ORIGINAL ARTICLE

Polymorphisms in the *HBB* gene relate to individual cardiorespiratory adaptation in response to endurance training

Z He, Y Hu, L Feng, Y Lu, G Liu, Y Xi, L Wen, X Xu, K Xu

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See end of article for authors' affiliations

Correspondence to: Yang Hu, Section of Exercise Biochemistry, Department of Sport and Human Sciences, Beijing Sport University, Beijing 100084, China; bsugene@yahoo.com

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Objective: The crucial role of haemoglobin in endurance performance has been well documented. We examined whether polymorphisms in the *HBB* gene modified aerobic capacity.
Methods: 102 recruits were trained by running 5000 m three times per week for 18 weeks. Exercise intensity progressively increased from an initial heart rate corresponding to 95% of the individual baseline ventilatory threshold during the first 10 weeks to 105% during the last 8 weeks. The phenotypes measured were running economy and VO_2max. Running economy was determined by measuring submaximal VO_2 for 5 min at a constant running speed of 12 km·h^{-1} and VO_2max was obtained during an incremental test to exhaustion. Genomic DNA was extracted from white cells of peripheral blood and the −551C/T, intron2,+16C/G and +340 A/T genotypes were examined relative to the TAA site variants by PCR-RFLP.
Results: Genotype distributions were in Hardy-Weinberg equilibrium at three loci. None of the running economy and VO_2max-related traits were associated with the three polymorphisms or haplotypes at baseline, while the training response of running economy was associated with −551C/T and intron2,+16C/G polymorphisms. Subjects homozygous for intron2,+16C/C or −551C/C had decreased oxygen cost of running compared to the other individuals.
Discussion: It was concluded that the −551C/C or intron2,+16C/C genotype might explain part of the individual variation in the cardiorespiratory adaptation to endurance training.

Haemoglobin is a protein-based component of red blood cells and is primarily responsible for transferring oxygen from the lungs to respiring tissues. The crucial role of haemoglobin in exercise has been well documented.[1–4] An acute reduction in blood haemoglobin concentration, even when the circulating blood volume is maintained, results in lower maximal oxygen uptake (VO_2max) and endurance performance due to the reduction in the oxygen carrying capacity of the blood.[4] Conversely, an increase in haemoglobin concentration is associated with enhanced VO_2max and endurance capacity, which is also proportional to the increase in the oxygen carrying capacity of the blood; the effects on endurance capacity appear more pronounced and prolonged than on VO_2max.[1] Compared to VO_2max, less research has focused on studying the factors that determine running economy, which is one of the main indicators of submaximal aerobic capacity. Recent investigations nevertheless suggest that some strategies originally intended to enhance blood oxygen transport capacity (for example, intermittent hypoxic exposure) might also improve running economy.[5] On the other hand, the VO_2 cost of leg pedalling exercise decreases with increasing blood haemoglobin concentration, at least in elite endurance cyclists.[6] Since haemoglobin is an excellent buffer, an increase in the circulating haemoglobin mass helps improve the blood buffering capacity.[7] A higher blood buffering capacity could facilitate lactate[8] and proton release from the active muscle and increase the capacity to produce energy via the anaerobic pathways.

The haemoglobin molecule has been proposed as an ideal O_2 sensor.[9–11] The O_2 sensing properties of the haemoglobin molecule appear to involve conformational changes resulting from O_2 desaturation.[12] On desaturation, red blood cells could elicit vasodilatation through four independent mechanisms: release of adenosine 5′-triphosphate (ATP),[13] release of *S*-nitrosylated molecules originally bound to β93 cysteine residues of oxyhaemoglobin,[14 15] deoxygenated haemoglobin acting as a nitrite reductase and conversion to nitric oxide via the reaction with haemoglobin at the site of ferric (Fe^{3+}) and ferrous (Fe^{2+}) haemoglobin.[16–19]

