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Genotype scores in energy and iron-metabolising genes are higher in elite endurance athletes than in non-athlete controls

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Novelty bullets: points that summarize the key findings in the work:	Genetic profile in energy generation and iron-metabolising genes in elite endurance athletes is different than non-athlete´s., There is an implication of an "optimal" genetic profile in the selected genes favouring endurance sporting performance.
Keyword:	physical endurance, sports performance, sport, genetic profile, AMPD1 protein, human HFE protein, human PPARGC1A protein
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1 **i) Title**

2 Genotype scores in energy and iron-metabolising genes are higher in elite endurance
3 athletes than in non-athlete controls

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22 ***Background***: Information about the association of energy and iron-metabolising genes
23 with endurance performance is scarce. The objective of this investigation was to
24 compare the frequencies of polymorphic variations of genes involved in energy
25 generation and iron metabolism in elite endurance athletes vs. non-athlete controls.
26 ***Methods***: Genotype frequencies in 123 male elite endurance athletes (75 professional
27 road cyclists and 48 elite endurance runners) and 122 male non-athlete participants were
28 compared by assessing four genetic polymorphisms: *AMPD1* c.34C/T (rs17602729),
29 *PPARGC1A* c.1444G/A (rs8192678) HFE_{H63D} c.187C/G (rs1799945) and HFE_{C282Y}
30 c.845G/A (rs1800562). A weighted genotype score (w-TGS: from 0 to 100 arbitrary
31 units; a.u.) was calculated by assigning a corresponding weight to each polymorphism.
32 ***Results***: In the non-athlete population, the mean w-TGS value was lower
33 (39.962±14.654 a.u.) than in the group of elite endurance athletes (53.344±17.053 a.u).
34 The binary logistic regression analysis showed that participants with a w-TGS>38.975
35 a.u had an odds ratio of 1.481 (95%CI: 1.244-1.762; p<0.001) for achieving elite athlete
36 status. ***Conclusions***: The genotypic distribution of polymorphic variations involved in
37 energy generation and iron metabolism was different in elite endurance athletes vs.
38 controls. Thus, an optimal genetic profile in these genes might contribute to physical
39 endurance in athlete status.

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41 **Keywords:** physical endurance; sports performance, sport; genetic profile; AMPD1
42 protein; human HFE protein; human PPARGC1A protein.

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45 **Novelty**

46 1. Genetic profile in energy generation and iron-metabolising genes in elite endurance
47 athletes is different than non-athlete´s.

48 2. There is an implication of an "*optimal*" genetic profile in the selected genes favouring
49 endurance sporting performance.

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1 Introduction

Endurance performance is related to a complex phenotype, influenced by a myriad of intrinsic and extrinsic factors (Lundby et al., 2017). Among the intrinsic factors, the likelihood of becoming an endurance athlete is influenced by the athlete's skeletal muscle fibre composition, maximal cardiac output and oxygen uptake (VO_{2max}) during exercise, metabolic efficiency and total haemoglobin mass (Joyner & Coyle, 2008). Interestingly, most of these traits are strongly influenced by genetics while some of them can be positively modified with endurance exercise training. Thus, the determinism of endurance athlete status is explained by the optimal combination of genetic predisposition and adequate physical conditioning (Eynon et al., 2013).

It has been shown that at least 120 genetic markers are linked to elite athlete status (Ahmetov & Fedotovskaya, 2015) and almost all chromosomes contain at least one gene associated with sport performance. From these genetic markers of performance, more than 70 are associated with endurance-type sports activities although only about a dozen genes have shown positive associations with elite athlete status in three or more studies (Ahmetov et al., 2016; Ahmetov & Fedotovskaya, 2015; Ahmetov et al., 2009; Varillas Delgado et al., 2019). Interestingly, most of the genetic variants associated with endurance performance codified proteins related to cellular metabolism (Ahmetov et al., 2009; Varillas Delgado et al., 2019) and muscle and cardiovascular function (Ahmetov & Fedotovskaya, 2015).

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Studies on genetic variants which influence elite endurance performance have shown that several genes associated with metabolic efficiency might entail an improvement in endurance capacity through decreased oxidative stress (Al-Khelaifi et al., 2018; Fikenzer et al., 2018; Lee et al., 2017; Petibois et al., 2002). Nevertheless, the whole

92 metabolic genetic profile related to an elite endurance athlete's status is not completely
93 understood. The Adenosine Monophosphate Deaminase isoform 1 (*AMPD1*) is an
94 important regulator of energy metabolism in the muscle fibre that shifts the equilibrium
95 of the myokine reactions towards ATP production by converting AMP into inosine
96 monophosphate (IMP) (Fedotovskaya et al., 2013; Gineviciene et al., 2014;
97 Maciejewska-Skrendo et al., 2019). Previous investigations have found that carrying the
98 T allele in one polymorphism in the *AMPD1* gene (c.34C/T; rs17602729) might reduce
99 the likelihood of being an elite endurance athlete (Cieszczyk et al., 2011; Gronek et al.,
100 2018) because it might be associated with a reduced VO_{2max} and lower response to
101 endurance training (Thomaes et al., 2011). Moreover, the peroxisome proliferator
102 activated receptor γ coactivator 1α (PGC1α) is a transcriptional coactivator of the
103 peroxisome proliferator-activated receptor (PPAR) family, which regulates the
104 expression of several genes associated with substrate oxidation, mitochondrial
105 biogenesis and muscle fibre conversion (Peplonska et al., 2017). PGC1α is encoded by
106 the *PPARGC1A* gene, and recent meta-analyses have shown that endurance athletes had
107 a higher frequency of the Gly/Gly genotype in one common polymorphism (rs8192678)
108 of the *PPARGC1A* gene, suggesting that this polymorphism might facilitate endurance
109 performance (Chen et al., 2019; Petr et al., 2019; Tharabenjasin et al., 2019). Finally,
110 genetics play a significant role in interindividual differences in serum iron parameters.
111 The homeostatic iron regulator protein (HFE), codified by the *HFE* gene, regulates iron
112 reabsorption (Grealy et al., 2015; Janssen & Swinkels, 2009; Ruiz et al., 2009).
113 Individuals with C/G or GG genotypes in the c.187C/G variant (rs1799945) of this gene
114 possessed higher circulating iron concentrations which ultimately produce a higher
115 haemoglobin concentration (Barbara et al., 2016). A recent investigation has found that

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116 the frequencies of the *HFE* CG/GG genotypes were higher in endurance athletes and
117 were associated with greater VO_{2max} in men athletes (Semenova et al., 2020).

118 There is a consensus in the scientific community about the importance of the combined
119 influence of several genetic variants, rather than the existence of one "endurance gene",
120 for excelling in endurance performance. The complex interaction of genetic variants
121 (Pickering et al., 2019) might help to explain individual variations in human endurance
122 performance and thus, the possession of an optimal polygenic profile seems necessary
123 to succeed in endurance sports (Guth & Roth, 2013; Moran & Pitsiladis, 2017;
124 Sarzynski et al., 2017). A previous investigation that calculated a potentially 'perfect'
125 polygenic score in endurance athletes, by accounting the number of favourable alleles in
126 seven candidate genes (including *AMPD1, PPARGC1A* and *HFE*), found that elite
127 endurance athletes had a higher polygenic score than the control population (Ruiz et al.,
128 2009). This outcome highlights the necessity of having several favourable alleles in
129 candidate genes for achieving elite athlete status, at least in endurance exercise.

