Are elite endurance athletes genetically predisposed to lower disease risk?

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Gómez-Gallego F, Ruiz JR, Buxens A, Altmäe S, Artieda M, Santiago C, González-Freire M, Verde Z, Artea D, Martínez A, Tejedor D, Lao JI, Arenas J, Lucia A. Are elite endurance athletes genetically predisposed to lower disease risk? Physiol Genomics 41: 82–90, 2010. First published December 22, 2009; doi:10.1152/physiolgenomics.00183.2009.—We compared a polygenic profile that combined 33 disease risk-related mutations and polymorphisms among nonathletic healthy control subjects and elite endurance athletes. The study sample comprised 100 healthy Spanish male nonathletic (sedentary) control subjects and 100 male elite endurance athletes. We analyzed 33 disease risk-related mutations and polymorphisms. We computed a health-related total genotype score (TGS) as first proposed by Williams and colleagues (1094-8341/10 $8.00 Copyright© 2010 American Physiological Society

Evidence suggests that genetic factors are likely to modify the risk of having the most prevalent diseases and the main causes of death in adults living in the Western world, i.e., cardiovascular disease, diabetes, dyslipidemia, obesity, and insulin resistance, and different types of cancer. Gene-environment interactions and lifestyle factors, particularly physical activity levels and exercise participation, can also modify the risk of having the aforementioned diseases.

For instance, humans with long-lasting participation in strenuous aerobic exercise and with very high levels of cardiorespiratory fitness, such as male former elite endurance athletes, have lower risk of all-cause mortality and longer life expectancy than matched sedentary control subjects. Increased life span was also reported in university endurance athletes, i.e., oarsmen who participated in the first 40 years of the Oxford-Cambridge boat race and nonprofessional ice skaters able to finish ultraendurance (200 km) races. These observed differences could be due to lifestyle or genetic factors. The documented association between high levels of endurance exercise (or cardiorespiratory fitness) and longer life expectancy raises a question of public health relevance: Is prescription of the highest possible levels of aerobic exercise, after obvious habituation, useful to maximize health benefits in the general population?

It is also known that the former athletes smoked less, consumed less alcohol, and had a healthier diet than their referents (22). However, whether the association between high levels of endurance exercise and decreased disease risk is biased because of genetic selection remains to be elucidated. With regard to this, genetic factors modify not only the risk for many diseases but also the association between regular exercise, fitness, and disease. It could be that elite endurance athletes are endowed with a more favorable genotype profile for disease risk and life expectancy than the general population of which they are not representative (37).

We compared a polygenic profile (i.e., by calculating the total genotype score (TGS) as first proposed by Williams and Folland (74)) that combined a total of 33 mutations and polymorphisms among two groups of men of the same ethnic origin (Spanish Caucasians): 1) healthy, nonathletic control subjects and 2) world-class elite endurance athletes. The genetic variations we studied have been shown to be associated with the most prominent causes of mortality in adult males of the Western world, i.e., cardiovascular disease and related disorders and cancer.

A list of genes, their variants, and genotypes is provided (in alphabetical order) in Table 1. A description summary of the putative influence(s) of the studied genetic variants in men’s disease/mortality risk or in important disease phenotypes is provided below.

The renin-angiotensin-aldosterone system (RAAS) plays an important role in blood pressure homeostasis and in the regulation of cardiovascular phenotypes. Two important genes of the RAAS are those encoding angiotensin-converting enzyme (ACE) and angiotensinogen (AGT). The ACE 287-bp Ins/Del(D) polymorphism (rs1799752) is associated with coronary artery disease (CAD). A recent meta-analysis on 118 studies (involving 43,733 cases with CAD and 82,606 controls) showed that, compared with II individuals, the DD genotype is associated with an increased risk for CAD [odds ratio (OR) 1.25; 95% confidence interval (CI) 1.16–1.35] (78). The DD genotype is also a risk factor for ischemic stroke (60). The 235Thr allele of the AGT Met235Thr (rs699) polymorphism

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increases the risk of CAD severity independently of other cardiovascular risk factors (39).

