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Quantitative trait loci for maximal exercise capacity phenotypes and their responses to training in the HERITAGE Family Study

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Rico-Sanz, J., T. Rankinen, T. Rice, A. S. Leon, J. S. Skinner, J. H. Wilmore, D. C. Rao, and C. Bouchard. Quantitative trait loci for maximal exercise capacity phenotypes and their responses to training in the HERITAGE Family Study. *Physiol Genomics* 16: 256–260, 2004. First published November 18, 2003; 10.1152/physiolgenomics.00035.2003.—The purpose of this study was to identify regions of the human genome linked to maximal oxygen uptake ($\dot{V}O_{2\,max}$) and maximal power output (MPO), and their response to a standardized 20-wk endurance-training program in sedentary black and white subjects. A total of 509 polymorphic markers covering the 22 autosomes were used in the genome-wide linkage scan. Baseline phenotypes were adjusted for age, sex, and body mass, whereas the training responses were adjusted for age, sex, and the baseline values. Regression-based single- and multipoint linkage analyses were used. In the sedentary state, a total of 351 and 102 sibling pairs were available for whites and blacks, respectively, and 329 and 90 sibling pairs, respectively, for the training response phenotypes. Baseline $\dot{V}O_{2\,max}$ showed promising linkage ($P < 0.0023$) with 11p15.1 (whites), and suggestive evidence of linkage ($0.01 > P > 0.0023$) was found on 1p31, 7q32, and 7q36 (blacks). Baseline MPO exhibited promising linkage on 10q23 and suggestive evidence of linkage on 13q33 and 18q11-q12 (whites). $\dot{V}O_{2\,max}$ training response yielded promising linkages with markers on 1p31 (blacks) and suggestive on 4q27, 7q34, and 13q12 (whites) and on 16q22 and 20q13.1 (blacks). Training-induced changes in MPO showed promising linkages on 5q23 (whites) and suggestive on 1q21, 4p15.1, and 4p13 (whites) and on 1q22 and 13q11 (blacks). In conclusion, the strongest evidence of linkage was found on chromosomal regions 11p15 and 10q23 for $\dot{V}O_{2\,max}$ and MPO in the sedentary state and on chromosomes 1p31 and 5q23 for their responsiveness to training. These chromosomal regions harbor several candidate genes that deserve further investigation.

aerobic power; candidate genes; endurance capacity

CARDIORESPIRATORY FITNESS is commonly evaluated on the basis of maximal oxygen uptake ($\dot{V}O_{2\,max}$) obtained during a progressive intensity test leading to volitional exhaustion. Low levels of cardiorespiratory fitness have been associated with increased risks for cardiovascular disease, type 2 diabetes, and premature death (3, 15, 17, 28, 29).

Evidence of a significant familial component for $\dot{V}O_{2\,max}$ has been obtained from twin and family studies (5, 7). The results of the HERITAGE Family Study showed a heritability estimate for $\dot{V}O_{2\,max}$ in the sedentary state of about 50% of the phenotypic variance adjusted for age, sex, body mass, and body composition (5). Furthermore, when these subjects were exposed to a 20-wk endurance-training program, the heritability estimate of the $\dot{V}O_{2\,max}$ response to training reached 47% of the variability in the response (4).

A search for genes affecting interindividual variation in $\dot{V}O_{2\,max}$ and its responsiveness to training is currently underway. One approach initially utilized is the identification of quantitative trait loci (QTLs). Recently, a genome-wide scan for $\dot{V}O_{2\,max}$ and its response to training in the white population of the HERITAGE Family study was performed using a map of 289 polymorphic markers covering all 22 autosomes (8). In the present study, the genome-wide linkage scan was extended to maximal power output and its responsiveness to training utilizing a denser map of 509 markers both in the white and black families of the HERITAGE Family Study.