130 Thus, the scientific information that interrelates the influence of the *AMPD1,*
131 *PPARGC1A* and *HFE* genes on endurance performance is scarce, and further
132 confirmation is needed to clearly depict the requirement of possessing several
133 favourable alleles in candidate genes to achieve elite athlete status in endurance exercise
134 disciplines. The main objective of this study was to compare the frequencies of
135 polymorphic variations of genes involved in energy generation and iron metabolism in
136 elite endurance athletes vs. non-athlete controls.

137 2 Materials and methods

138 2.1 *Study population*

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139 The study involved 123 elite endurance athletes (75 professional road cyclists and 48
140 elite endurance runners) and 122 men non-athlete participants (sedentary controls). All
141 participants in the group of endurance athletes and sedentary controls were male. An
142 analysis of the influence of liver-metabolising genes on elite athlete status has been
143 published elsewhere with this same sample (Varillas Delgado et al., 2019). All the elite
144 endurance athletes (25.8±4.2 years, range = 18-42 years) had tested negative for doping
145 substances in controls made by the World Anti-doping Agency. The elite runners had a
146 validated high level and elite sports records in endurance competitions: five athletes ran
147 the marathon in less than 2h 10 min, 12 athletes ran the half-marathon in less than 1h 03
148 min and the remaining 31 athletes participated in competitions of 10000 m and 5000 m
149 recording times below 30 min and 14 min respectively. Some of the athletes achieved
150 finalist positions in the marathon and the 10000 m in the European Championships,
151 with gold and silver medals in the European Cross-Country Championship. The
152 professional cyclists had participated in the *Union Cycliste Internationale* (UCI) World-
153 Tour events, including Grand Tours, classic cycle races, other one-day races or stage
154 races (often in all of them). Ten of the cyclists reached one of the top five positions in
155 the Grand Tours: *Tour de France*, *Giro d´Italia* and *Vuelta a España*. Both runners and
156 cyclists were men, due to the small number of high-level women athletes in Spain who
157 met the inclusion criteria. The sample of non-athlete controls was composed of healthy
158 men matched by age to the athletes (27.9±5.1 years, range = 19-42 years); they were
159 non-smokers and did not suffer from chronic or acute illnesses at the time of sampling.
160 Informed consent was obtained from all the participants in the study. The study protocol
161 was approved by the Committee of Institutional Ethics (University of Valladolid) and
162 complied with the Declaration of Helsinki for Human Research of 1974 (last modified
163 in 2000). Participants' rights and confidentiality were protected during the whole

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164 experiment, and the genetic information was used only for the purposes included in this
165 investigation.

166 2.2 *Genotypes*

167 2.2.1 Target genes

168 In this case-control investigation, the following functional single nucleotide
169 polymorphisms (SNPs) were genotyped:

170 - c.34C/T polymorphism (p.Gln12X) of *AMPD1* gene (location: 1p13) contributing to
171 the appearance of a premature stop codon, which leads to some related metabolic
172 muscle diseases due to the AMPD activity deficiency (Feng et al., 2017; Fischer et al.,
173 2005). Lack of the muscle-specific isoform of AMPD can cause a metabolic myopathy,
174 with exercise-induced muscle symptoms such as early fatigue, cramps and/or myalgia
175 (Gross, 1997).

176 - c.1444G/A polymorphism (p.Gly482Ser) of *PPARGC1A* gene (location: 4p15.2) is a
177 transcriptional coactivator of many different transcription factors and nuclear receptors.
178 It can act through direct interaction with a transcription factor, control energy
179 expenditure and regulate fat oxidation as well as non-oxidative glucose metabolism
180 (Maciejewska-Skrendo et al., 2019). It is responsible for the induction of reactive
181 oxygen species (ROS). Because ROS have been implicated as contributors to both the
182 onset and the progression of insulin resistance, this gene might play a role in the
183 development of type 2 diabetes mellitus (T2DM) and obesity (Baar, 2004).

184 - c.187C/G polymorphism (p.His63Asp) of *HFE* gene (HFE_{H63D}) (location: 6p21.3)
185 causes a heterogenic metabolic syndrome which is due to the unchecked transfer of iron
186 into the bloodstream and its toxic effects on parenchymatous organs (Barbara et al.,

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187 2016), inducing liver iron overload, are related to the risk of hepatocellular carcinoma in
188 otherwise predisposed patients (Ropero et al., 2007) and a risk factor for nephropathy in
189 type 2 diabetic patients (Moczulski et al., 2001).

190 - c.845G/A polymorphism (p.Cys282Tyr) of *HFE* gene (HFE_{C282Y}) (location: 6p21.3),
191 causes an excessively increased absorption of dietary iron and affects the normal
192 activity of another protein, hepcidin, a negative regulator of iron homeostasis (Katsarou
193 et al., 2019), causing liver cirrhosis and severe liver disease (Grosse et al., 2018;
194 Juzenas et al., 2016) and is related to various tumour types; colorectal (Chen et al.,
195 2013) and breast (Liu et al., 2013).

196 2.2.2 Deoxyribonucleic acid (DNA) extraction and genotyping

197 - Nucleic acid purification

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198 Genomic DNA was obtained from ethylenediaminetetraacetic acid (EDTA) anti-
199 coagulated blood samples according to standard phenol-chloroform procedures,
200 followed by precipitation with ethanol.

201 - Genotyping

202 *AMPD1*, *PPARGC1A* and *HFE* genotyping were carried out by direct polymerase chain
203 reaction (PCR) amplification and subsequent agarose gel electrophoresis in 2% agarose
204 gel, followed by specific restriction fragment analysis, as previously described
205 (Anderson et al., 2000; Steffensen et al., 1998; Su et al., 2008). All PCR reactions were
206 carried out in 20μl of the total volume, with DNA concentrations between 125-250μgr.

207 *AMPD1* genotyping

208 The *AMPD1* (c.34C/T) p.Gln12X was genotyped by PCR using an Eppendorf thermal
209 cycler, using the forward primer 5′-CTTCATACAGCTGAAGAGACA-3′ and the

210 reverse primer 5′-GAATCCAGAAAAGCCATGAGC-3′. The PCR mixture and
211 thermal-time profile were denatured at 94 °C for 5 min. The amplification step
212 consisted of 30 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C, with a final
213 extension of 5 min at 72 °C. After restriction enzyme digestion by NspI (ThermoFisher
214 Scientific, USA), the restriction products were separated by electrophoresis on a 2%
215 agarose gel.

216 *PPARGC1A* genotyping

217 The *PPARGC1A* (c.1444G>A) p.Gly482Ser was genotyped by PCR using an Eppendorf
218 thermal cycler, using the forward primer 5'-CAAGTCCTCCAGTCCTCAC-3′ and the
219 reverse primer 5'-GGGGTCTTTGAGAAAATAAGG-3'. The PCR mixture and
220 thermal-time profile were denatured at 94 °C for 5 min. The amplification step
221 consisted of 38 cycles of 45 s at 95 °C, 45 s at 60 °C and 45 s at 72 °C, with a final
222 extension of 10 min at 72 °C. After digestion by MspI (ThermoFisher Scientific, USA),
223 electrophoresis was carried out with separation of the restriction fragments in a 2 %
224 agarose gel.

