Physiol Genomics 10: 57–62, 2002.
First published June 5, 2002; 10.1152/physiolgenomics.00043.2002.

Genome-wide linkage scan for exercise stroke volume and cardiac output in the HERITAGE Family Study

TUOMO RANKINEN,[1] PING AN,[2] LOUIS PÉRUSSE,[3] TREVA RICE,[2]
YVON C. CHAGNON,[3] JACQUES GAGNON,[4] ARTHUR S. LEON,[5] JAMES S. SKINNER,[6]
JACK H. WILMORE,[7] D. C. RAO,[2,8] AND CLAUDE BOUCHARD[1]
[1]*Pennington Biomedical Research Center, Human Genomics Laboratory, Baton Rouge, Louisiana 70808;* [2]*Division of Biostatistics, Washington University School of Medicine, St. Louis 63110;* [3]*Physical Activity Sciences Laboratory, Laval University, Ste-Foy G1K 7P4;* [4]*Laboratory of Molecular Endocrinology, Laval University, Ste-Foy, Quebec, Canada G1V 4G2;* [5]*School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55455;* [6]*Department of Kinesiology, Indiana University, Bloomington, Indiana 46405;* [7]*Department of Health and Kinesiology, Texas A & M University, College Station, Texas 77843-4243; and* [8]*Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, Missouri 63110-1093*

Received 15 April 2002; accepted in final form 4 June 2002

Rankinen, Tuomo, Ping An, Louis Pérusse, Treva Rice, Yvon C. Chagnon, Jacques Gagnon, Arthur S. Leon, James S. Skinner, Jack H. Wilmore, D. C. Rao, and Claude Bouchard. Genome-wide linkage scan for exercise stroke volume and cardiac output in the HERITAGE Family Study. *Physiol Genomics* 10: 57–62, 2002. First published June 5, 2002; 10.1152/physiolgenomics.00043.2002.—A genome-wide linkage scan was performed for genes affecting submaximal exercise cardiac output (Q) and stroke volume (SV) in the sedentary state and their responses to a standardized 20-wk endurance training program. A total of 509 polymorphic markers were used, and 328 pairs of siblings from 99 white nuclear families and 102 sibling pairs from 105 black family units were available. Q and SV were measured in relative steady state during exercise at 50 W (Q50 and SV50, respectively). Baseline phenotypes were adjusted for age, sex, and body surface area (BSA), and the training responses (post-training − baseline, Δ) were adjusted for age, sex, baseline BSA, and baseline value of the phenotype. Three analytical strategies were used: a multipoint variance components linkage analysis using all the family data, and regression-based single- and multipoint linkage analyses using pairs of siblings. In whites, baseline SV50 and ΔSV50 showed promising linkages ($P < 0.0023$) with markers on chromosomes 14q31.1 and 10p11.2, respectively. Suggestive evidence of linkage ($0.01 > P > 0.0023$) for ΔSV50 and ΔQ50 was detected on chromosome 2q31.1 and for baseline SV50 and Q50 on chromosome 9q32-q33. In blacks, markers on 18q11.2 showed promising linkages with baseline Q50. Suggestive evidence of linkage was found in three regions for baseline SV50 (1p21.3, 3q13.3, 12q13.2) and one for baseline SV50 and Q50 (10p14). All these chromosomal regions include several potential candidate genes and therefore warrant further studies in the HERITAGE cohort and other studies.

genomic scan; exercise training; linkage analysis

THE COMPLETION of the Human Genome Project holds great promise for the development of new insights into biological mechanisms contributing to interindividual differences in responsiveness to acute exercise and exercise training. It has been reported that genetic factors account for a significant proportion of variability in exercise-related phenotypes, such as maximal oxygen uptake ($V_{O_2\,max}$) and exercise blood pressure, both in the sedentary state and in response to endurance training (2, 4–6). Identification of the genes and mutations responsible for these genetic effects would lead to a better understanding of the biology of adaptation to exercise and, ultimately, enable individualized prescription of exercise training for performance enhancement and for prevention and treatment of several public health problems.