METHODS

Subjects. The study design, inclusion criteria, and protocol have been previously described (6). Subjects were required to be in good physical health and able to complete a 20-wk exercise program. A total of 351 sibling pairs in the sedentary state and 329 pairs for the response to training were available in whites. It has been brought to our attention that the maximal number of sibling pairs was reported as 415 in a previous paper (8). This was an error for which a corrigendum will be published in the *Journal of Applied Physiology*. In the sample of blacks, the maximum number of sibling pairs is 102 in the sedentary state and 90 for the training response phenotypes. Subjects were required to be sedentary, defined as not having been involved in regular physical activity over the previous 6 mo. The study protocol had been approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

$\dot{V}O_{2\,max}$ and MPO measurements. Two maximal exercise tests were performed on a cycle ergometer on two separate days in the sedentary state and again on two separate days after training. The tests were conducted on a SensorMedics 800S (Yorba Linda, CA) cycle ergometer connected to a SensorMedics 2900 metabolic measurement cart.

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Table 1. *Age, weight, and height of the HERITAGE Family Study subjects used in the linkage analyses*

Variable	Group	Blacks			Whites		
		n	Mean	SD	*n*	Mean	SD
Age	Sons	64	28	7	146	25	6
	Daughters	132	28	8	163	25	6
Weight, kg	Sons	64	86.2	19.3	146	82.5	16.7
	Daughters	132	72.7	17.5	163	64.3	13.1
Height, cm	Sons	64	177.1	7.2	146	179.2	6.1
	Daughters	132	162.2	6.5	163	164.6	6.5

The criteria for $\dot{V}O_{2\,max}$ were respiratory exchange ratio (RER) > 1.1, plateau in $\dot{V}O_2$ (change of <100 ml/min in the last three 20-s intervals), and a heart rate within 10 beats/min of the maximal heart rate predicted for age. In the first test, subjects cycled at a power output (PO) of 50 W for 3 min, with increments of 25 W every 2 min until volitional exhaustion. For older or smaller individuals, the test was started at 40 W, with increases of 10–20 W every 2 min. In the second test, subjects exercised for 8–12 min at an absolute PO of 50 W, rested 4 min, and exercised for 8–12 min at a relative PO equivalent to 60% of $\dot{V}O_{2\,max}$. This was followed by 3 min at 80% of $\dot{V}O_{2\,max}$. The test then progressed to a maximal level of exertion. If both $\dot{V}O_{2\,max}$ values were within 5% of each other, then the average $\dot{V}O_{2\,max}$ from these two tests was taken as the $\dot{V}O_{2\,max}$ and used in the linkage analysis. If these differed by more than 5%, then the higher $\dot{V}O_{2\,max}$ value was used. The $\dot{V}O_{2\,max}$ response was defined as the difference (ml O_2/min) between posttraining $\dot{V}O_{2\,max}$ and baseline $\dot{V}O_{2\,max}$. The MPO (watts) attained at the point of volitional exhaustion was recorded. The responsiveness to training of MPO was defined as the difference (watts) between posttraining MPO and baseline MPO. Each of the pre- and posttraining MPO values were the mean of two measurements.

Exercise training program. The training was conducted on cycle ergometers (Universal Aerobicycle, Cedar Rapids, IA). Subjects were endurance trained, three times a week, for 20 wk. The intensity of the exercise progressively increased from a heart rate corresponding to 55% of $\dot{V}O_{2\,max}$ during the first 4 wk to 75% during the last 8 wk. The duration was also progressively increased from 30 min/day during the first 2 wk to 50 min/day, which was maintained from the 14th week to the end of the program. A more detailed description of the training program can be found elsewhere (25). To maintain constant training heart rates, the ergometers were interfaced with a Mednet computer system (Universal Gym Mednet, Cedar Rapids, IA) to adjust automatically the PO to the individuals' heart rates. Trained exercise specialists supervised all training sessions on site.