225 *HFE* genotyping

226 In the HFE gene we studied two polymorphisms: (c.187C/G) p.His63Asp (HFE_{H63D})
227 and (c.845G/A) p.Cys282Tyr (HFE_{C282Y}). For the His63Asp polymorphism, the forward
228 primer 5′-ACATGGTTAAGGCCTGTTGC-3′ and reverse primer 5′-
229 GCCACATCTGGCTTGAAATT-3′ were used, and for p.Cys282Tyr polymorphism
230 (HFEC282Y) forward primer 5′-CAATGGGGATGGGACCTACC-3′ and reverse
231 primer 5′-GCTCTCATCAGTCACATACCCCAG-3′. The PCR mixture and thermal-
232 cycle profile were first denatured at 94 °C for 3 min. The amplification step consisted of
233 40 cycles of 30 s at 94 °C, 30 s at 60 °C (for HFE_{H63D}) and 30 s at 64 ºC (for HFE_{C282Y})

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234 and 30 s at 72 °C, with a final extension of 8 min at 72 °C. After restriction enzyme
235 digestion by BclI (ThermoFisher Scientific, USA) for HFE_{H63D} and MspI
236 (ThermoFisher Scientific, USA) for HFE_{C282Y}, the restriction fragments were separated
237 by electrophoresis on a 2% agarose gel.

238 2.3 *Polygenic potential for endurance performance in the Spanish population*

239 The combined influence of the four polymorphisms studied was calculated using a
240 weighted total genotype score (w-TGS). Initially, genotypes from each SNP were coded
241 according to the number of alleles with potential benefits for endurance performance
242 (Table 1; (Ruiz et al., 2009; Semenova et al., 2020)). For this codification, we used an
243 additive model (Williams & Folland, 2008) as follow: a score of 2 was assigned to the
244 "*optimal*" or preferable endurance genotype (i.e., homozygosity for the allele previously
245 associated to endurance performance), a score of 1 was assigned to heterozygote
246 genotype, while a score of 0 was assigned to the less optimal genotype. Afterwards,
247 these scores were weighted by using β-coefficients for each SNP (Table 1), based on the
248 assumption that each SNP of interest have independent effects and contribute in an
249 additive manner on endurance performance. To calculate the β-coefficient of each SNP,
250 a multivariable regression analysis was conducted to assess the partial contribution of
251 each SNP to the status of elite endurance athlete (coded as 1) or to control (coded as 0).
252 The relative contribution of each SNP in relation to the status of elite endurance athlete
253 was calculated as follows:

254 SNP partial contribution = ([β-coefficient for SNP] / Σ [of all β-coefficient])

255 The score within each SNP (i.e., 2, 1 and 0) was then weighted by its partial
256 contribution and a weighted genotype score was obtained for each SNP (w-GS).
257 Afterwards, all w-GS were summed to obtain a unique w-TGS for each participant

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258 (theoretical range: 0–8 a.u.). Lastly, this value was transformed to a 0-100 a.u. scale to
259 improve the comparison with previous investigations with a different number of SNP
260 investigated (Ruiz et al., 2009; Varillas Delgado et al., 2019) using the following
261 formulae:

262 $$\text{w-TGS} = ((\text{w-GS}\,\mathit{AMPD1} + \text{w-GS}\,\mathit{PPARGC1A} + \text{w-GS}\,\mathit{HFE}_{H63D} + \text{w-GS}\,\mathit{HFE}_{C282Y}) \times 100)/8$$

263

264 With this approach, a w-TGS of 100 (a.u.) represents the "*perfect*" polygenic profile for
265 endurance performance and a w-TGS of 0 a.u. would be the "*worst*" possible profile for
266 endurance performance.

267 2.4 *Statistical analysis*

268 Compliance of Hardy-Weinberg Equilibrium (HWE) in each SNP was tested using $\chi 2$
269 tests. The statistical average and kurtosis were calculated using the Statistical Package
270 for the Social Sciences (SPSS), v.21.0 for Windows (*IBM Corp. Released 2012. IBM*
271 *SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp*). The probability of
272 having an "*optimal*" endurance genotype for one to four polymorphisms between elite
273 endurance athletes and non-athletics was calculated using the $\chi 2$ test with fixed α error
274 of 0.05. The genotypic frequencies of the polymorphisms in *AMDP1, PPARGC1A,*
275 HFE_{H63D} and HFE_{C282Y} variants were compared between elite endurance athletes and
276 non-athletes, using a $\chi 2$ test with fixed α error of 0.05. The ability of w-TGS to correctly
277 distinguish potential elite endurance athletes from non-athletes (0 = non-athlete, 1 =
278 elite) was assessed using receiver operating characteristic (ROC) curves (Zweig &
279 Campbell, 1993). With that purpose, the area under the ROC curve (AUC) was
280 calculated with confidence intervals of 95% (95%CI). Finally, a binary logistic

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281 regression model was used to study the relationship between w-TGS and the athletic
282 status.

283 **3 Results**

284 3.1 *Single SNP analysis*

285 All the SNPs analysed met the HWE. In the *AMPD1* variant, the group of endurance
286 athletes showed a higher frequency in the "optimal" genotype (C/C 79.67%) when
287 compared to the non-athlete group (C/C 66.39%; p=0.019). For *PPARGC1A*, the
288 "*optimal*" genotype in elite endurance athletes (G/G 62.61%) was higher than in the
289 non-athlete population (G/G 53.29%; p=0.011). In the HFE_{H63D}, the distribution of the
290 genotypes was different in elite endurance athletes and non-athletes (p<0.001).
291 Specifically, a higher frequency in the "*optimal*" genotype was found in athletes (G/G
292 6.51%) vs. non-athletes (G/G 0.00%; Table 2). However, there were no between-group
293 differences in the genotype frequencies of the HFE_{C282Y} gene (p=0.986; Table 2). In any
294 case, there was no statistically significant differences in the genotypic distribution
295 between elite endurance cyclists and elite endurance runners in any gene (data not
296 shown).

297 *3.2 Weighted-total genotype score*

298 The mean value of the w-TGS was lower in the control population (39.962±14.654 a.u.,
299 statistical kurtosis: -0.672±0.435) than in the group of elite endurance athletes
300 (53.344±17.053 a.u., statistical kurtosis: -0.234±0.433; p<0.001). The distributions of
301 frequency of individuals according to their w-TGS is represented in Figure 1. The w-
302 TGS distribution of elite endurance athletes was shifted right with respect to the
303 distribution of non-athletes (p=0.001). ROC analysis showed significant discriminatory

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304 accuracy of w-TGSs in the identification of elite endurance athletes (AUC=0.721; 305 95%CI: 0.658-0.785; p<0.001) with a sensitivity of 0.837 and a specificity of 0.574 306 (Figure 2). The corresponding w-TGS value at this point was 38.975 a.u. Binary logistic 307 regression analysis showed that participants with a w-TGS higher than 38.975 a.u. had 308 an odds ratio (OR) of 1.481 (95%CI: 1.244-1.762; p<0.001) of being elite endurance 309 athletes, compared to those with a w-TGS below this cut-off value.