Exercise-related phenotypes are typically multifactorial, i.e., they are affected by both environmental and genetic factors. The genetic effect is usually polygenic, i.e., it is determined by a combination of several individual genes, each having a small to moderate effect. Moreover, potential gene-gene and gene-environment interactions further complicate the dissection of the phenotypic variance. Genome-wide linkage scan is a powerful method to identify genomic regions harboring genes that contribute to phenotypic variation. This approach has been used to identify genes for several chronic diseases, e.g., type 2 diabetes (15), and the first genomic scan for $V_{O_2\,max}$ was recently published (8). To fully understand the genetic makeup of multifactorial traits, including exercise-related phenotypes, it is necessary to investigate their intermediate phenotypes, i.e., traits that contribute to physiological pathways regulating the main phenotype of interest. This strategy has been proposed for and utilized in genetic re-

Article published online before print. See web site for date of publication (http://physiolgenomics.physiology.org).

Address for reprint requests and other correspondence: T. Rankinen, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124 (E-mail: rankint@pbrc.edu).

search of several chronic diseases, such as hypertension (17).

Cardiac output (Q) and stroke volume (SV) are central indicators of cardiac function. They also serve as excellent intermediate phenotypes for other exercise-related phenotypes, such as maximal and submaximal oxygen consumption and blood pressure. In the HERITAGE Family Study, maximal heritabilities of 41% and 42% were reported for SV and Q, respectively, measured during steady-state submaximal exercise at 50 W in 99 sedentary white nuclear families. The corresponding heritability estimates for submaximal exercise SV and Q responses to a 20-wk endurance training program were 29% and 38% (1). These observations provide a good justification to start looking for genomic regions and individual genes that are responsible for the genetic effects on exercise Q and SV. The purpose of this study was to perform a genome-wide linkage scan for submaximal exercise Q and SV measured in the sedentary state and also in response to a 20-wk endurance program using the data from the HERITAGE Family Study.

METHODS

Subjects. The study cohort consists of 483 white subjects (233 men and 250 women) from 99 nuclear families and 259 black subjects (88 men and 171 women) from 105 family units. The complete training response data were available for 450 whites (216 men and 234 women) and 251 blacks (88 men and 163 women). The maximum number of sib-pairs available was 328 and 102 in whites and blacks, respectively. The mean age of fathers was 50.9 (range 39.3–65.9) and 53.6 (44.4–64.3) yr, of mothers 47.2 (37.5–64.8) and 52.1 (42.4–65.2) yr, of sons 28.4 (17.0–45.8) and 25.4 (17.0–40.2) yr, and of daughters 25.4 (16.4–48.1), and 25.4 (17.2–40.9) yr in blacks and whites, respectively. The study design and inclusion criteria have been described previously (7). To be eligible, the individuals were required to be in good health, i.e., free of diabetes, cardiovascular diseases or other chronic diseases that would prevent their participation in an exercise training program. Subjects were also required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 mo. Individuals with resting systolic blood pressure greater than 159 mmHg and/or diastolic blood pressure more than 99 mmHg were excluded. 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.

Submaximal exercise cardiac output and stroke volume. Before and after the 20-wk training program, each subject completed three cycle ergometer (SensorMedics Ergo-Metrics 800S, Yorba Linda, CA) exercise tests conducted on separate days: a maximal exercise test (Max), a submaximal exercise test (Submax), and a submaximal/maximal exercise test (Submax/Max) (32). The Submax test was performed at 50 W and at 60% of the initial maximal oxygen consumption ($V_{O_2\,max}$). Subjects exercised for 8–12 min at each work rate, with a 4-min period of seated rest between exercise periods. The Submax/Max test was started with the Submax protocol. After exercising at 60% $V_{O_2\,max}$, subjects also exercised for 3 min at 80% $V_{O_2\,max}$. The test was then progressed to a maximal level of exertion. Heart rate (HR) and Q were determined twice at 50 W (HR50 and Q50, respectively), and the values presented in this paper represent the mean of the responses for the two submaximal tests (i.e., four individual measurements), both before and after training. Q50 was determined using the Collier CO_2 rebreathing technique (11), as described by Wilmore et al. (33). SV at 50 W (SV50) was derived by dividing Q50 by HR50 (measured with ECG) at the time of the Q50 determination (i.e., SV50 = Q50/HR50). Q50 and SV50 training responses (Δ) were calculated as posttraining values minus pretraining values. The reproducibility of measurements was high, with coefficients of variation and intraclass correlation coefficients ranging from 4.4 to 7.6 and 0.76 to 0.93, respectively (35).