Molecular studies. A total of 509 markers with an average spacing of 6.0 Mb across the 22 autosomes were used. PCR conditions and genotyping methods have been described in detail previously (9). Automatic DNA sequencers from LI-COR were used to detect the PCR products, and genotypes were scored automatically using the software SAGA. Incompatibilities with Mendelian inheritance were checked, and markers were regenotyped completely if incompatibilities were found. Microsatellite markers were selected from different sources but mainly from the Marshfield panel version 8a. The panel of markers included also some candidate genes for relevant HERITAGE phenotypes. Map locations were taken from the Genetic Location DataBase of Southampton, UK (**http://cedar.genetics.soton.ac.uk**).

Linkage analysis. Baseline $\dot{V}O_{2\,max}$ and MPO were adjusted for age, gender, and body mass using step-wise multiple regressions (e.g., 23). Training response phenotypes were adjusted for age, sex, and the baseline value of the phenotype. The residuals from the regression were then standardized to zero mean and unit variance within each subgroup and constituted the analysis variable. Single- and multipoint linkage analyses were performed with the sibling-pair linkage procedure (12, 13), as implemented in the SIBPAL program of the SAGE 4.0 Statistical Package (26). Both single- and multipoint estimates of allele sharing identical by descent were generated using the GENIBD program of the SAGE 4.0 package. All analyses were conducted separately for whites and blacks. The alpha level used here to identify promising results ($P < 0.0023$) represents, on average, one false positive per scan for experiments involving ~400 markers (22). Empirical *P* values (a maximum of 1,000,000 replicates) were calculated for all markers with nominal *P* values 0.01 or less.

RESULTS

Table 1 shows the basic characteristics of the subjects available for the genome-wide linkage scans. Table 2 summarizes the subjects' unadjusted baseline $\dot{V}O_{2\,max}$ and MPO and their responses to training.

Tables 3 and 4 describe the chromosomal regions and map positions of markers showing promising ($P < 0.0023$) and suggestive linkages ($0.01 > P > 0.0023$) with $\dot{V}O_{2\,max}$ and MPO in the sedentary state for whites and blacks, respectively. In whites, baseline $\dot{V}O_{2\,max}$ showed promising linkage with markers on chromosome 11p15.1, while suggestive evidence of linkage with $\dot{V}O_{2\,max}$ was found on 1p31 and 7q32 and 7q36 in blacks. The genome-wide scan yielded promising linkage on 10q23 and suggestive evidence of linkage on 13q33 and 18q11–12 for baseline MPO only in whites.

Tables 5 and 6 present the promising and suggestive linkages with $\dot{V}O_{2\,max}$ and MPO training responses in whites and blacks, respectively. Suggestive evidence of linkage was found for $\dot{V}O_{2\,max}$ training response on 4q27, 7q34, and 13q12 in whites, along with a promising linkage on 1p31, and suggestive evidence of linkage on 16q22 and 20q13.1 in blacks. The search for MPO training response QTLs revealed a promising linkage on 5q23 and suggestive evidence of linkage on 1q21, 4p15.1, and 4p13 in whites, while suggestive evidence of linkage was found on 1q22 and 13q11 in blacks.

DISCUSSION

In the present study, genome-wide linkage scans were performed to identify chromosomal regions that might be associated with the phenotypic variability of $\dot{V}O_{2\,max}$ and MPO and their responsiveness to training in whites and blacks. This study is an extension of a previous genome-wide scan for $\dot{V}O_{2\,max}$ and its responsiveness to training performed in whites

Table 2. *Unadjusted mean and SD values for baseline $\dot{V}O_{2max}$ and MPO and their responses to training*

Variable	Group	Blacks			Whites		
		n	Mean	SD	*n*	Mean	SD
		Baseline					
$\dot{V}O_{2max}$, ml/min	Sons	64	2,931	436	146	3,292	499
	Daughters	132	1,816	351	163	2,061	300
MPO, W	Sons	64	212	34	146	247	48
	Daughters	132	130	28	163	156	28
		Training response					
$\dot{V}O_{2max}$, ml/min	Sons	63	425	211	138	492	245
	Daughters	115	320	150	156	373	191
MPO, W	Sons	63	61	23	138	69	32
	Daughters	114	38	17	156	43	19

$\dot{V}O_{2max}$, maximal oxygen uptake; MPO, maximal power output.