310 **4 Discussion**

311 Previous research has been satisfactory in finding links between potential genetic 312 markers associated with enhanced physiological functioning and elite endurance 313 performance (Ahmetov & Fedotovskaya, 2015; Ruiz et al., 2009; Varillas Delgado et 314 al., 2019). Interestingly, most of the genes previously associated with endurance 315 performance codify proteins related to cellular metabolism and muscle and 316 cardiovascular function. However, the information about the association of energy and 317 iron-metabolising genes with elite endurance athlete status is unknown. This 318 investigation represents the first attempt, using a polygenic model, to determine whether 319 polymorphic variations in energy and iron-metabolising genes had a joint effect on the 320 probability of becoming an elite endurance athlete. The main outcome of this 321 investigation is that there is a significant '*favourability*' in the genetic profile studied for 322 elite endurance athletes versus non-athletes, which is represented in the single 323 comparisons of the distribution of three out of the four genes studied (Table 2). 324 However, the addition of all the genes investigated, estimated by the total genotype 325 score, was even clearer to determine the polygenic influence of these genes on the 326 endurance athlete status. Thus, these results suggest that there is an endurance-specific 327 polygenic profile in energy metabolism and iron modulation variants that is more 328 suitable for human endurance exercise performance.

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329 Although outstanding endurance exercise performance in sports such as cycling and
330 running might be facilitated by an optimal polygenic profile in numerous key genes, the
331 current analysis indicates that the influence of the genes investigated here is strong
332 enough to differentiate elite athletes from non-athletes (Ahmetov et al., 2009). Perhaps,
333 the clear differentiation between the group of elite endurance athletes and the control
334 group in the genotypic distribution of the genes under investigation, and in the w-TGS,
335 even with this low number of genes, is due to the high-performance status of endurance
336 athletes. Elite athletes with a pure endurance-oriented phenotype and world-class
337 performance, like the ones studied here, are seldom gathered together in
338 genotype:phenotype association studies, and the majority of studies in the field have
339 typically focused on endurance-related phenotype traits (Grealy et al., 2015; Yvert et al.,
340 2016). Of note, the results of the current investigation should not be translated to other
341 forms of exercise and sports because the "*optimum*" genotype profile probably does
342 differ between endurance- and more power-oriented or intermittent sports (Al-Khelaifi
343 et al., 2018).

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344 The current analysis shows a higher w-TGS in elite endurance athletes than in non-
345 athlete controls. The cut-off value of 38.975 a.u. in the 0-100 w-TGS scale was effective
346 to discriminate the likelihood of being an endurance athlete with respect to non-athletes.
347 However, these results also suggest the unlikely nature of finding an individual with a
348 polygenic profile equivalent to 100 w-TGS, even elite endurance athletes and in a w-
349 TGS made with only four genes. Interestingly, the Ruiz et al. study (2009) also found a
350 difference in the w-TGS profile of Spanish elite endurance athletes (runners and
351 cyclists) and non-athlete controls when investigating 7 different genes associated with
352 performance. The current investigation is innovative because it confirms a more
353 favourable w-TGS in elite endurance athletes, even when using a lower number of

354 genes, while it might be more accurate because eliminates some SNPs that have been
355 discarded as influential for endurance performance (Del Coso et al., 2019). In any case,
356 the outcomes of this investigation confirms some of the findings by Ruiz et al. study
357 (Ruiz et al., 2009) and clearly depict that elite endurance performance might be
358 obtained without a w-TGS score close to 100 a.u.

359 While previous investigations have found that the *AMPD1* C allele may help athletes to
360 attain elite status in sprint/power-based sports (Gineviciene et al., 2014; Thomaes et al.,
361 2011), the current investigation suggests that this allele might also benefit endurance
362 performance. Interestingly, 79.7% of the elite endurance athletes were homozygous for
363 the *AMPD1* C allele. Although it has been found that heterozygosity in this
364 polymorphism does not impede outstanding endurance performance (Rubio et al.,
365 2005), the results of the current analysis suggest that C/C homozygosity in the *AMPD1*
366 gene might be the optimal genotype to excel in endurance sport (Grealy et al., 2015;
367 Lucia et al., 2009; Rubio et al., 2005; Ruiz et al., 2009).

368 The distribution of the *PPARGC1A* genotype was different between elite athletes and
369 the non-athlete population. However, a high proportion of non-athletes contained the
370 optimal G/G genotype for this gene (Table 2). The importance of the G/G genotype in
371 the *PPARGC1A* gene has been previously found when comparing samples of elite
372 Turkish and Brazilian athletes with control populations (Guilherme et al., 2018; Tural et
373 al., 2014) and its influence on performance has been associated with the induction of
374 enhanced mitochondrial biogenesis associated with endurance training (Baar, 2004).
375 However, the importance of this gene is not exclusive to endurance sports because a
376 higher than expected proportion of the G/G genotypes was also present in strength
377 based sports (Guilherme et al., 2018; Peplonska et al., 2017). The current analysis is

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378 innovative because it interrelates the optimal *PPARGC1A* genotype with other genes
379 associated with metabolism. This outcome suggests that, although the sole presence of
380 the G/G genotype does not guarantee outstanding endurance performance, it might
381 favour this phenotype in the presence of other optimal genetic profiles of genes key for
382 performance.

383 The tendency of endurance athletes to develop iron deficiency can trigger anaemia over
384 time. For this reason, special care usually should be taken to avoid the mechanisms that
385 cause this deficiency in elite athletes (Burden et al., 2015; Coates et al., 2017;
386 Nikolaidis et al., 2018). The mutation of HFE_{H63D} is associated with a higher capacity
387 for iron absorption without causing hemochromatosis. However, the polymorphism
388 HFE_{C282Y} is more related to hemochromatosis (Chicharro et al., 2004; Zoller & Vogel,
389 2004). In a study by Chicharro et al., (Chicharro et al., 2004) carried out with Spanish
390 elite athletes, the frequency of G/G homozygotes for the HFE_{H63D} variant was 3.7% in
391 athletes and 3.1% in non-athletes, these frequencies being similar to our research (6.5%
392 and 0.0% for athletes and non-athletes, respectively, Table 2). Low frequencies were
393 also present in the G/A heterozygosity for HFE_{C282Y} polymorphism with 3.1% in
394 athletes and 4.5% in controls (Chicharro et al., 2004), comparable to the 7.3% and 7.4%
395 found in our study. These results indicate that the proportion of elite endurance athletes
396 with optimal genotype profiles for the *HFE* gene is low while heterozygosity in either
397 HFE_{H63D} or HFE_{C282Y} polymorphic variants is more present in elite athletes than in the
398 control population (Hermine et al., 2015). Accordingly, although the likelihood of
399 having an optimal profile in the two polymorphisms of the *HFE* gene is minimal even in
400 elite endurance athletes, heterozygosity might confer an intermediate phenotype in
401 terms of iron absorption that might favour endurance performance.