Normal adult haemoglobin (molecular weight, 64 500) consists of two α and two β polypeptide chains, each bound to a haeme group. The human α-globin gene cluster is located on chromosome 16 (16p13.3), spans about 30 kb and includes seven loci: 5′-ζ – pseudo ζ – μ – pseudo α-1 – α-2 – α-1 – θ-3′. The α-2 (HBA2) and α-1 (HBA1) coding sequences are identical. The order of the genes in the β-globin cluster is 5′-ε – γ-G – γ-A – δ – β-3′, which are located on 11p15.5. These genes are arranged in the order of their expression during development. The α (HBA) and β (HBB) loci determine the structure of the two types of polypeptide chains in adult haemoglobin (Hb). Two α chains plus two β chains constitute HbA, which in the normal adult constitutes about 97% of the total haemoglobin. α Chains combine with δ chains to form HbA-2, which with HbF (fetal haemoglobin) makes up the remaining 3% of adult haemoglobin. Data from the Heritage Family Study suggest that linkages at p values of 0.01 and better are observed with markers on 4q, 8q, 11p15.1 and 14q for VO_2max before training and with markers on 1p, 2p, 4q, 6p and 11p14 for the change in VO_2max in response to a 20 week standardised endurance training program.[20] In this regard, the *HBB* gene might be viewed as a functional and positional candidate gene for endurance capacity. To the best of our knowledge, however, no information is available concerning the association between *HBB* gene polymorphisms and aerobic capacity. Therefore, the principal aim of this

Abbreviations: ATP, adenosine 5′-triphosphate; LBM, lean body mass; RER, respiratory exchange ratio; TBM, total body mass; UTR, untranslated region

Table 1 Primers and PCR-RFLP conditions for polymorphism analysis of the *HBB* gene

RefSNP ID	Primers (5′→3′)	Position	Variation	Tm	$MgCl_2$	Restriction enzyme
	Up: ctt tgg gtt gta agt ga Down: ttg gga tat gta gat gg	−551,	C/T	60°C	2.5 mM	*RsaI*
Rs10768683	Up: tta ggc tgc tgg tgg tc Down: caa tca ttc gtc tgt ttc c	+16, intron2	C/G	58°C	2 mM	*AvaII*
	Up: tta ggc tgc tgg tgg tc Down: caa tca ttc gtc tgt ttc c	+340 relative to the TAA site, 3′-UTR	A/T	56°C	2.5 mM	*HinfI*

study was to examine the genetic association between the *HBB* gene and VO_2max and running economy in an untrained state (baseline) and in response to aerobic training (changes) in a young Chinese Han population.

METHODS

Subjects

Written informed consent was obtained from each subject prior to commencement of the training regimen. The study was approved by the local institutional ethics committee and was carried out in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. A total of 181 biologically unrelated male recruits from a local military police force volunteered to participate in our study and filled in a questionnaire designed by a professor experienced in statistics. The recruits were healthy, untrained and of Chinese Han origin in Northern China with similar lifestyles; untrained status was defined as not having engaged in regular vigorous physical activity in the past 2 years (less than two 20 (or more) minute sessions/week of vigorous exercise (running, cycling, or swimming)). The questionnaire also sought background information (for example, nationality, birth place, lifestyle, physical activity, smoking, blood glucose level, and blood lipids, etc). However, only 102 subjects completed both pre- and post-training tests (see below) and the entire exercise training program. Their physical characteristics were (mean ± SD): age 18.8 ± 0.9 years, height 171.7 ± 5.8 cm, and body mass 60.3 ± 6.5 kg. No subjects took any medications before or during the program. During the program, all subjects followed the same type of diet and the same daily schedule in terms of training and rest hours. Apart from this program, the subjects were only engaged in military training without other endurance training.

Endurance training program

Subjects ran 5000 m on a 400 m track three times per week for 18 weeks and were supervised by an exercise specialist. Subjects underwent adaptive training for 2 weeks prior to formal training. The intensity of the exercise progressively increased from an initial heart rate corresponding to 95% of the individual baseline ventilatory threshold during the first 10 weeks to 105% during the last 8 weeks. To maintain target heart rate, subjects wore a heart rate monitor (S810 Polar; Polar, Kempele, Finland) during each running session.