Table 3. *Promising ($P < 0.0023$) and suggestive ($0.01 > P > 0.0023$) linkages with $\dot{V}O_{2max}$ and MPO in the sedentary state in whites adjusted for age, sex, and body mass*

Marker	Chr	Map Position, Mb	Trait	SIBPAL (*P* Value) Multipoint	SIBPAL (*P* Value) Single point
D10S677	10q23	96.1	MPO	0.0014	0.0019
SUR	11p15.1	21.2	$\dot{V}O_{2max}$	0.0014	0.024
D13S796	13q33	104.5	MPO	0.0098	0.052
D18S1107	18q11.1	23.5	MPO	0.0095	0.025
D18S866	18q11.2	24.7	MPO	0.0077	0.28
D18S478	18q12	28.8	MPO	0.0064	0.17

SIBPAL is a program of the SAGE 4.0 statistical package. Chr, chromosome.

Table 5. *Promising ($P < 0.0023$) and suggestive ($0.01 > P > 0.0023$) linkages with the responsiveness to training of $\dot{V}O_{2max}$ and MPO in whites adjusted for age, sex, and the baseline value*

Marker	Chr	Map Position, Mb	Trait	SIBPAL (*P* Value) Multipoint	SIBPAL (*P* Value) Single point
S100STUI	1q21	154.0	MPO	0.0091	0.009
D4S2397	4p15.1	32.2	MPO	0.0075	0.0024
D4S1627	4p13	47.2	MPO	0.0062	0.040
FABP2	4q27	127.8	$\dot{V}O_{2max}$	0.0086	0.0093
D5S1505	5q23	135.6	MPO	0.002	0.078
D7S495	7q34	149.8	$\dot{V}O_{2max}$	0.0089	0.012
D13787	13q12	19.4	$\dot{V}O_{2max}$	0.0087	0.018
D13S1243	13q12	19.5	$\dot{V}O_{2max}$	0.0088	0.017

only (8) using a less dense map. In the latter scan, two analytical procedures were used: a single-point linkage procedure using the original Haseman-Elston regression model (13), and a multipoint variance components approach using all the family data. In the present study, 220 additional markers were added, reducing the intermarker distance from 11 cM to about 6 Mb on average. MPO was added to the $\dot{V}O_{2\,max}$ phenotypes, and the scan was performed in whites and blacks. We also used both single- and multipoint linkage procedures using the revised Haseman-Elston model (12) as implemented in the SAGE 4.0 software package. This model has been suggested to be more powerful than their original model in detecting QTLs for moderately heritable traits. In the previous scan, only suggestive linkages were found, whereas in the present scan, several promising linkages for $\dot{V}O_{2\,max}$ and MPO were detected. Differences in the analytical techniques and number of markers (289 vs. 509) utilized might explain the differences between the two reports.

Sedentary state. The $\dot{V}O_{2\,max}$ represents the optimal coordinated performance of the respiratory, cardiovascular, hormonal, and neuromuscular systems under maximal exercise stress. During the present graded exercise protocol, MPO coincided with $\dot{V}O_{2\,max}$ and is presumed to depend primarily on generation of muscle power at maximal oxidative metabolism plus an additional anaerobic energy production by muscles. The correlation between the unadjusted $\dot{V}O_{2\,max}$ and MPO in the sedentary state was 0.94 in both whites and blacks. However, the correlations between the adjusted phenotypes (i.e., those used in the linkage analyses) decrease to 0.78 in blacks and 0.81 in whites. Thus the lack of common linkage signals between these two traits is somewhat surprising, and there is probably no single explanation for it.