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402 The current analysis presents some limitations that should be discussed to adequately 403 understand the scope of the investigation. The relatively small sample of endurance 404 athletes precludes us from drawing definite conclusions. Yet, due to the limited nature 405 of the population under investigation, we believe this limitation is justifiable as there are 406 hardly better endurance specialists in Spain. Numerous genetic variants that have not 407 been included in the model are likely to appear in the foreseeable future, which can also 408 explain individual variations in the potential for attaining elite endurance athletic status. 409 In addition, this study has only focused on genetic data while it does not contain 410 information that associates the genotype-phenotype in these athletes, which will need to 411 be completed in subsequent research. Future research is also necessary in elite 412 endurance women, as the influence of some polymorphisms might differ between sexes 413 in Spanish Caucasian elite athletes.

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414 *Conclusions*

415 The genotypic distribution of polymorphic variations involved in energy generation and 416 iron metabolism was different in elite Spanish endurance athletes vs. controls. These 417 results confirm the beneficial influence of an optimal genetic profile to obtain elite 418 athlete status and widen the importance of genetics to become an elite endurance 419 athlete.

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425

426 **DECLARATIONS**

427 • **Ethics approval and consent to participate**

428 Informed consent was obtained from all the participants in the study. The study protocol
429 was approved by the Committee of Institutional Ethics (University of Valladolid) and
430 complied with the Declaration of Helsinki for Human Research of 1974 (last modified
431 in 2000).

432 • **Consent for publication**

433 Not applicable

434 • **Availability of data and material**

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435 All data generated or analysed during this study are included in this published article
436 (and its supplementary information files).

437 • **Competing interests**

438 David Varillas Delgado, Juan del Coso, Diana Monge Martín and Juan José Tellería
439 Orriols declare that they have no competing interests.

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443 *performance in endurance sports*" (15/UPB10/08).

444 • **Authors' contributions**

445 DVD carried out the genetic study and recruitment of participants, as well as the
446 statistical study, forming part of his doctoral thesis.

447 JJTO helped to search for the genes involved, collaborated in writing and advise for its
448 edition and in the improvement of the methodological aspects.

449 DMM collaborated in writing of paper.

450 JDC, as the senior author, reviewed the work, collaborated in writing, and advise for its
451 edition and in the improvement of the methodological aspects.

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454 **References**

455 Ahmetov, II, Egorova, E. S., Gabdrakhmanova, L. J., & Fedotovskaya, O. N. (2016). Genes and
456 Athletic Performance: An Update. *Med Sport Sci, 61*, 41-54.
457 https://doi.org/10.1159/000445240
458 Ahmetov, II, & Fedotovskaya, O. N. (2015). Current Progress in Sports Genomics. *Adv Clin*
459 *Chem, 70*, 247-314. https://doi.org/10.1016/bs.acc.2015.03.003
460 Ahmetov, II, Williams, A. G., Popov, D. V., Lyubaeva, E. V., Hakimullina, A. M., Fedotovskaya, O.
461 N., Mozhayskaya, I. A., Vinogradova, O. L., Astratenkova, I. V., Montgomery, H. E., &
462 Rogozkin, V. A. (2009). The combined impact of metabolic gene polymorphisms on
463 elite endurance athlete status and related phenotypes. *Hum Genet, 126*(6), 751-761.
464 https://doi.org/10.1007/s00439-009-0728-4
465 Al-Khelaifi, F., Diboun, I., Donati, F., Botre, F., Alsayrafi, M., Georgakopoulos, C., Suhre, K.,
466 Yousri, N. A., & Elrayess, M. A. (2018). A pilot study comparing the metabolic profiles
467 of elite-level athletes from different sporting disciplines. *Sports Med Open, 4*(1), 2.
468 https://doi.org/10.1186/s40798-017-0114-z
469 Anderson, J. L., Habashi, J., Carlquist, J. F., Muhlestein, J. B., Horne, B. D., Bair, T. L., Pearson, R.
470 R., & Hart, N. (2000). A common variant of the AMPD1 gene predicts improved
471 cardiovascular survival in patients with coronary artery disease. *J Am Coll Cardiol,*
472 *36*(4), 1248-1252. https://doi.org/10.1016/s0735-1097(00)00850-0
473 Baar, K. (2004). Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and
474 2, and PPAR alpha in the adaptive response to endurance exercise. *Proc Nutr Soc,*
475 *63*(2), 269-273. https://doi.org/10.1079/pns2004334
476 Barbara, K. H., Marcin, L., Jedrzej, A., Wieslaw, Z., Elzbieta, A. D., Malgorzata, M., Ewa, M., &
477 Jacek, K. J. (2016). The impact of H63D HFE gene carriage on hemoglobin and iron
478 status in children. *Ann Hematol, 95*(12), 2043-2048. https://doi.org/10.1007/s00277-
479 016-2792-x
480 Burden, R. J., Morton, K., Richards, T., Whyte, G. P., & Pedlar, C. R. (2015). Is iron treatment
481 beneficial in, iron-deficient but non-anaemic (IDNA) endurance athletes? A systematic
482 review and meta-analysis. *Br J Sports Med, 49*(21), 1389-1397.
483 https://doi.org/10.1136/bjsports-2014-093624
484 Chen, W., Zhao, H., Li, T., & Yao, H. (2013). HFE gene C282Y variant is associated with
485 colorectal cancer in Caucasians: a meta-analysis. *Tumour Biol, 34*(4), 2255-2259.
486 https://doi.org/10.1007/s13277-013-0766-3
487 Chen, Y., Wang, D., Yan, P., Yan, S., Chang, Q., & Cheng, Z. (2019). Meta-analyses of the
488 association between the PPARGC1A Gly482Ser polymorphism and athletic
489 performance. *Biol Sport, 36*(4), 301-309. https://doi.org/10.5114/biolsport.2019.88752
490 Chicharro, J. L., Hoyos, J., Gomez-Gallego, F., Villa, J. G., Bandres, F., Celaya, P., Jimenez, F.,
491 Alonso, J. M., Cordova, A., & Lucia, A. (2004). Mutations in the hereditary
492 haemochromatosis gene HFE in professional endurance athletes. *Br J Sports Med,*
493 *38*(4), 418-421. https://doi.org/10.1136/bjsm.2002.003921
494 Cieszczyk, P., Eider, J., Ostanek, M., Leonska-Duniec, A., Ficek, K., Kotarska, K., & Girdauskas, G.
495 (2011). Is the C34T polymorphism of the AMPD1 gene associated with athlete
496 performance in rowing? *Int J Sports Med, 32*(12), 987-991. https://doi.org/10.1055/s-
497 0031-1283186
498 Coates, A., Mountjoy, M., & Burr, J. (2017). Incidence of Iron Deficiency and Iron Deficient
499 Anemia in Elite Runners and Triathletes. *Clin J Sport Med, 27*(5), 493-498.
500 https://doi.org/10.1097/jsm.0000000000000390