Running economy, VO_2max and ventilatory threshold measurements

Subjects undertook a battery of anthropometric and physiological tests prior to and after the 18 week exercise training program. Body composition was determined by anthropometric measurement combined with bioelectrical impedance analysis (BIA-Tanita; Tanita, Tokyo, Japan). Running economy was determined by measuring submaximal VO_2 for a 5 min period while running on a treadmill (Treadmill E6; Jaeger, Hoechberg, Germany) at a constant speed (12 $km \cdot h^{-1}$).[21] Gas exchange data were continuously measured with an automated open-circuit system (Mijnhault Oxycon Champion; Jaeger). VO_2, pulmonary ventilation, respiratory exchange ratio (RER) and heart rate were measured during the running economy test. VO_2max was measured following the running economy test and during an incremental test. Starting at 12 $km \cdot h^{-1}$ (0.5% incline), treadmill speed and incline were increased every minute by 0.5 $km \cdot h^{-1}$ and 2%, respectively, until subjects' volitional exhaustion. VO_2max was reached if the two following criteria were met simultaneously[20]: (a) RER $\geqslant$1.10; (b) change in VO_2 $\leqslant$5% or 100 $ml \cdot min^{-1}$ in the last three 30 s intervals. Running economy and VO_2max were expressed in $l \cdot min^{-1}$, $ml \cdot min^{-1} \cdot$total body mass (TBM) $^{-1}$ in kg, and $ml \cdot min^{-1} \cdot$ lean body mass (LBM) $^{-1}$ in kg. The ventilatory threshold was concurrently determined by three validated methods[22]: (a) the ventilatory equivalent method; (b) the excess carbon dioxide method; and (c) the modified V-slope method using 30 s averaged data. Visual evaluation to determine ventilatory threshold was carried out independently by two experienced investigators using these three methods. Training response was calculated as [Δ = (post-training minus baseline)/baseline×100%]. The reliability of the techniques used for LBM, VO_2max and running economy determination have been previously corroborated by our group, with intra-class correlation for repeated measurements ranging between 0.93 (LBM) and 0.97 (submaximal VO_2) ($p<0.001$).

Genotype classifications

Briefly, genomic DNA was extracted from white cells of peripheral blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). −551C/T, intron2,+16C/G and +340 A/T relative to the TAA site were selected. The −551C/T polymorphism is in the region which functions as a silencer in transient expression.[23 24] Intron2,+16C/G is in the alternative splicing region of mRNA, which might lead to differential expression. +340 A/T is in the 3′-untranslated region (3′-UTR) which is critical for normal β-globin mRNA stability.[25–28] The primers were designed by Primer 5.0 software based on the sequence NM_000518. The primer sequence and polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) conditions are shown in table 1.

Statistical analyses

Data were expressed as mean ± SD. A χ^2 analysis was employed to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium. The associations between endurance performance phenotypes and the three genetic markers were analysed by ANOVA using SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA). Lewontin's D′ and r^2 were estimated using SHEsis software.[29] Haplotype associations were estimated using Haplo.Score.[30] The post hoc power analysis was computed by G*Power.[31]

Table 2 Running economy at baseline and response to training according to *HBB* genotype

	−551C/T			Intron2,16C/G			+340A/T in 3′-UTR		
	CC (n = 28)	*TC* (n = 48)	*TT* (n = 26)	*GG* (n = 26)	*CG* (n = 51)	*CC* (n = 25)	*TT* (n = 41)	*AT* (n = 49)	*AA* (n = 12)
Baseline									
RE(HR) (bpm)	168±9	167±10	169±9	171±9	165±10	169±10	169±10	166±9	169±10
RE(VE) (l·min^{-1})	75.14±9.61	75.69±12.08	76.99±7.29	77.10±8.47	73.22±10.19	80.00±11.03	78.00±8.69	73.85±11.20	76.83±10.83
RE(VO_2) (l·min^{-1})	2.74±2.34	2.64±2.98	2.71±2.14	2.69±0.20	2.61±0.26	2.82±0.28	2.70±2.17	2.66±3.05	2.74±0.23
RE(VO_2·TBM^{-1}) (ml·min^{-1}·TBM^{-1} in kg)	45.13±3.91	44.41±3.81	44.90±2.54	45.10±3.15	44.22±3.26	45.39±4.39	44.57±3.07	44.82±3.65	44.93±4.75
RE(VO_2·LBM^{-1}) (ml·min^{-1}·LBM^{-1} in kg)	72.53±5.74	71.26±5.69	72.25±4.19	72.81±4.67	70.92±4.90	72.80±6.60	71.96±4.55	71.76±5.38	71.95±7.79
Training response (%)									
ΔRE(HR)	−4.38±3.40	−2.17±6.55	−2.73±4.21	−3.66±4.00	−1.78±6.50	−4.49±2.91*	−2.86±5.72	−2.58±5.31	−4.53±3.80
ΔRE(VE)	−15.27±8.27	−13.52±12.00	−14.50±9.21	−15.76±10.84	−11.98±10.63	−17.29±8.30*	−15.60±9.87	−12.51±11.34	−16.74±6.20
Δ RE(VO_2)	−8.69±5.89	−6.80±6.91	−7.72±5.83	−8.68±6.08	−6.34±6.59	−8.86±5.94	−7.48±6.22	−7.06±6.42	−9.83±6.69
ΔRE(VO_2·TBM^{-1})	−11.40±5.58*	−8.38±6.55	−9.08±5.30	−9.59±6.53	−8.20±5.81	−11.49±5.75*	−8.60±6.35	−9.24±5.61	−12.27±6.60
ΔRE(VO_2·LBM^{-1})	−7.14±3.56*	−5.24±4.09	−5.64±3.28	−5.94±4.05	−5.15±3.64	−7.17±3.67*	−5.35±3.94	−5.80±3.55	−7.67±4.14