In contrast with other linked polymorphisms (−922A/G and −1468T/A) that are not associated with changes in gene transcription, the T→C mutation of the NOS3 −786 T/C polymorphism (rs2070744) results in significantly reduced gene promoter activity and reduced endothelial nitric oxide (NO) synthesis (45). The NOS3 −786 T/C polymorphism was originally associated with coronary vasospasm (45) and myocardial infarction in Japanese patients (46); a recent large meta-analysis revealed a per-allele OR of 1.17 (95% CI 1.07–1.28) for this polymorphism with regard to CAD risk (10).

β-Adrenergic receptors (βARs) are members of a family of G protein-coupled receptors that are stimulated by naturally occurring catecholamines. In humans, three βARs are known: β₁AR, β₂AR, and β₃AR. The genes that encode these receptors are associated with metabolic and cardiovascular disease phenotypes. While the Arg389Gly variation (rs1801253) in the β₁AR gene (ADRB1) influences an important health-related phenotype as peak oxygen uptake in cardiac patients (18, 69), the Gly16Arg (rs1042713) and Gln27Glu (M/N) (rs1042714) polymorphisms in β₂AR gene (ADRB2) contribute to metabolic syndrome susceptibility in men, with the OR being 1.83 (95% CI 1.10–3.05) and 2.43 (95% CI 1.19–4.95) in men bearing the Gly/Arg and Arg/Arg genotypes, respectively, and 1.67 (95% CI 0.84–3.33) in those bearing the Gln/Gln genotype (16). The Arg allele of the ADRB3 Thrp64Arg polymorphism (rs4994) is linked with increased risk of weight gain in obese people (11).

Hypertension is a common disorder of multifactorial origin that constitutes a major risk factor for cardiovascular events such as stroke and myocardial infarction. The AGT Met235Thr variant (rs699) is associated with the risk of hypertension, with the 235Thr allele being an independent risk factor for this disease even after adjustment for age, sex, and ethnicity (OR 1.33, 95% CI 1.04–1.70) and with a linear relation between AGT 235Thr allele number (“dose”) and blood pressure (48).