https://mc06.manuscriptcentral.com/apnm-pubs

501 Del Coso, J., Hiam, D., Houweling, P., Perez, L. M., Eynon, N., & Lucia, A. (2019). More than a
502 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for
503 injuries. *Eur J Appl Physiol, 119*(1), 49-60. https://doi.org/10.1007/s00421-018-4010-0
504 Eynon, N., Hanson, E. D., Lucia, A., Houweling, P. J., Garton, F., North, K. N., & Bishop, D. J.
505 (2013). Genes for elite power and sprint performance: ACTN3 leads the way. *Sports*
506 *Med, 43*(9), 803-817. https://doi.org/10.1007/s40279-013-0059-4
507 Fedotovskaya, O. N., Danilova, A. A., & Akhmetov, II. (2013). Effect of AMPD1 gene
508 polymorphism on muscle activity in humans. *Bull Exp Biol Med, 154*(4), 489-491.
509 https://doi.org/10.1007/s10517-013-1984-9
510 Feng, A. F., Liu, Z. H., Zhou, S. L., Zhao, S. Y., Zhu, Y. X., & Wang, H. X. (2017). Effects of AMPD1
511 gene C34T polymorphism on cardiac index, blood pressure and prognosis in patients
512 with cardiovascular diseases: a meta-analysis. *BMC Cardiovasc Disord, 17*(1), 174.
513 https://doi.org/10.1186/s12872-017-0608-0
514 Fikenzer, K., Fikenzer, S., Laufs, U., & Werner, C. (2018). Effects of endurance training on serum
515 lipids. *Vascul Pharmacol, 101*, 9-20. https://doi.org/10.1016/j.vph.2017.11.005
516 Fischer, S., Drenckhahn, C., Wolf, C., Eschrich, K., Kellermann, S., Froster, U. G., & Schober, R.
517 (2005). Clinical significance and neuropathology of primary MADD in C34-T and G468-T
518 mutations of the AMPD1 gene. *Clin Neuropathol, 24*(2), 77-85.
519 Gineviciene, V., Jakaitiene, A., Pranculis, A., Milasius, K., Tubelis, L., & Utkus, A. (2014). AMPD1
520 rs17602729 is associated with physical performance of sprint and power in elite
521 Lithuanian athletes. *BMC Genet, 15*, 58. https://doi.org/10.1186/1471-2156-15-58
522 Grealy, R., Herruer, J., Smith, C. L., Hiller, D., Haseler, L. J., & Griffiths, L. R. (2015). Evaluation of
523 a 7-Gene Genetic Profile for Athletic Endurance Phenotype in Ironman Championship
524 Triathletes. *PLoS One, 10*(12), e0145171.
525 https://doi.org/10.1371/journal.pone.0145171
526 Gronek, P., Gronek, J., Lulinska-Kuklik, E., Spieszny, M., Niewczas, M., Kaczmarczyk, M., Petr,
527 M., Fischerova, P., Ahmetov, II, & Zmijewski, P. (2018). Polygenic Study of Endurance-
528 Associated Genetic Markers NOS3 (Glu298Asp), BDKRB2 (-9/+9), UCP2 (Ala55Val),
529 AMPD1 (Gln45Ter) and ACE (I/D) in Polish Male Half Marathoners. *J Hum Kinet, 64*, 87-
530 98. https://doi.org/10.1515/hukin-2017-0204
531 Gross, M. (1997). Clinical heterogeneity and molecular mechanisms in inborn muscle AMP
532 deaminase deficiency. *J Inherit Metab Dis, 20*(2), 186-192.
533 https://doi.org/10.1023/a:1005352605421
534 Grosse, S. D., Gurrin, L. C., Bertalli, N. A., & Allen, K. J. (2018). Clinical penetrance in hereditary
535 hemochromatosis: estimates of the cumulative incidence of severe liver disease
536 among HFE C282Y homozygotes. *Genet Med, 20*(4), 383-389.
537 https://doi.org/10.1038/gim.2017.121
538 Guilherme, J., Bertuzzi, R., Lima-Silva, A. E., Pereira, A. D. C., & Lancha Junior, A. H. (2018).
539 Analysis of sports-relevant polymorphisms in a large Brazilian cohort of top-level
540 athletes. *Ann Hum Genet, 82*(5), 254-264. https://doi.org/10.1111/ahg.12248
541 Guth, L. M., & Roth, S. M. (2013). Genetic influence on athletic performance. *Curr Opin Pediatr,*
542 *25*(6), 653-658. https://doi.org/10.1097/MOP.0b013e3283659087
543 Hermine, O., Dine, G., Genty, V., Marquet, L. A., Fumagalli, G., Tafflet, M., Guillem, F., Van
544 Lierde, F., Rousseaux-Blanchi, M. P., Palierne, C., Lapostolle, J. C., Cervetti, J. P., Frey,
545 A., Jouven, X., Noirez, P., & Toussaint, J. F. (2015). Eighty percent of French sport
546 winners in Olympic, World and Europeans competitions have mutations in the
547 hemochromatosis HFE gene. *Biochimie, 119*, 1-5.
548 https://doi.org/10.1016/j.biochi.2015.09.028
549 Janssen, M. C., & Swinkels, D. W. (2009). Hereditary haemochromatosis. *Best Pract Res Clin*
550 *Gastroenterol, 23*(2), 171-183. https://doi.org/10.1016/j.bpg.2009.02.004