Exercise training program. The exercise training program has been described in detail previously (32). Briefly, the exercise intensity of the 20-wk training program was customized for each participant based on the HR-V_{O_2} relationship measured at baseline. During the first 2 wk, the subjects trained at a HR corresponding to 55% of the baseline $V_{O_2\,max}$ for 30 min per session. Duration and intensity of the training sessions were gradually increased to 50 min and 75% of the HR associated with baseline $V_{O_2\,max}$, which were then sustained for the last 6 wk. Training frequency was three times per week and all training was performed on cycle ergometers in the laboratory. HR was monitored during all training sessions by a computerized cycle ergometer system (Universal FitNet System), which adjusted ergometer resistance to maintain the target HR. Trained exercise specialists supervised all exercise sessions.

Data adjustment. Both SV50 and Q50 increase as a function of body size, tend to decrease with aging, and are greater in men than in women. Therefore, baseline Q50 and SV50 were adjusted for the effects of sex, age, and body surface area (BSA) using step-wise multiple regression (25). The pretraining levels of SV50 and Q50 were strong determinants (10–35% of the total variance) of the respective training responses. Therefore, training response phenotypes were adjusted also for baseline value of the phenotype. In summary, Q50 and SV50 phenotypes were regressed on baseline BSA, baseline Q50 or SV50 (for training responses only), and up to a 3rd degree polynomial in age, separately within race-by-sex-by-generation subgroups. Only significant terms (5% level) were retained (i.e., the model did not need to be saturated). The residuals from this regression (or the raw score, if no BSA or age terms were significant) were then standardized to zero mean and unit variance within each subgroup and constituted the analysis variable.

Molecular studies. A total of 509 markers with an average spacing of 6.0 Mb were used. PCR conditions and genotyping methods have been fully outlined previously (10). Automatic DNA sequencers from LI-COR were used to detect the PCR products, and genotypes were scored automatically using the software SAGA. Incompatibilities of Mendelian inheritance were checked, and markers showing incompatibilities were regenotyped completely (<10% were retyped). Microsatellite markers were selected mainly from the Marshfield panel version 8a. The panel of markers included also some candidate genes for relevant HERITAGE phenotypes, including blood pressure. Map locations were taken from the Genetic Location DataBase of Southampton, UK (**http://cedar.genetics.soton.ac.uk**).

Linkage analyses. Linkage analysis was performed using a multipoint variance components model as implemented in SEGPATH (22). Under this model, a phenotype is influenced by the additive effects of a trait locus (g), a residual familial background modeled as a pseudo-polygenic component (G_R), and a residual nonfamilial component (r). The effects of the trait locus and the pseudo-polygenic component on the phe-

notype represent the locus-specific (h^2_g) and residual genetic (h^2_r) heritabilities. Allele sharing probabilities (at each marker location for each sib pair) were used as input data for the SEGPATH linkage model. These multipoint probabilities were derived using the program MAPMAKER/SIBS (18). Other parameters in the model include spouse (u) and additional sibling (b) resemblance, and the mean and variance of the phenotype in the offspring.

The linkage hypothesis is tested by restricting the trait locus heritability to be zero. A likelihood ratio test contrasts the null hypothesis ($h^2_g = 0$) with the alternative (h^2_g estimated). The difference in $-2 \ln L$ (minus twice the log likelihood) between the null and alternate hypotheses is asymptotically distributed as a 50:50 mixture of a χ_1^2 and a point mass at zero, and the P value is one-half of that associated with the χ^2 value with 1 degree of freedom (30). The LOD score is $[\chi^2/(2 \times \log_e 10)]$. The alpha level used here to identify promising results ($P < 0.0023$, corresponding to a LOD score of 1.75) represents, on average, one false-positive per scan for experiments involving ~400 markers (24).

In addition to the SEGPATH analyses, both single-point and multipoint linkage analyses were performed with the sib-pair linkage procedure (12, 14) as implemented in the SIBPAL2 program of the SAGE 4.0 Statistical Package (28). Briefly, if there is a linkage between the marker locus and a putative gene influencing the phenotype, then sibs sharing a greater proportion of alleles identical-by-descent (IBD) at the marker locus also will show a greater resemblance in the phenotype. Phenotypic resemblance of the sibs, modeled as the mean-corrected trait cross-product of the sibs' trait values, is linearly regressed on the estimated proportion of alleles that the sib-pair shares IBD at each marker locus. Both single- and multipoint estimates of allele sharing IBD were generated using the GENIBD program of the SAGE 4.0 package. All analyses were conducted separately for blacks and whites.