*p<0.05.
LBM, lean body mass; RE, running economy; ΔRE(HR), the training response of heart rate at RE; ΔRE(VO_2), the training response of VO_2 (l·min^{-1}) at RE; ΔRE(VO_2·LBM^{-1}), the training response of VO_2 (ml·min^{-1}·LBM^{-1} in kg) at RE; ΔRE(VO_2·TBM^{-1}), the training response of VO_2 (ml·min^{-1}·TBM^{-1} in kg) at RE; RE(HR), heart rate at RE; RE(VE), ventilatory threshold at RE; RE(VO_2), VO_2 at RE expressed as l·min^{-1}; RE(VO_2·LBM^{-1}), VO_2 at RE expressed as ml·min^{-1}·LBM^{-1} in kg; RE(VO_2·TBM^{-1}), VO_2 at RE expressed as ml·min^{-1}·TBM^{-1} in kg; TBM, total body mass.

RESULTS

After training, the oxygen cost of running decreased from 2.69±0.26 to 2.48±0.25 l·min^{-1} (p<0.001) with the per cent change ranging from −25.0% to 8.0%. The VO_2max increased from 3.44±0.38 to 3.49±0.40 l·min^{-1} (p<0.05) with the percent change ranging from −13.6% to 24.1%.

Genotype distributions were in Hardy-Weinberg equilibrium at three loci (p>0.05). By pair-wise linkage analysis, the Lewontin's D′ and r^2 were 0.737 and 0.513 between −551C/T and intron2,+16C/G, 0.670 and 0.231 between −551C/T and +340A/T, and 0.933 and 0.474 between intron2,+16C/G and +340A/T, respectively. When the three polymorphisms were considered together, four haplotypes were identified (>5%): T_{-C551T}-C_{+C16G}-A_{+A340T} (30%), C_{-C551T}-C_{+C16G}-T_{+A340T} (13%), C_{-C551T}-G_{+C16G}-T_{+A340T} (8%), and T_{-C551T}-G_{+C16G}-T_{+A340T} (41%).

Table 2 displays running economy-related traits stratified by genotype at baseline and their response to training. None of the three polymorphisms was significantly associated with baseline running economy-related traits. Nevertheless, the training response of running economy was associated with −551C/T and intron2,+16C/G polymorphisms, and individuals homozygous for intron2,+16C/C or −551C/C exhibited a lower cost of running (when VO_2 was expressed as ml·min^{-1}·TBM^{-1} in kg and ml·min^{-1}·LBM^{-1} in kg), that is, better running economy, than the other subjects. In addition, there was no significant association with haplotypes at baseline or trainability.

The VO_2max data are shown in table 3 at baseline and their response to training; no significant difference was observed among different genotypes or haplotypes.