551 Joyner, M. J., & Coyle, E. F. (2008). Endurance exercise performance: the physiology of
552 champions. *J Physiol, 586*(1), 35-44. https://doi.org/10.1113/jphysiol.2007.143834
553 Juzenas, S., Kupcinskas, J., Valantiene, I., Sumskiene, J., Petrenkiene, V., Kondrackiene, J.,
554 Kucinskas, L., Kiudelis, G., Skieceviciene, J., & Kupcinskas, L. (2016). Association of HFE
555 gene C282Y and H63D mutations with liver cirrhosis in the Lithuanian population.
556 *Medicina (Kaunas), 52*(5), 269-275. https://doi.org/10.1016/j.medici.2016.09.004
557 Katsarou, M. S., Papasavva, M., Latsi, R., & Drakoulis, N. (2019). Hemochromatosis: Hereditary
558 hemochromatosis and HFE gene. *Vitam Horm, 110*, 201-222.
559 https://doi.org/10.1016/bs.vh.2019.01.010
560 Lee, E. C., Fragala, M. S., Kavouras, S. A., Queen, R. M., Pryor, J. L., & Casa, D. J. (2017).
561 Biomarkers in Sports and Exercise: Tracking Health, Performance, and Recovery in
562 Athletes. *J Strength Cond Res, 31*(10), 2920-2937.
563 https://doi.org/10.1519/jsc.0000000000002122
564 Liu, X., Lv, C., Luan, X., & Lv, M. (2013). C282Y polymorphism in the HFE gene is associated with
565 risk of breast cancer. *Tumour Biol, 34*(5), 2759-2764. https://doi.org/10.1007/s13277-
566 013-0833-9
567 Lucia, A., Martin, M. A., Esteve-Lanao, J., San Juan, A. F., Rubio, J. C., Olivan, J., & Arenas, J.
568 (2009). C34T mutation of the AMPD1 gene in an elite white runner. *BMJ Case Rep,*
569 *2009*. https://doi.org/10.1136/bcr.07.2008.0535
570 Lundby, C., Montero, D., & Joyner, M. (2017). Biology of VO2 max: looking under the
571 physiology lamp. *Acta Physiol (Oxf), 220*(2), 218-228.
572 https://doi.org/10.1111/apha.12827
573 Maciejewska-Skrendo, A., Cieszczyk, P., Chycki, J., Sawczuk, M., & Smolka, W. (2019). Genetic
574 Markers Associated with Power Athlete Status. *J Hum Kinet, 68*, 17-36.
575 https://doi.org/10.2478/hukin-2019-0053
576 Moczulski, D. K., Grzeszczak, W., & Gawlik, B. (2001). Role of hemochromatosis C282Y and
577 H63D mutations in HFE gene in development of type 2 diabetes and diabetic
578 nephropathy. *Diabetes Care, 24*(7), 1187-1191.
579 https://doi.org/10.2337/diacare.24.7.1187
580 Moran, C. N., & Pitsiladis, Y. P. (2017). Tour de France Champions born or made: where do we
581 take the genetics of performance? *J Sports Sci, 35*(14), 1411-1419.
582 https://doi.org/10.1080/02640414.2016.1215494
583 Nikolaidis, P. T., Veniamakis, E., Rosemann, T., & Knechtle, B. (2018). Nutrition in Ultra-
584 Endurance: State of the Art. *Nutrients, 10*(12). https://doi.org/10.3390/nu10121995
585 Peplonska, B., Adamczyk, J. G., Siewierski, M., Safranow, K., Maruszak, A., Sozanski, H.,
586 Gajewski, A. K., & Zekanowski, C. (2017). Genetic variants associated with physical and
587 mental characteristics of the elite athletes in the Polish population. *Scand J Med Sci*
588 *Sports, 27*(8), 788-800. https://doi.org/10.1111/sms.12687
589 Petibois, C., Cazorla, G., Poortmans, J. R., & Deleris, G. (2002). Biochemical aspects of
590 overtraining in endurance sports: a review. *Sports Med, 32*(13), 867-878.
591 https://doi.org/10.2165/00007256-200232130-00005
592 Petr, M., Maciejewska-Skrendo, A., Zajac, A., Chycki, J., & Stastny, P. (2019). Association of Elite
593 Sports Status with Gene Variants of Peroxisome Proliferator Activated Receptors and
594 Their Transcriptional Coactivator. *Int J Mol Sci, 21*(1).
595 https://doi.org/10.3390/ijms21010162
596 Pickering, C., Kiely, J., Grgic, J., Lucia, A., & Del Coso, J. (2019). Can Genetic Testing Identify
597 Talent for Sport? *Genes (Basel), 10*(12). https://doi.org/10.3390/genes10120972
598 Ropero, P., Briceno, O., Lopez-Alonso, G., Agundez, J. A., Gonzalez Fernandez, F. A., Garcia-Hoz,
599 F., Villegas Martinez, A., Diaz-Rubio, M., & Ladero, J. M. (2007). [The H63D mutation in
600 the HFE gene is related to the risk of hepatocellular carcinoma]. *Rev Esp Enferm Dig,*

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601 *99*(7), 376-381. https://doi.org/10.4321/s1130-01082007000700002 (La mutacion
602 H63D del gen HFE se asocia con un riesgo aumentado de carcinoma hepatocelular .)
603 Rubio, J. C., Martin, M. A., Rabadan, M., Gomez-Gallego, F., San Juan, A. F., Alonso, J. M.,
604 Chicharro, J. L., Perez, M., Arenas, J., & Lucia, A. (2005). Frequency of the C34T
605 mutation of the AMPD1 gene in world-class endurance athletes: does this mutation
606 impair performance? *J Appl Physiol (1985), 98*(6), 2108-2112.
607 https://doi.org/10.1152/japplphysiol.01371.2004
608 Ruiz, J. R., Gomez-Gallego, F., Santiago, C., Gonzalez-Freire, M., Verde, Z., Foster, C., & Lucia, A.
609 (2009). Is there an optimum endurance polygenic profile? *J Physiol, 587*(Pt 7), 1527-
610 1534. https://doi.org/10.1113/jphysiol.2008.166645
611 Sarzynski, M. A., Ghosh, S., & Bouchard, C. (2017). Genomic and transcriptomic predictors of
612 response levels to endurance exercise training. *J Physiol, 595*(9), 2931-2939.
613 https://doi.org/10.1113/jp272559
614 Semenova, E. A., Miyamoto-Mikami, E., Akimov, E. B., Al-Khelaifi, F., Murakami, H., Zempo, H.,
615 Kostryukova, E. S., Kulemin, N. A., Larin, A. K., Borisov, O. V., Miyachi, M., Popov, D. V.,
616 Boulygina, E. A., Takaragawa, M., Kumagai, H., Naito, H., Pushkarev, V. P., Dyatlov, D.
617 A., Lekontsev, E. V., Pushkareva, Y. E., Andryushchenko, L. B., Elrayess, M. A.,
618 Generozov, E. V., Fuku, N., & Ahmetov, II. (2020). The association of HFE gene H63D
619 polymorphism with endurance athlete status and aerobic capacity: novel findings and
620 a meta-analysis. *Eur J Appl Physiol*. https://doi.org/10.1007/s00421-020-04306-8
621 Steffensen, R., Varming, K., & Jersild, C. (1998). Determination of gene frequencies for two
622 common haemochromatosis mutations in the Danish population by a novel
623 polymerase chain reaction with sequence-specific primers. *Tissue Antigens, 52*(3), 230-
624 235. https://doi.org/10.1111/j.1399-0039.1998.tb03037.x
625 Su, Y., Peng, S. B., Li, Z. Q., & Huang, Q. Y. (2008). [Association study between PPARGC1A
626 Thr394Thr/ Gly482Ser polymorphisms and type 2 diabetes]. *Yi Chuan, 30*(3), 304-308.
627 https://doi.org/10.3724/sp.j.1005.2008.00304
628 Tharabenjasin, P., Pabalan, N., & Jarjanazi, H. (2019). Association of PPARGC1A Gly428Ser
629 (rs8192678) polymorphism with potential for athletic ability and sports performance:
630 A meta-analysis. *PLoS One, 14*(1), e0200967.
631 https://doi.org/10.1371/journal.pone.0200967
632 Thomaes, T., Thomis, M., Onkelinx, S., Fagard, R., Matthijs, G., Buys, R., Schepers, D.,
633 Cornelissen, V., & Vanhees, L. (2011). A genetic predisposition score for muscular
634 endophenotypes predicts the increase in aerobic power after training: the CAREGENE
635 study. *BMC Genet, 12*, 84. https://doi.org/10.1186/1471-2156-12-84
636 Tural, E., Kara, N., Agaoglu, S. A., Elbistan, M., Tasmektepligil, M. Y., & Imamoglu, O. (2014).
637 PPAR-alpha and PPARGC1A gene variants have strong effects on aerobic performance
638 of Turkish elite endurance athletes. *Mol Biol Rep, 41*(9), 5799-5804.
639 https://doi.org/10.1007/s11033-014-3453-6
640 Varillas Delgado, D., Telleria Orriols, J. J., & Martin Saborido, C. (2019). Liver-Metabolizing
641 Genes and Their Relationship to the Performance of Elite Spanish Male Endurance
642 Athletes; a Prospective Transversal Study. *Sports Med Open, 5*(1), 50.
643 https://doi.org/10.1186/s40798-019-0227-7
644 Williams, A. G., & Folland, J. P. (2008). Similarity of polygenic profiles limits the potential for
645 elite human physical performance. *J Physiol, 586*(1), 113-121.
646 https://doi.org/10.1113/jphysiol.2007.141887
647 Yvert, T., Miyamoto-Mikami, E., Murakami, H., Miyachi, M., Kawahara, T., & Fuku, N. (2016).
648 Lack of replication of associations between multiple genetic polymorphisms and
649 endurance athlete status in Japanese population. *Physiol Rep, 4*(20).
650 https://doi.org/10.14814/phy2.13003

651 Zoller, H., & Vogel, W. (2004). Iron supplementation in athletes--first do no harm. *Nutrition,*
652 *20*(7-8), 615-619. https://doi.org/10.1016/j.nut.2004.04.006
653 Zweig, M. H., & Campbell, G. (1993). Receiver-operating characteristic (ROC) plots: a
654 fundamental evaluation tool in clinical medicine. *Clin Chem, 39*(4), 561-577.