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene</th>
<th>Variant (rs)</th>
<th>Disease- and Mortality-Related Phenotypes (references)</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin I-converting enzyme</td>
<td>287-bp Ins/Del(D) (rs1799752)</td>
<td>CAD (55, 78), ischemic stroke (60), type 2 diabetes (77)</td>
<td>0 = II, 1 = ID, 2 = DD</td>
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<tr>
<td>ADRB1</td>
<td>Adrenergic β₁-receptor</td>
<td>Arg389Gly (rs1801253)</td>
<td>Cardiorespiratory fitness in heart disease (18, 69)</td>
<td>0 = GG, 1 = GA, 2 = AA</td>
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<td>ADRB2</td>
<td>Adrenergic β₂-receptor</td>
<td>Gly16Arg (rs1042713)</td>
<td>Metabolic syndrome (16)</td>
<td>0 = AA, 1 = AG, 2 = GG</td>
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<tr>
<td>ADRB3</td>
<td>Adrenergic β₃-receptor</td>
<td>Trp64Arg (rs4994)</td>
<td>Metabolic syndrome (16)</td>
<td>0 = NN, 1 = NM, 2 = MM</td>
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<td>AGT</td>
<td>Angiotensinogen</td>
<td>Met235Thr (rs699)</td>
<td>Hypertension (32) and CAD severity (39)</td>
<td>0 = TT, 1 = TA, 2 = AA</td>
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<tr>
<td>APOA1</td>
<td>Apolipoprotein A-I</td>
<td>−75 G/A (rs5070)</td>
<td>Blood lipids (52) and blood pressure (54)</td>
<td>0 = GG, 1 = GA, 2 = AA</td>
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<td>APOB</td>
<td>Apolipoprotein B</td>
<td>Arg3500Gln</td>
<td>Hypercholesterolemia (31, 49)</td>
<td>0 = AA, 1 = AG, 2 = GG</td>
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<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>Arg138Cys (rs7412)</td>
<td>Hyperlipoproteinemia (50, 67)</td>
<td>0 = AA, 1 = AC, 2 = CC</td>
</tr>
<tr>
<td>DSG2</td>
<td>Desmoglein 2</td>
<td>Arg45Gln</td>
<td>Arhythmogenic right ventricular dysplasia (6)</td>
<td>0 = AA, 1 = AG, 2 = GG</td>
</tr>
<tr>
<td>GNB3</td>
<td>Guanine nucleotide binding protein β</td>
<td>Arg48His</td>
<td>Hypertension (7, 59)</td>
<td>0 = AA, 1 = AH, 2 = HH</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione S-transferase π1</td>
<td>Thr305Ter (M/N)</td>
<td>Lung cancer (1)</td>
<td>0 = II, 1 = IV, 2 = VV</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Glutathione S-transferase θ1</td>
<td>Cys506Tyr</td>
<td>Lung cancer (70)</td>
<td>0 = AA, 1 = AV, 2 = VV</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin-6</td>
<td>Gly811Cys</td>
<td>Myelodysplastic syndrome (15)</td>
<td>0 = presence, 2 = absence</td>
</tr>
<tr>
<td>NAT2</td>
<td>N-acetyltransferase 2</td>
<td>Ser275Ser (M/N) (rs1799931)</td>
<td>Colorectal cancer (40)</td>
<td></td>
</tr>
<tr>
<td>NOS3</td>
<td>Nitric oxide synthase 3</td>
<td>Gly286Glu (M/N) (rs1799931)</td>
<td>Blood glucose (30)</td>
<td>0 = CC, 1 = GC, 2 = GG</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
<td>Cys112Arg (rs429358)</td>
<td>Longevity (19)</td>
<td></td>
</tr>
<tr>
<td>OGG1</td>
<td>8-Oxoguanine DNA glycosylase</td>
<td>Ala16Val (rs4880)</td>
<td>Pancreatic cancer (41, 73)</td>
<td>0 = AA, 1 = AV, 2 = VV</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease. For genotypes, 2 = highest disease risk or shorter life expectancy.
Heterotrimeric G proteins couple receptors for diverse extracellular signals to effector enzymes or ion channels; common polymorphisms in the GNB3 gene (which encodes the heterotrimeric protein guanine nucleotide binding protein β) have been associated with multigenic disorders, e.g., hypertension (72). The T allele of the 825C/T variation (rs5443) is associated with alternative splicing of the gene and formation of a truncated but functionally active β3-subunit (59). Carriers of the T allele have a significantly increased risk for essential hypertension. A recent meta-analysis including 14,094 hypertensive cases and 17,760 controls found that the TT vs. CC + CT contrast yielded an overall OR of 1.08 (95% CI 1.01–1.15) and the contrast of TT + CT vs. CC an OR of 1.17 (95% CI 1.06–1.29) (7).

The apolipoprotein A-I gene (APOAI) –75G/A (rs5070) polymorphism influences blood pressure, with the A allele being associated with higher diastolic and systolic pressure levels (54).

Apolipoproteins act as enzyme cofactors, receptor ligands, and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues. Numerous polymorphisms have been described in apolipoprotein genes with functional consequences. The apolipoprotein A-I (APOAI) –75G/A (rs5070) polymorphism is associated with the high-density lipoprotein cholesterol (HDLc) response to regular exercise (52, 63).

Apolipoprotein B is the chief protein component of the low-density lipoprotein cholesterol (LDLc) and serves as the ligand for the removal of LDLc from the circulation by the LDL receptor. Familial hypercholesterolemia increases the risk for premature ischemic heart disease; there are three ligand-defective mutations in the apolipoprotein B-100 (APOB) gene that cause familial defective apolipoprotein B and subsequent hypercholesterolemia: Arg3500Gln (65), Arg3531Cys (49), and Arg3500Trp (24). Notably, the APOB Arg3500Gln mutation causes severe familial hypercholesterolemia (OR 78, 95% CI 1.41–3.33), including smokers (OR 1.58, 95% CI 1.14–2.19) (70).