DISCUSSION

The noteworthy finding from this study was that the −551C/T and intron2,+16C/G polymorphisms in the *HBB* gene were associated with aerobic capacity at submaximal intensities, that is, running economy. Cardiorespiratory fitness (which is often quantified through VO_2max determination) is as strong a predictor of mortality as other conventional risk factors such as hypercholesterolaemia, cigarette smoking and hypertension.[32 33] However, most daily activities are executed at submaximal exercise intensities, and thus submaximal aerobic capacity is an important variable. Running economy is typically defined as the energy demand for a given velocity of submaximal intensity.[5] In our study, the −551C/T and intron2,+16C/G polymorphisms in the *HBB* gene were associated with running economy training response. Only one study has investigated the heritability of running economy at running speed of 5, 6, 7 and 8 km·h^{-1} by using the monozygotic and dizygotic twin method in an untrained state, but the researchers did not detect any genetic component in running economy (nor in VO_2max relative to

Table 3 VO_2max at baseline according to *HBB* genotypes

	−551C/T			Intron2,+16C/G			+340A/T in 3′-UTR		
	CC (n = 28)	*TC* (n = 48)	*TT* (n = 26)	*GG* (n = 26)	*CG* (n = 51)	*CC* (n = 25)	*TT* (n = 41)	*AT* (n = 49)	*AA* (n = 12)
Baseline									
VO_2max (l·min^{-1})	3.53±0.35	3.40±0.41	3.43±0.36	3.39±3.60	3.40±3.63	3.57±4.27	3.44±3.75	3.42±3.85	3.51±4.16
VO_2max·TBM^{-1} (ml·min^{-1}·TBM^{-1} in kg)	57.69±4.26	57.10±4.26	56.59±3.93	56.56±3.82	57.44±4.20	57.10±4.51	56.50±3.82	57.58±4.18	57.48±5.20
VO_2max·LBM^{-1} (ml·min^{-1}·LBM^{-1} in kg)	93.02±7.10	91.64±6.70	91.45±7.43	91.55±6.89	92.28±6.68	91.78±7.81	91.44±6.53	92.40±6.78	92.01±9.36
Training response (%)									
ΔVO_2max (l·min^{-1})	2.73±6.91	0.91±6.01	1.25±9.24	0.49±7.96	1.55±7.10	2.43±6.57	1.55±7.94	1.19±6.57	2.55±7.19
ΔVO_2max·TBM^{-1} (ml·min^{-1}·TBM^{-1} in kg)	−2.29±6.56	−2.84±6.46	−2.98±7.54	−2.66±7.76	−3.02±6.19	−2.18±6.83	−1.94±8.05	−3.62±5.14	−1.71±7.47
ΔVO_2max·LBM^{-1} (ml·min^{-1}·LBM^{-1} in kg)	1.04±5.89	1.03±6.38	1.06±8.57	0.87±8.03	0.97±6.67	1.37±5.97	1.65±8.07	0.27±5.75	2.03±6.38

*p<0.05.
LBM, lean body mass; TBM, total body mass; VO_2max, maximal oxygen uptake; VO_2max·LBM^{-1}, VO_2max expressed as ml·min^{-1}·LBM^{-1} in kg; VO_2max·TBM^{-1}, VO_2max expresses as ml·min^{-1}·TBM^{-1} in kg. VO_2max (l·min^{-1}), the training response of VO_2max (l·min^{-1}); ΔVO_2max·LBM^{-1} (ml·min^{-1}·LBM^{-1} in kg), the training response of VO_2max·LBM^{-1} (ml·min^{-1}·LBM^{-1} in kg); ΔVO_2max·TBM^{-1} (ml·min^{-1}·TBM^{-1} in kg), the training response of VO_2max·TBM^{-1} (ml·min^{-1}·TBM^{-1} in kg).

body mass).[34] The heritability of VO_2max, on the other hand, has been well documented in previous research.[20 35] Therefore, it was postulated that the report of Rodas *et al* was questionable due, possibly, to the limited sample size.[34] Moreover, these alleles might contribute to the trainability of running economy but do not appear to influence running economy in the untrained state. The *HBB* gene would be a new gene associated with the training response of running economy.