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1 **TABLES**

2 **Table 1**. Studied polymorphisms, score assigned to each genotype for the calculation of the total genotype score, and genotype frequencies in the
3 Spanish population obtained from a public data base.

Symbol	Gene	Polymorphism	Genotype score	β-coefficient	Weighted genotype score	Iberian population (%)
AMPD1	Adenosine monophosphate deaminase 1	c.34C>T (p.Gln12X)	0=TT 1=CT 2=CC	0.675	0=TT 1.0=CT 2.0=CC	0 28 72
PPARGC1A	Peroxisome Proliferator Activated Receptor γ Coactivator α	c.1444G>A (p.Gly482Ser)	0=AA 1=GA 2=GG	0.383	0=AA 0.6=GA 1.2=GG	12 53 35
HFE_{H63D}	Hemochromatosis variant H63D	c.187C>G (p.His63Asp)	0=CC 1=GC 2=GG	1.425	0=CC 2.2=GC 4.4=GG	58 34 8
HFE_{C282Y}	Hemochromatosis variant C282Y	c.845G>A (p.Cys282Tyr)	0=GG 1=GA 2=AA	0.149	0=GG 0.2=GA 0.4=AA	92 8 0

4 Data for Spanish population have been obtained in www.ensembl.org.

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1 **Table 2**. Distribution of elite endurance athletes and non-athletes in the polymorphisms studied.

	Elite endurance athletes (n=123)	**Non-athletes (n=122)**	**p value**
AMPD1* *rs17602729			
T/T	0 (0.00%)	0 (0.00%)	0.019
C/T	25 (20.33%)	41 (33.61%)	
C/C	98 (79.67%)	81 (66.39%)	
PPARGC1A* *rs8192678			
A/A	0 (0.00%)	8 (6.55%)	0.011
G/A	46 (37.39%)	49 (40.16%)	
G/G	77 (62.61%)	65 (53.29%)	
HFE$_{H63D}$* *rs1799945			
C/C	47 (38.21%)	88 (72.13%)	<0.001
G/C	68 (55.28%)	34 (27.87%)	
G/G	8 (6.51%)	0 (0.00%)	
HFE$_{C282Y}$* *rs1800562			
G/G	114 (92.68%)	113 (92.62%)	0.986
G/A	9 (7.32%)	9 (7.38%)	
A/A	0 (0.00%)	0 (0.00%)	

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1 **FIGURE CAPTIONS**

2 **Figure 1**. Distribution of individuals according to their weighted total genotype score in
3 elite endurance athletes and in non-athlete control population.

4 (*) The distribution is different from the distribution of non-athletes at $p<0.001$.

5 **Figure 2**. ROC curve summarizing the ability of the weighted total genotype score to
6 distinguish potential elite endurance athletes from non-athletes.

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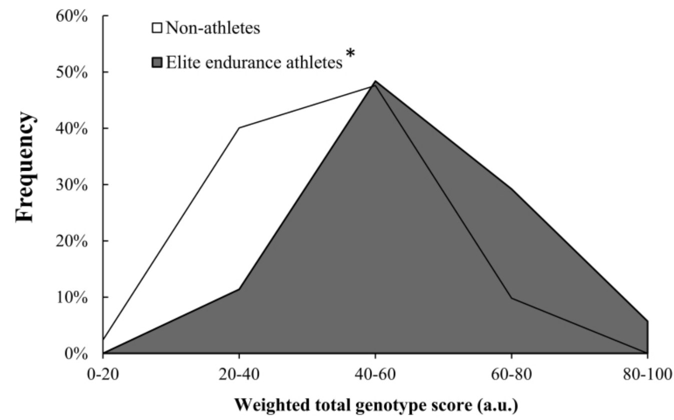

Figure 1. Distribution of individuals according to their weighted total genotype score in elite endurance athletes and in non-athlete control population.
(*) The distribution is different from the distribution of non-athletes at p<0.001.

199x100mm (600 x 600 DPI)

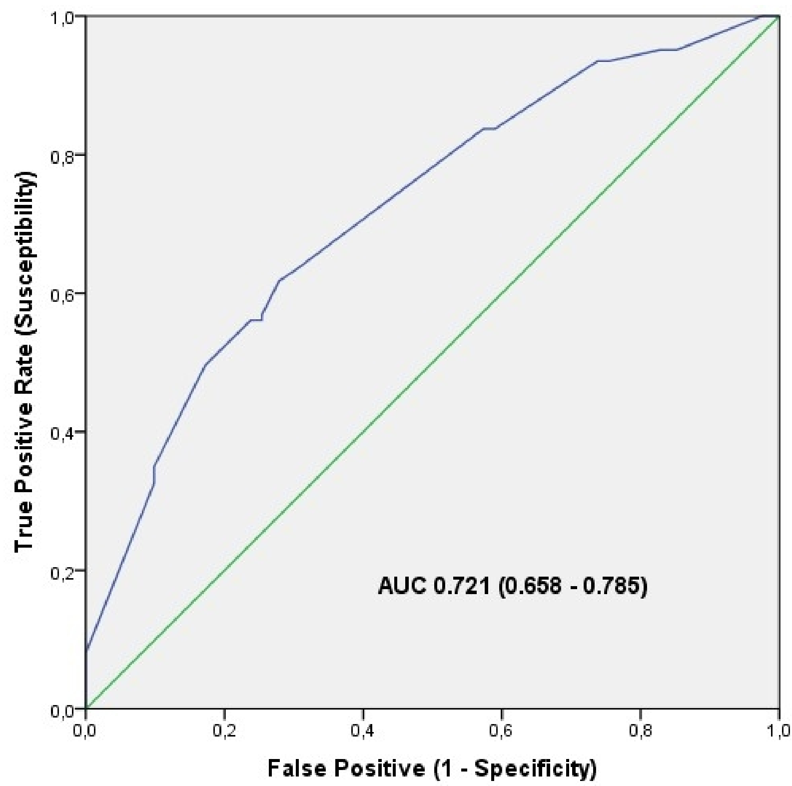

Figure 2. ROC curve summarizing the ability of the weighted total genotype score to distinguish potential elite endurance athletes from non-athletes.

166x133mm (96 x 96 DPI)