RESULTS

The means and standard deviations for cardiac output and stroke volume at 50 W (Q50 and SV50, respectively) and their responses to endurance training are summarized in Table 1. These results have been described and discussed in detail elsewhere (34). In whites, markers on chromosomes 10p11.2 and 14q31.1 showed promising linkages with SV50 training response and baseline SV50, respectively (Table 2). In addition, suggestive evidence for quantitative trait loci (QTL) for both ΔSV50 and ΔQ50 were detected on chromosome 2q31.1. Markers on chromosome 9q32-q33 showed suggestive linkages with baseline SV50 and Q50. These QTLs were detected with all linkage methods. In addition, SEGPATH provided suggestive evidence of linkage for baseline SV50 and Q50 on chromosomes 7q35 and 17p13.1, respectively, and for ΔQ50 in 11q13.1 and 20q13.33. However, SIBPAL2 showed only modest support ($P = 0.01$–0.05) for these QTLs.

In blacks, markers on chromosome 18q11.2 showed promising evidence of linkage for baseline Q50 (Table 3). Three regions with suggestive linkages were detected for baseline SV50 (1p21.3, 3q13.3, 12q13.2), and one suggestive QTL (10p14) was found for baseline Q50 (Table 3). One of the QTLs (10p15-p13) was common for both baseline phenotypes. In addition, SIBPAL2 provided suggestive evidence of linkage for ΔQ50 on chromosome 19q13.43, but SEGPATH provided only modest support ($P = 0.026$).

DISCUSSION

Based on the evidence from quantitative genetic studies, it is reasonable to undertake a search for QTLs and, ultimately genes, affecting submaximal exercise Q and SV both in the sedentary state and in response to exercise training. Identifying these QTLs and resolving them in terms of genes and mutations would benefit not only our understanding of basic human exercise physiology but also would contribute to our

Table 1. *Unadjusted baseline stroke volume and cardiac output and their responses to training*

		Blacks			Whites		
Variable	Group	*n*	Mean	SD	*n*	Mean	SD
			Baseline				
SV50, ml/beat	Fathers	25	104.8	14.8	90	104.6	17.2
	Mothers	51	84.9	13.2	91	84.2	12.9
	Sons	65	113.9	14.3	141	111.3	17.1
	Daughters	131	86.9	14.3	153	86.7	14.8
Q50, l/min	Fathers	25	11.6	1.2	90	10.9	1.6
	Mothers	51	11.4	1.4	91	10.6	1.4
	Sons	65	12.1	1.4	141	11.8	1.5
	Daughters	131	11.5	1.3	153	10.9	1.3
			Training response				
ΔSV50, ml/beat	Fathers	25	+8.5	13.7	85	+2.4	10.6
	Mothers	46	+8.5	9.2	88	+4.1	9.2
	Sons	63	+5.3	11.4	131	+3.2	14.0
	Daughters	117	+3.2	11.0	146	+3.0	10.2
ΔQ50, l/min	Fathers	25	−0.2	1.3	85	−0.7	1.2
	Mothers	46	−0.2	1.0	88	−0.7	0.9
	Sons	63	−0.3	1.2	131	−0.6	1.2
	Daughters	116	−0.7	1.1	146	−0.7	1.0

SV50, stroke volume at 50 W; Q50, cardiac output at 50 W.