By virtue of its ability to bind to lipoprotein receptors, apolipoprotein E plays a key role in the metabolism of triglyceride-rich lipoproteins in the plasma. The apolipoprotein E family contains three major isoforms (ApoE2, E3, and E4). The Arg158Cys (rs7412) variant in ApoE2 (50) and the Cys112Arg (rs429358) variant in ApoE3 are associated with type III hyperlipoproteinemia (67).

The Leu7 allele is also associated with increased risk for type 2 diabetes, owing to a higher insulin resistance among Pro7 carriers (66). The D allele of the ACE I/D polymorphism is also associated with a 14% increased risk of type II diabetes relative to the I variant (OR 1.14, 95% CI 1.04–1.24) (77). Interleukin-6 (IL-6) is a multifunctional cytokine; a functional G/C polymorphism at position –174 (rs1800795) has been described in the 5’-flanking region of IL6 (21), with the G allele being associated with increased transcriptional response (21). The –174 G/C variant influences numerous disease and disease-related phenotype traits, with the C allele being associated with lower fasting glucose levels and decreased type 2 diabetes risk (30) and the GG genotype decreasing the possibility of reaching high longevity (100 yr) compared with C-allele carriers (OR 0.49, 95% CI 0.31–0.80) (19).

Sudden cardiac death associated with exertion is a prominent manifestation of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), an inherited disorder characterized by fibrofatty replacement of cardiac myocytes that typically manifests in the right ventricle (6, 44). In particular, the following four mutations in DSG2, the gene encoding desmoglein-2 [an essential transmembrane member of the desmosome (58)] are associated with ARVD/C: Arg45Gln, Arg48Gln, Trp305Ter (M/N), Cys561Tyr, and Gly811Cys (6).

Cancer is a complex disease, and the task of identifying human cancer predisposition genes is difficult. There are, however, some genetic variants that are strong candidates to influence cancer risk and prognosis, e.g., polymorphisms in the genes encoding enzymes involved in detoxification or DNA repair such as N-acetyltransferase 2 (NAT2) or glutathione S-transferases π1 and θ (GSTP1 and GSTTI).

The main cause of cancer mortality in men worldwide is lung cancer. The GSTP1 enzyme is a detoxification enzyme with substrate specificity for both exogenous carcinogens and chemotherapy agents. Genetic polymorphisms in GSTP1 exon 5 (Ile105Val) and exon 6 (Ala114Val) influence this enzyme’s activity. A recent pooled analysis showed that the Ile105Val (rs1695) variant in GSTP1 is associated with lung cancer; an overall association (OR 1.11, 95% CI 1.03–1.21) was found between lung cancer and carriage of the GSTP1 Val/Val or Ile/Val genotype compared with those carrying the Ile/Ile genotype (13). The variant Val allele of the exon 6 polymorphism is also associated with lung cancer risk in men (OR 2.17, 95% CI 1.41–3.33), including smokers (OR 1.58, 95% CI 1.14–2.19) (70).

Human 8-oxoguanine DNA glycosylase 1 (OGG1) has a major role in the repair of 8-hydroxyguanine, a major promutagenic DNA lesion. The rs1052133 polymorphism in the OGG1 gene leads to substitution of the amino acid at codon 326 from Ser to Cys and a decrease in enzyme activity. This polymorphism was originally linked with increased lung cancer susceptibility (61). A recent meta-analysis showed that the homozygous Cys/Cys genotype is significantly associated with increased lung cancer risk compared with Ser allele carrier status (OR 1.31, 95% CI 1.02–1.69); the association was strongest for the most aggressive form of the disease, i.e., small-cell carcinoma (OR 2.40, 95% CI 1.32–4.49) (47).