Table 4. *Suggestive ($0.01 > P > 0.0023$) linkages with $\dot{V}O_{2max}$ in the sedentary state in blacks adjusted for age, sex, and body mass*

Marker	Chr	Map Position, Mb	Trait	SIBPAL (*P* Value) Multipoint	SIBPAL (*P* Value) Single point
LEPR	1p31	87.8	$\dot{V}O_{2max}$	0.01	0.036
LEPMSAT	7q32	134.3	$\dot{V}O_{2max}$	0.0068	0.054
D7S3070	7q36	162.9	$\dot{V}O_{2max}$	0.0083	0.0046
NOS3	7q36	164.3	$\dot{V}O_{2max}$	0.003	0.20

The most promising sedentary state $\dot{V}O_{2\,max}$ QTL was found in whites on chromosome 11p15 ($P = 0.0014$). The linkage was detected with a sulfonyl urea receptor (SUR) gene marker. This is similar to what was found in a previous genome scan using 289 markers (8), although the linkage in the present scan attained a higher significance level. The SUR gene product is involved in the regulation of insulin secretion. However, we did not observe an association of the SUR marker with $\dot{V}O_{2\,max}$, which suggests that another gene is involved. Close to the SUR locus is the KCNJ11 gene, which forms ATP-sensitive potassium channels together with SUR in pancreatic β-cells and plays a role in the coupling of cell metabolism to membrane potential in heart and skeletal muscle. This QTL also contains the muscle LIM protein (MLP) gene. MLP is a regulator of myogenesis found primarily in the vicinity of the Z disk. It interacts with α-actinin in cardiac and slow-twitch (ST) skeletal muscles (1). Other genes under the linkage peak include the MYOD1 gene, a transcription factor and controller of skeletal muscle differentiation and repair (18), which is expressed preferentially in fast-twitch (FT) muscle (14), and the LDHA gene, which encodes a key enzyme of the glycolytic pathway.

Several MPO QTLs were identified in whites. The most promising for sedentary state MPO was found on 10q23, near the myoferlin gene. Myoferlin is expressed in the plasma and nuclear membranes of cardiac and skeletal muscles, binding calcium and phospholipids (10). Also encoded on 10q23 is the HIF1AN gene, whose product inhibits hypoxia-inducible factor (HIF1A)-mediated transcription of genes, whose proteins

Table 6. *Promising ($P < 0.0023$) and suggestive ($0.01 > P > 0.0023$) linkages with the responsiveness to training of $\dot{V}O_{2max}$ and MPO in blacks adjusted for age, gender, and the baseline value*

Marker	Chr	Map Position, Mb	Trait	SIBPAL (*P* Value) Multipoint	SIBPAL (*P* Value) Single point
LEPR	1p31	87.8	$\dot{V}O_{2max}$	0.0017	0.0013
D1S198	1p31	88.6	$\dot{V}O_{2max}$	0.01	0.30
D1S398	1q22	167.8	MPO	0.0033	0.12
D13S175	13q11	16.2	MPO	0.0055	0.021
RADI	16q22	72.6	$\dot{V}O_{2max}$	0.0041	0.017
D16S2624	16q22	77.7	$\dot{V}O_{2max}$	0.01	0.099
D20S857	20q13.1	59.1	$\dot{V}O_{2max}$	0.0028	0.03
D20839	20q13.1	59.1	$\dot{V}O_{2max}$	0.0031	0.021

either increase erythropoiesis and angiogenesis or glycolytic metabolism. Other genes involved in oxidative phosphorylation, the NADH-ubiquinone oxidoreductase 1β, subcomplex 8 gene, and the COX assembly protein gene, are also encoded under the 10q23 linkage peak.