Table 2. *Promising ($P < 0.0023$) and suggestive ($P < 0.01$) linkages with submaximal exercise stroke volume and cardiac output phenotypes in the sedentary state and in response to endurance training in whites*

Marker	Chr	Map Position, Mb	Trait	SIBPAL2 (*P* values)		SEGPATH	
				Multipoint	Singlepoint	LOD	*P* value
D2S305	2p23.3	28.889	ΔQ50	0.027	0.062	1.27	0.008
D2S335	2q31.1	180.056	ΔSV50	0.006	0.0043	1.46	0.0047
			ΔQ50	0.0018	0.006	1.63	0.003
D2S1391	2q32.1	193.234	ΔSV50	0.006	0.015	1.01	0.012
LEP	7q31.33	134.321	SV50	0.011	0.004	1.30	0.007
D7S495	7q34	149.829	SV50	0.027	0.136	1.20	0.009
D7S2195	7q35	156.380	SV50	0.028	0.058	1.44	0.005
D9S58	9q32	115.027	Q50	0.0057	0.0064	0.99	0.016
			SV50	0.011	0.029	1.56	0.0037
D9S106	9q32	117.423	SV50	0.011	0.008	1.44	0.005
			Q50	0.005	0.0024	0.74	0.032
D9S930	9q33.1	119.816	Q50	0.0094	0.0066	0.59	0.05
D9S51	9q33.1	121.137	SV50	0.028	0.112	1.37	0.006
D9S155	9q33.1	121.961	SV50	0.021	0.098	1.35	0.006
D9S154	9q33.1	123.013	Q50	0.007	0.0051	0.51	0.63
D9S934	9q33.2	123.662	SV50	0.009	0.025	1.62	0.003
			Q50	0.0026	0.027	0.81	0.026
D9S250	9q33.3	127.945	SV50	0.044	0.246	1.42	0.0053
D10S2325	10p14	14.077	SV50	0.007	0.026	0.54	0.058
D10S197	10p14	20.312	ΔSV50	0.0055	0.021	1.13	0.011
D10S601	10p12.3	21.762	ΔSV50	0.0027	0.00015	1.07	0.013
D10S1732	10p12.3	22.826	ΔSV50	0.0046	0.061	0.99	0.016
D10S1666	10p11.2	35.285	ΔSV50	0.00005	0.00097	1.96	0.0013
D10S1768	10p11.2	38.415	ΔSV50	0.0018	0.176	1.20	0.009
D11S1889	11q13.1	75.347	ΔQ50	0.014	0.058	1.39	0.0056
D14S53	14q31.1	82.701	SV50	0.0019	0.0004	1.73	0.0024
D17S974	17p13.1	12.330	Q50	0.027	0.018	1.35	0.0063
D20S171	20q13.33	67.196	ΔQ50	0.015	0.036	1.35	0.006

All baseline phenotypes are adjusted for age, sex, and baseline body surface area (BSA). Training response (Δ) phenotypes are adjusted also for baseline value of the response phenotype. Chr, chromosome.

understanding of the genetic basis of cardiovascular regulation. The present study is the first attempt to localize such genomic regions, and our results indicate suggestive evidence for QTLs affecting submaximal exercise Q and SV on chromosomes 2q, 10p, and 14q in whites and on chromosomes 1p and 18q in blacks. However, none of the evidence for any QTL was consistent between the two race groups, which may reflect the smaller sample size and lower statistical power to detect linkages in black families.

By definition, a genetic linkage is a property of a chromosomal locus and does not refer to an allele (a specific mutation) in a given gene. Therefore, a genome-wide linkage analysis can be used to identify chromosomal regions, which harbor genes and mutations affecting the phenotype, but a significant linkage result does not indicate that the genetic marker in question is associated with the trait. Identification of a gene responsible for the linkage signal requires further characterization of the QTL region by typing additional microsatellite markers and single nucleotide polymorphisms, using a combination of linkage and association methods, sequencing steps, and other technologies. Furthermore, replication of the QTLs in other populations is desirable to gain further support for the relevance of the chromosomal region for a given phenotype. Unfortunately, similar data on submaximal exercise SV and Q phenotypes from family studies have not been reported yet, and therefore the comparison of our findings with those from other populations is not possible. However, a comparison with linkage scans for resting hemodynamic phenotypes reveals some common chromosomal areas. The submaximal exercise SV and Q training response QTL in chromosome 2q31 in the present study maps to the same region where previous studies have reported QTLs for familial dilated cardiomyopathy (31) and for resting diastolic blood pressure in Old Order Amish (16) and in a geographically defined subgroup of Finnish dizygotic twins (21). The linkage with baseline SV50 and Q50 in chromosome 10p14 coincides with a QTL for arrhythmogenic right-ventricular dysplasia (20) and a linkage region for resting systolic blood pressure in the Quebec Family Study (26). Finally, the linkages between baseline SV50 and markers on chromosomes 1p21.3 and 12q13 in blacks map close to QTLs reported for autosomal recessive polymorphic ventricular tachycardia induced by catecholamines or vigorous exercise in Bedouin families (19) and for left ventricular contractility in whites of the HyperGEN study (3), respectively. Interestingly, the QTLs for SV50 and Q50 are localized on chromosomal regions different from those for submaximal exercise blood pressure (23) and maximal oxygen uptake (8), suggesting that these physiologically related traits may not share a common genetic background.