Common NAT2 polymorphisms determine an individual’s acetylation capacity for carcinogens and drugs. A recent study on a large Spanish population showed that, compared with NAT2 rapid or intermediate acetylators, NAT2 slow acetylators [i.e., individuals homozygous for the slow allele of the Arg64Cln (rs1801279), Tyr94Tyr (M/N) (rs1041983), Ile114Thr (rs1801280), Leu161Leu (M/N) (rs1799929), Arg197Gln (rs1799930), Arg268Lys (rs1208), or Gly286Glu (M/N) (rs1799931) polymorphisms] had an increased overall risk of bladder cancer (OR 1.4, 95% CI 1.2–1.7) (25). Meta-analyses also showed that the overall association for NAT2 was robust (P < 0.0001) (25).
The GSTT1 enzyme mediates exposure to various cytotoxic and genotoxic agents, including those associated with increased risk of the myelodysplastic syndrome (MDS). The GSTT1 gene has a “null” variant allele, in which the entire gene is absent; inheritance of the GSTT1 null genotype confers a significantly higher risk of MDS (OR 4.3, 95% CI 2.5–7.4) (15). The null genotype is also associated with increased risk of one of the commonest types of cancer worldwide, i.e., colorectal cancer (e.g., in Caucasians OR 1.39, 95% CI 1.21–1.50) (40).

Exogenous reactive oxygen species (ROS) induce DNA damage. Manganese superoxide dismutase (SOD2) is one of the major enzymes responsible for the defense against oxidative damage. The alanine-to-valine polymorphism (rs4880) at codon 16 (Ala16Val) in the SOD2 gene has been associated with increased cancer susceptibility; particularly strong is the evidence for the association between the Val/Val genotype and pancreatic cancer (adjusted OR 1.96, 95% CI 1.0–3.8; P = 0.04) (73).

It should be noted that some of the variants we chose for our study are also associated with elite endurance athletic status, and thus could be considered as potential confounders. The ACE I/D polymorphism of ACE gene is arguably the most extensively studied genetic variation with regard to exercise-related phenotypes. It is related to cardiovascular and skeletal muscle function. While the I allele has been associated with elite endurance performance (29, 64), controversy exists, with some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2). Data are overall less controversial for some authors finding no association (51) or even a beneficial effect of the D allele (2).

The ADRB2 Gly16Arg (76) and ADRB3 Trp64Arg polymorphisms are associated with elite endurance performance status (56). Finally, besides its putative influence in cardiovascular and metabolic disease phenotypes, the C825T polymorphism in GNB3 is also associated with elite endurance athlete status (vs. sprint athlete status) (20).

Methods

Subjects. The studied sample comprised 100 healthy male nonathletic control subjects (not enrolled in competitive sports; age 19–32 yr) and 100 male elite endurance athletes (age 20–39 yr) who competed within the last 10 yr. Fifty athletes were elite endurance runners, having all participated in at least one Olympic, and some were Olympic finalists or Europe/World Champions. The remaining 50 athletes were professional road cyclists who have all been Tour de France finishers, including top 3 finishers. All participants were of the same Caucasian (Spanish) descent for at least three generations. The study protocol was approved by the institutional ethics committee of Universidad Europea de Madrid and was in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000). Our study was in accordance with stringent recommendations for replicating human genotype-phenotype association studies (14).

Genotyping methods. We extracted DNA from saliva or blood samples between 2004 and 2008. All genotyping was performed in the same laboratory (Progenika Biopharma) with a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation, and analysis of the arrays were performed following procedures described elsewhere with minor modifications (62). The results of polymorphisms ACE Ins/Del (rs1799752), AGT Met235Thr (rs699), and IL6 −174C>G (rs1800795) were corroborated in a second laboratory (Universidad Europea de Madrid) with a different methodology.