The molecular mechanism underlying the association observed here remains to be elucidated. −551C/T is in strict linkage disequilibrium with (AT)x(T)y between nucleotides −610 and −490 relative to the cap site. The (AT)x(T)y is associated with the binding capacity of the β protein (BP1), which is a negative regulatory transacting factor, so −551C/T may be involved in negative regulation.[23 24] The −551T/T motif might bind the putative BPI repressor more strongly than the −551C/T and −551C/C motifs did. Intron2,+16C/G is in the splicing region. The possible significance of intron2,+16C/G was predicted by NNSPLICE and SpliceView computer programs.[36 37] Intron2+16G, but not intron2+16C, might contain two possible branch sites, and the new branch site might induce a truncated protein. Hence, individuals who are homozygous for −551C/C or intron2,+16C/C might have a higher haemoglobin concentration than normal. In addition, the *HBB* gene is located close to the sulfonylurea receptor (*SUR*) gene and the voltage-gated potassium channel gene (*KCNA11*).[20] SUR is expressed in pancreatic β-cells where it forms ATP sensitive potassium channels together with KCNA11, and is thereby involved in the regulation of insulin secretion. KCNA11 is expressed in several tissues, including heart and skeletal muscle, where it plays a role in the coupling of cell metabolism to membrane potential. Thus, the −551C/T and intron2,+16C/G polymorphisms might be in linkage disequilibrium with some other functional polymorphisms and/or might be one of the accumulated genes responsible for aerobic capacity at submaximal intensity in humans. An obvious next step is comprehensive sequencing of this region and functional studies to clarify how the *HBB* gene polymorphisms contribute to aerobic capacity.

What is already known on this topic

- The crucial role of haemoglobin in exercise has been well documented.
- The blood haemoglobin concentration is related to maximal oxygen uptake (VO_2max) and endurance performance.
- New information suggests that the haemoglobin molecule is an ideal O_2 sensor.

What this study adds

- No information is available concerning the association between *HBB* gene polymorphisms and endurance performance.
- Our data indicate that individuals homozygous for the −551C or intron2,+16C allele might have a greater capacity to improve running economy in response to endurance exercise training.

Our study has some limitations. One potential limitation of our investigation was the method (BIA-Tanita, Japan) we used to estimate LBM since the algorithms used with this methodology were not designed for a Chinese population. Although the intra-class correlation coefficient for repeated tests was 0.93 ($p<0.001$), our method has not been validated against other accepted methods (for example, under water weighing or magnetic resonance imaging). Thus, there may be errors predicting LBM using BIA-Tanita. Finally, the addition of a group of approximately 100 controls not undergoing any training and being tested before and after the same time period as our study subjects would have strengthened our investigation.

In summary, our data indicate that individuals homozygous for the −551C or intron2,+16C allele might have a greater capacity to improve running economy in response to endurance exercise training. Thus, it is suggested that *HBB* genotypes might explain some of the individual variation in the cardiorespiratory adaptation induced by aerobic training, at least in a male Chinese Han population. Further research on larger populations and/or of different ethnic origin is necessary to fully determine causality between *HBB* genotypes and individual differences in physiological adaptations to endurance training.

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Authors' affiliations

Z He, L Feng, Y Lu, Biology Center, China Institute of Sport Science, Beijing, China
Y Hu, G Liu, X Xu, K Xu, Department of Sport and Human Sciences, Beijing Sport University, Beijing, China
Y Xi, L Wen, Department of Sport and Human Sciences, Tianjin Institute of Physical Education, Tianjin, China

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COMMENTARY

In humans, maximal aerobic capacity (VO_2max) is largely limited by the amount of oxygen supplied to working muscles. Although less research has focused on the factors that determine submaximal aerobic capacity (for example, running economy), recent investigations suggest that some strategies originally designed to enhance blood oxygen transport capacity (for example, intermittent hypoxic exposure) might also improve running economy. This study analyses the possible association between *HBB* gene polymorphisms and both the baseline levels and trainability of VO_2max and running economy in Chinese military police recruits. Much of what we know in biomedical sciences (including sports sciences) comes from research performed on Caucasian individuals living in western societies. Unfortunately, these populations follow a sedentary lifestyle that is not in accordance with our genetic makeup. Thus, scientific studies (including genotype data) on other populations are welcome to improve our knowledge and understanding of human adaptation to exercise.

A Lucia
Universidad Europea de Madrid, Spain; alejandro.lucia@uem.es