The constantly improving sequence map of the human genome provides us with an opportunity to iden-

Table 3. *Promising ($P < 0.0023$) and suggestive ($P < 0.01$) linkages with submaximal exercise stroke volume and cardiac output phenotypes in the sedentary state and in response to endurance training in blacks*

Marker	Chr	Map Position, Mb	Trait	SIBPAL2 (*P* values)		SEGPATH	
				Multi-point	Single-point	LOD	*P* value
D1S1588	1p21.3	102.149	SV50	0.0033	0.012	1.42	0.005
D1S1631	1p21.23	106.988	SV50	0.0038	0.0076	1.42	0.005
D1S404	1q44	260.870	Q50	0.0089	0.0072	0.72	0.034
D1S2842	1q44	261.449	Q50	0.0064	0.0063	0.79	0.029
D1S2682	1q44	262.355	Q50	0.0027	0.144	0.94	0.019
D2S1384	2q33.2	212.118	Q50	0.0078	0.016	0.90	0.021
D3S3045	3q13.31	129.480	SV50	0.0078	0.021	1.25	0.008
D3S3045	3q13.31	129.480	ΔQ50	0.008	0.014	1.00	0.016
D10S585	10p14	13.301	Q50	0.056	0.186	1.30	0.007
D10S2325	10p14	14.077	SV50	0.022	0.019	1.25	0.0082
D10S2325	10p14	14.077	Q50	0.009	0.012	1.48	0.0045
D10S1725	10p13	16.097	Q50	0.009	0.101	1.15	0.011
D12S398	12q13.13	62.030	SV50	0.018	0.052	1.38	0.006
D12S359	12q13.13	62.172	SV50	0.030	0.089	1.20	0.009
D12S1604	12q13.13	62.230	SV50	0.039	0.0042	1.26	0.008
D12S1724	12q13.2	62.671	SV50	0.010	0.0054	1.55	0.0038
D12S90	12q14.2	71.552	SV50	0.040	0.040	1.40	0.0056
D18S542	18p11.21	13.690	Q50	0.0087	0.058	1.11	0.012
D18S480	18q11.1	22.965	Q50	0.012	0.015	1.23	0.0086
D18S1107	18q11.2	23.468	Q50	0.007	0.006	1.28	0.0076
D18S866	18q11.2	24.674	Q50	0.0015	0.110	1.79	0.002
D18S819	18q11.2	27.043	Q50	0.0022	0.019	1.57	0.0036
D18S1151	18q12.1	31.423	Q50	0.0077	0.155	1.26	0.008
D18S56	18q12.3	41.443	Q50	0.009	0.0076	1.01	0.016
D19S254	19q13.43	63.697	ΔQ50	0.0048	0.010	0.82	0.026

All baseline phenotypes are adjusted for age, sex, and baseline BSA. Training response (Δ) phenotypes are adjusted also for baseline value of the response phenotype.

tify the strongest candidate genes for the linkage region before pursuing positional cloning efforts. For example, in the present study, the marker D10S1666 on chromosome 10p11.2 showed promising linkage with SV50 training response. Within 1.0 Mb of this marker is located a gene encoding integrin-β1 (ITGB1). Integrins are transmembrane receptors composed of α- and β-subunits, and they serve as an important link between extracellular matrix and intracellular structures and functions. In the heart, one role of the integrins is to function as mechanotransducers during normal development and in response to several physiological signals (27). Considering that integrin-β1 is the major β-subunit isoform expressed in cardiac myocytes, the ITGB1 would be a good candidate gene for exercise training-induced changes in cardiac phenotypes, such as stroke volume.