Total genotype score and genotype score per disease category/subcategory. We assumed that each individual polymorphism would have limited power to influence disease risk or the association between fitness and disease. We therefore computed the combined influence of all the studied polymorphisms, following the TGS procedure recently applied to report the association between polygenic profiles and complex phenotypes such as cardiovascular risk (35) or the probability of becoming an endurance athlete champion (53, 74). We constructed a health-related genotype score assuming an additive model (equaling 0, 1, or 2), that is, on the basis of the number of unfavorable alleles associated with disease risk that were carried by each subject for each polymorphism (Table 1). The health-related TGS was transformed into a scale of 0–100 for easier interpretation, i.e., TGS = 100/66 × Σ of the 33 genotype scores (53, 74).

Following the aforementioned methodology, we also computed specific TGSs for the following disease categories: 1) global “cardiometabolic” risk TGS [including ACE I/D, AGT Met235Thr, APOAI −75G/A, ADRB2 Gly16Arg, ADRB2 Gln27Glu (M/N), ADRB3 Trp64Arg, APOB Arg3500Gln, APOB Arg3531Cys, APOB Arg3500Trp, APOE Arg158Cys, APOE Cys112Arg, DSG2 Arg454Gln, DSG2 Arg484Gln, DSG2 Trp305Ter (M/N), DSG2 Cys506 Tyr, DSG2 Gly811Cys, GNB3 825CT, IL6 −174G/C, NPY Leu7Pro and NOS3 −786T/C]; 2) global cancer TGS [GSTP1 Ile105Val, GSTP1 Ala114Val, NAT2 Arg64Gln, NAT2 Tyr94Tyr (M/N), NAT2 Ile114Thr, NAT2 Leu116Leu (M/N), NAT2 Arg197Gln, NAT2 Arg268Lys, NAT2 Gly286Glu (M/N), GSTT1 “null” allele, OGCG1 Cys326Ser and SOD2 Ala16Val]; and 3) TGS for polymorphisms that, besides their effect on disease susceptibility, can be also associated with elite endurance athletic status (ACE I/D, ADRB2 Gly16Arg, ADRB3 Trp64Arg and GNB3 C825T).

We also computed TGSs for each disease subcategory (≥3 variants each): 1) cardiovascular disease and stroke (ACE I/D, AGT Met235Thr and NOS3 −786 T/C); 2) hypertension (AGT Met235Thr, APOAI 75 G/A and GNB3 825CT); 3) dyslipidemia (APOAI −75G/A, APOB Arg3500Gln, APOB Arg3531Cys, APOB Arg3500Trp, APOE Arg158Cys, APOE Cys112Arg and NPY Leu7Pro); 4) insulin resistance (ACE I/D, IL6 −174 G/C and NPY Leu7Pro); 5) risk of sudden cardiac death (ARDVCD) [DSG2 Arg454Gln, DSG2 Arg484Gln, DSG2 Trp305Ter (M/N), DSG2 Cys506 Tyr and DSG2 Gly811Cys]; 6) lung cancer (GSTP1 Ile105Val, GSTP1 Ala114Val, OGG1 Cys326Ser); and 7) other types of cancer [NAT2 Arg64Gln, NAT2 Tyr94 Tyr (M/N), NAT2 Ile114Thr, NAT2 Leu116Leu (M/N), NAT2 Arg197Gln, NAT2 Arg268Lys, NAT2 Gly286Glu (M/N), GSTT1 “null” allele, and SOD2 Ala16Val]. For each of the aforementioned categories and subcategories, TGS was computed as 100/total number of alleles × Σ of the total number of genotype scores (53, 74), e.g., TGS = 100/20 × Σ of the 10 genotype scores for a category including 10 genetic variants in total.

Statistical analysis. We used unpaired Student’s t-test to compare the “global” health-related TGS as well as the TGS by disease categories/subcategories among groups (control subjects vs. athletes). We also compared the genotype and allelic frequency for each polymorphism between the groups with the χ2-test with α set at 0.05 and corrected for multiple comparisons (28). We compared frequencies of having a “disease risk”-related genotype (also called “disease-trait genotype”) for 0 up to 33 variants among control subjects versus athletes with a χ2-test. All analyses were performed with the Statistical Package for Social Sciences (SPSS, v. 16.0 for Windows; SPSS, Chicago, IL).
RESULTS

Genotyping success rate was >99.99% (only 1 missing polymorphism in 1 subject from the control group). All genotype distributions in the study subjects were in Hardy-Weinberg equilibrium, except four in the athlete group (rs1799752 \((P = 0.018)\), rs1042714 \((P = 0.043)\), rs5443 \((P = 0.013)\), and rs2070744 \((P = 0.048)\)).