Recently, a genome scan for loci associated with aerobic running capacity in untrained rats was published (27). The authors identified one region on rat chromosome 16 that is thought to be syntenic with the QTL found on 13q33 in the present study. Human chromosome 13q33 contains, among others, the insulin receptor substrate-2 gene (IRS2). The IRS2 gene product plays a major role in β-cell development and insulin-mediated glucose disposal (32). The $IRS2^{-/-}$ mice exhibit significant insulin resistance in skeletal muscle and liver (33).

In summary, in the sedentary state, human variation in $\dot{V}O_{2\,max}$ is potentially influenced by a locus on chromosome 11p15. On the other hand, loci on 10q23 and 13q33 may contribute to MPO.

Training response. Exercise training increases the capacity to deliver oxygen and substrates to the working muscle. The training protocol employed in the present study, which led to increases in $\dot{V}O_{2\,max}$ and MPO, has been shown to increase stroke volume and cardiac output, as well as the maximal activities of enzymes of the aerobic and anaerobic energy delivery pathways (24, 30). The correlations between the unadjusted $\dot{V}O_{2\,max}$ and MPO training responses were 0.57 and 0.52 for blacks and whites, respectively. These correlations decreased to 0.49 in blacks and 0.43 in whites when computed with the change scores as adjusted for the linkage analyses. This suggests that the two training response phenotypes are substantially different from one another.

The analysis identified several $\dot{V}O_{2\,max}$ training response QTLs. The most promising linkage was found in blacks on 1p31. The QTL on 1p31 was identified with markers in the leptin receptor genes. Among other roles, leptin enhances muscle fatty acid oxidation and glucose uptake, effects that have been suggested to be mediated by activation of AMP-activated protein kinase (AMPK) through the leptin receptor (19, 20). The α2-catalytic subunit of AMPK, abundant in all skeletal muscle types (11) and cardiac muscle (21), is localized on 1p31 near the leptin receptor gene. Mice deficient in muscle α2-AMPK activity show reduced glucose uptake and glycogen content and reduced voluntary wheel running activity (21). Chronic activation of α2-AMPK has been shown to enhance oxidative enzyme expression and mitochondrial biogenesis (2, 31).

The most promising QTL for the training-induced changes in MPO was found in whites on 5q23, which harbors the calcium/calmodulin-dependent protein kinase IV (CaMK4) among others. Exercise activates AMPK (31). Results of experiments on transgenic mice showed that AMPK increased expression of CaMK4 (35), and CaMK4 activated the promoter of the PPAR-γ coactivator-1 gene (PPARGC1) (34). The PPARGC1 gene, under the 4p15 QTL linkage peak, is preferentially expressed in ST fibers, and its activation leads to mitochondrial biogenesis, upregulation of mitochondrial enzymes involved in fatty acid metabolism and electron transport, and reduced susceptibility to fatigue during repetitive contractions (16, 34). PPARGC1 overexpression in transgenic mice results in FT muscle fibers becoming redder, increasing expression of troponin I (slow) and myoglobin, and activating genes of mitochondrial oxidative metabolism (16).

In summary, human variation in $\dot{V}O_{2\,max}$ response to training in blacks is potentially influenced by a locus on chromosome 1p31. On the other hand, a locus on 5q23 may contribute to MPO response to training in whites.

Differences in sample size and marker heterozygosity between blacks and whites may have precluded a higher degree of concordance in QTLs between the two samples. Nonetheless, loci detected in each population harbor genes that are potential candidates for further investigation of human variation in $\dot{V}O_{2\,max}$ and MPO and their responses to endurance training.

In conclusion, multi- and single-point linkage analyses performed on whites and blacks of the HERITAGE Family Study showed promising linkages on chromosomal regions 11p15 and 10q23 with $\dot{V}O_{2\,max}$ and MPO in the sedentary state and on chromosomes 1p31 and 5q23 with their responsiveness to training. These chromosomal regions harbor several candidate genes that should be investigated further.

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