

6.7. Conclusions and future directions

The observed effect of the *FAAH* rs324420 is of the utmost importance for future research in elite athletes experiencing daily stressful training and competition events and potential sports injuries due to the mechanism of action of *FAAH* through the ECS applied to stress management, pain regulation and inflammation control. In addition to the promising character of *FAAH* for drug discovery in patients affected by inflammation and pain, its rare genetic variants may also improve mental discipline and physical performance among elite athletes. The review of the current literature presented in this article suggests that the role of genes coding for structural and biochemical components of brain areas related to psychological traits have been less investigated than athletes' phenotypes related to musculoskeletal and cardiovascular functions. Nevertheless, genetic research in the sports context has shown modest progress since gene-based association analyses need to be more robust in discovering several minor and cumulative gene effects. In the future, more

effective genomic-based research methodologies might speed up the discovery of genes associated with both mental and physical athletic performance and welfare. In addition, it will be critical to create cohorts of truly elite individuals with adequate specific physiological data to offer the requisite resolution, to investigate the findings using multidisciplinary methodologies and to enhance biological and clinical research on athletic ability, health and potential risk of disease or injury. This may impact teams' (e.g., athletes, coaches and sports medicine staff) daily work, which can benefit from individualised training programmes according to each athlete's sex, body composition, nutrition, previous injuries and environmental conditions, while helping to avoid eventual burnout and potential sport dropout.

6.8. References

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7 Conclusões finais e perspectivas futuras

7. Conclusões finais e perspectivas futuras

Dado que os resultados encontrados foram já apresentados e discutidos nos capítulos anteriores, parece-nos pertinente apresentar de seguida, as conclusões finais e as perspectivas futuras, decorrentes da investigação realizada, bem como, a nossa visão baseada na experiência no desporto de alto rendimento.

Assim, parece-nos possível concluir que o polimorfismo *FAAH* rs324420, que potencialmente afeta os processos mentais e as emoções via serotoninérgica, influencia a predisposição para o desempenho desportivo de alto rendimento.

O nosso trabalho mostra pela primeira vez que:

- > os jogadores de elite, quer sejam hoquistas ou voleibolistas, portadores do alelo A no polimorfismo *FAAH* rs324420, estão mais propensos a ser super-atletas (os hoquistas, três vezes mais, e os voleibolistas, duas vezes mais) do que os portadores do genótipo CC.

- > os hoquistas de elite, que sofreram mais lesões desportivas, apresentam quase quatro vezes mais tendência para serem super-atletas, do que os mais jovens, talvez devido à exigência elevada do regime de treino e da competição a este nível.

- > os voleibolistas de elite, que são mais velhos e que possuem um maior tamanho da mão dominante, demonstram quase três e seis vezes, respetivamente, mais tendência para serem super-atletas do que os mais novos e com a mão de menor tamanho.

- > dos sete polimorfismos genéticos estudados nos dois grupos de atletas, o polimorfismo *VDR* rs731236 foi o único, no qual se observaram diferenças entre os hoquistas, de acordo com o género, embora este não tenha apresentado associação com o desempenho desportivo.

De acordo com o exposto, os atletas portadores do alelo A no polimorfismo *FAAH* rs324420 poderão ter melhores desempenhos desportivos no treino e em competição, possivelmente associados a uma melhor disciplina mental em situações de stress, que requerem uma rápida tomada de decisão, e a uma melhor capacidade de resiliência e de lidar com as lesões desportivas, devido a uma eventual maior tolerância à dor.

No caso do hóquei em patins, os últimos quinze minutos da competição caracterizam-se habitualmente por situações decisivas para o resultado final do jogo, através da marcação de livres-diretos, de grandes penalidades e de inferioridade numérica. Estas situações são, do ponto de vista da componente física e mental do exercício, muito intensas e desgastantes, em particular para os guarda-redes. Para além disso, o facto de se tratar de um desporto de invasão e de elevado contacto físico, associado a uma elevada velocidade de deslocação dos jogadores, aumenta o risco de lesões desportivas.

Apesar de o jogo de voleibol não envolver limite temporal, nem ser um desporto de invasão, o efeito mecânico do impacto produzido no sistema músculo-esquelético, causado pelos saltos verticais e rápidas mudanças de direção, aumenta o risco de lesões desportivas.

Para além disso, a obrigatoriedade de se jogar o quinto *set* para decidir qual a equipa vencedora, em caso de empate, e de este último *set*, ter que ser obrigatoriamente jogado até aos quinze pontos, acarreta responsabilidade adicional e impulsividade à tomada de decisões, que podem ser geradoras de stress nos atletas.

Neste sentido, sugere-se que os treinadores e restantes membros das equipas técnica e clínica ponderem sobre o potencial uso de estratégias de relaxamento físico e cognitivo durante os períodos de treino, como forma de otimização do desempenho nos treinos e de preparação para as competições, e desta forma, minimizar-se o erro na prestação desportiva.

Por outro lado, parece importante promover-se o diálogo entre treinadores, atletas e a comunidade científica sobre a realização de testes genéticos para se caracterizar o perfil genómico dos atletas e potenciais resultados para o desempenho desportivo.

Dada a associação do polimorfismo *FAAH* rs324420 com a maior tolerância à dor e à diminuição da inflamação, propomos que essa caracterização deve incluir os polimorfismos analisados neste estudo, com particular ênfase no *FAAH* rs324420, em interação com outros indicadores de saúde e do perfil nutricional dos atletas, praticantes de outros desportos e envolvendo amostra maiores.

Apesar das questões éticas, que devem ser devidamente acauteladas, e que podem ser levantadas, relativamente à testagem do perfil genómico em atletas de alto rendimento e o uso desses resultados, realça-se a sua pertinência na prevenção e no tratamento de lesões, bem como, na promoção da recuperação do atleta, de forma mais eficaz, e consequentemente, minimizando-se o tempo de paragem parcial ou total do atleta e prevendo-se um regresso aos treinos potencialmente mais rápido.

8 Referências gerais

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