The genotype and allele frequencies of the studied polymorphisms in Spanish control subjects and athletes are depicted in Table 2. We observed significant differences in the ADRB3 Trp64Arg (rs4994) and SOD2 Ala16Val (rs4880) genotype and allele distributions among the study groups, yet after correction for multiple comparisons (28) statistical significance only remained of the rs4994 genotype \((P < 0.001)\).

There were no individuals with more than nine “disease-trait” genotypes (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3). The computed health-related TGS remained of the rs4994 genotype (Table 3).

DISCUSSION

We found no evidence that elite endurance athletes are genetically predisposed to have lower disease risk, which also suggests that this group is not predisposed to have longer life expectancy than matched sedentary control subjects.

Table 3. Distribution of Spanish (Caucasian) nonathletic control subjects and elite endurance athletes with a “disease-trait” genotype for 0–33 genetic variants

<table>
<thead>
<tr>
<th>Number of “Disease-Trait” Genotypes</th>
<th>Control Subjects ((n = 100))</th>
<th>Athletes ((n = 100))</th>
<th>(P)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.296</td>
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<tr>
<td>1</td>
<td>5</td>
<td>3</td>
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<td>2</td>
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<td>8</td>
<td>7</td>
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<tr>
<td>8</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Data are numbers of subjects with 0–9 “disease-trait” genotypes; there were no individuals with >9 “disease-trait” genotypes.

We observed significant differences in the ADRB3 Trp64Arg (rs4994) after adjusting for multiple comparisons (28). This polymorphism has a documented effect on both disease susceptibility [obesity (11)] and elite endurance athletic status (56). It is of note that we did not find significant between-group differences in the genotype or allele frequency of some variants that have been shown to be associated with elite endurance status in other cohorts, e.g., ACE I/D (29, 64), ADRB2 Gly16Arg (76), or GNB3 C825T (20). With regard to this,
there is growing complexity and controversy regarding elite athletic status and genetic polymorphisms as the number of genetic association studies grows. This is exemplified by the conflicting findings among different studies for the ACE gene, which is the most studied gene in the field of exercise genetics (9).

The studied athletes seem to be endowed with a more “favorable” polygenic profile for endurance performance per se, independent of disease risk, than the general population (53). In a recent study with the very best competitors (n = 46) of the present athlete cohort we indeed showed that a TGS computed with seven polymorphisms associated with endurance performance was significantly higher than that of nonathletic control subjects (53). It is of note that only one of the polymorphisms studied here, ACE I/D, was included in this purely “athletic-oriented” TGS. Thus the previously reported decrease in all-cause mortality in former world-class Caucasian endurance athletes (who competed in Olympic Games from 1920 to 1965) (38, 57) or in other endurance sportsman cohorts (26, 33, 68) is likely not biased by genetic selection. Rather, the aforementioned findings would provide further support for the dose-response health benefits of regular physical activity reported in over 100 prospective studies (42).

Our genetic makeup is shaped to support the balance between energy intake and energy expenditure that was common to Paleolithic hunter-gatherer societies, in which daily energy expenditure during physical activities was 4–6 MJ (or 1,000–1,500 kcal) (12). The daily energy expenditure during physical activity of most individuals in contemporary Western societies is only ~38% of that of our Paleolithic ancestors (12) and is, therefore, biologically abnormal, leading to chronic maladaptation and “diseases of civilization,” particularly cardiovascular disease. In contrast, physically active westerners, including elite endurance athletes, follow a lifestyle that is more in accordance with our genetic makeup and thus have a reduced risk of such diseases. Indeed, cardiorespiratory fitness, which is one of the main outcomes of regular endurance physical activity, is a strong, independent predictor of all-cause and cardiovascular disease mortality (8). That said, it is nevertheless of interest to note that genetic factors do interact with physical activity levels so that there is individual variability in the training responsiveness of phenotype traits indicative of cardiorespiratory fitness. For instance, research from the HERITAGE Family Study showed a major gene effect on the training response of an important fitness and health phenotype trait such as heart rate at submaximal exercise (3).

Our findings are limited by the fact that we only analyzed a snapshot of the numerous polymorphisms and mutations that can potentially affect disease risk. We also assumed that the effect of the genotype and the disease risk was additive, and we scored each polymorphism as 0, 1, and 2. With regard to this potential limitation, the results remained the same after repetition of the analysis assuming other models (i.e., dominant, recessive). On the other hand, all gene variants were given the same weight in the total score, but whether the selected genes and the gene variants explain the same proportion of the variance in the disease risks is not known. Our findings are also limited by the fact that we did not study genetic susceptibility to other types of diseases, notably infectious diseases. Finally, it must be recognized that our model does not take into account potential complex interactions between genetic variants that might not influence disease risk individually. Multiple rare alleles within an individual can also explain disease susceptibility. Future research is also necessary to assess whether the unique environmental factors of elite athletes (in particular, demanding training regimens since youth) can alter the expression of health-related genes during critical periods of development, i.e., through epigenetic mechanisms (71).

In summary, the findings of the present study suggest that there is no evidence that elite male endurance athletes are genetically predisposed to have lower disease risk than matched nonathletic control subjects. Thus the previously documented association between high levels of endurance exercise undertaken by humans and decrease in all-cause mortality is likely not biased by genetic selection. Keeping in mind the importance of other lifestyle factors, our data overall support the notion that prescription of regular, vigorous exercise (after obvious habituation) might be a potentially useful tool to improve the health status of the general population. As shown by Koch and Britton’s studies in rats (36, 75), it might be that an improved regulation of oxidative pathways in mitochondria (as could be the case in elite athletes) may be a common factor linking cardiorespiratory fitness to decreased disease risk.

**Table 4. Comparison of “global” health-related score and total genotype scores per disease category and subcategory in control subjects and elite endurance athletes**

<table>
<thead>
<tr>
<th>Health-related score</th>
<th>Control Subjects</th>
<th>Athletes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>23.8 ± 1.0</td>
<td>24.2 ± 0.8</td>
<td>0.553</td>
</tr>
<tr>
<td>Global “cardiometabolic” risk</td>
<td>24.2 ± 0.8</td>
<td>23.8 ± 1.0</td>
<td>0.553</td>
</tr>
<tr>
<td>Cardiovascular disease and stroke</td>
<td>56.7 ± 21.6</td>
<td>56.0 ± 21.9</td>
<td>0.829</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36.2 ± 18.4</td>
<td>37.4 ± 18.8</td>
<td>0.656</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5.0 ± 5.9</td>
<td>4.7 ± 5.3</td>
<td>0.718</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>35.2 ± 16.2</td>
<td>33.7 ± 18.9</td>
<td>0.548</td>
</tr>
<tr>
<td>Cancer (global)</td>
<td>29.3 ± 9.6</td>
<td>29.5 ± 8.6</td>
<td>0.897</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>18.7 ± 19.3</td>
<td>19.5 ± 14.6</td>
<td>0.731</td>
</tr>
<tr>
<td>Other types</td>
<td>32.9 ± 11.4</td>
<td>32.8 ± 10.8</td>
<td>0.972</td>
</tr>
<tr>
<td>Endurance athletic status</td>
<td>39.8 ± 17.0</td>
<td>42.8 ± 15.1</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 100 control subjects and 100 elite endurance athletes. Note: Data for the TGS corresponding to the risk of cardiac death (due to arrhythmogenic right ventricular dysplasia/cardiomyopathy) are not shown because no study participant carried a mutant allele for any of the studied DSG2 mutations. As such, TGS equaled 0.0 in both groups.