The marker D2S335 on chromosome 2q31 showed suggestive linkages with both Q50 and SV50 training responses. Interestingly, a gene encoding titin (TTN) is located in the same region with this marker. Titin is a structural protein in striated muscle cells and is a major determinant of elastic properties of muscle fibers. Consequently, titin seems to be a major contributor to the diastolic force of myocardium, and differential expression of titin isoforms has been suggested to contribute to the elastic diversity of atrial and ventricular myofibrils (9, 13). Furthermore, a mutation in the titin gene has been proposed to contribute to some forms of familial hypertrophic cardiomyopathies (29). Finally, marker D14S53, which showed the strongest evidence for linkage with baseline SV50 in whites, maps in intron 4 of the estrogen-related receptor-β (ESRRB) gene. The ESRRB is an orphan nuclear receptor that is homologous to the estrogen receptors, but is not activated by natural estrogens. Another interesting candidate on this region is the transforming growth factor-β3 (TGFB3) gene, which is located within 500 kb of the D14S53. Considering the growth-promoting properties of transforming growth factor-β, and that the expression of TGFB3 is increased in cardiac hypertrophy, TGFB3 is also a good candidate gene for this QTL. Thus all the QTLs identified in the current study harbor several potential candidate genes. Naturally, these hypotheses must be tested in future studies using positional cloning and fine mapping techniques.

In summary, these data from the HERITAGE Family Study provide evidence for several genomic regions that contain genes potentially affecting submaximal exercise Q and SV in the sedentary state and in response to endurance training in blacks and whites. These genomic regions should be explored further to identify the genes and characterize the mutations that contribute to observed interindividual variation in exercise Q and SV phenotypes.

The HERITAGE Family Study is supported by National Heart, Lung, and Blood Institute Grants HL-45670 (to C. Bouchard), HL-47323 (to A. S. Leon), HL-47317 (to D. C. Rao), HL-47327 (to J. S. Skinner), and HL-47321 (to J. H. Wilmore). A. S. Leon is partially supported by the Henry L. Taylor endowed Professorship in Exercise Science and Health Enhancement. C. Bouchard is partially supported by the George A. Bray Chair in Nutrition.

Some of the results of this paper were obtained by using the program package SAGE, which is supported by National Institutes of Health Research Resource Grant 1-P41-RR-03655.

REFERENCES

1. **An P, Rice T, Gagnon J, Leon AS, Skinner JS, Bouchard C, Rao DC, and Wilmore JH.** Familial aggregation of stroke volume and cardiac output during submaximal exercise: the HERITAGE Family Study. *Int J Sports Med* 21: 566–572, 2000.
2. **An P, Rice T, Perusse L, Borecki I, Gagnon J, Leon A, Skinner J, Wilmore J, Bouchard C, and Rao D.** Complex segregation analysis of blood pressure and heart rate measured before and after a 20-week endurance exercise training program: the HERITAGE Family Study. *Am J Hypertens* 13: 488–497, 2000.
3. **Arnett DK, Devereux RB, Kitzman D, Oberman A, Hopkins P, Atwood L, Dewan A, and Rao DC.** Linkage of left ventricular contractility to chromosome 11 in humans: the HyperGEN Study. *Hypertension* 38: 767–772, 2001.
4. **Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, and Rao DC.** Familial aggregation of $\dot{V}O_{2max}$ response to exercise training: results from the HERITAGE Family Study. *J Appl Physiol* 87: 1003–1008, 1999.
5. **Bouchard C, Daw EW, Rice T, Perusse L, Gagnon J, Province MA, Leon AS, Rao DC, Skinner JS, and Wilmore JH.** Familial resemblance for $VO_{2\ max}$ in the sedentary state: the HERITAGE family study. *Med Sci Sports Exerc* 30: 252–258, 1998.
6. **Bouchard C, Dionne FT, Simoneau JA, and Boulay MR.** Genetics of aerobic and anaerobic performances. *Exerc Sport Sci Rev* 20: 27–58, 1992.
7. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, and Gagnon J.** The HERITAGE family study. Aims, design, and measurement protocol. *Med Sci Sports Exerc* 27: 721–729, 1995.
8. **Bouchard C, Rankinen T, Chagnon YC, Rice T, Perusse L, Gagnon J, Borecki I, An P, Leon AS, Skinner JS, Wilmore JH, Province M, and Rao DC.** Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE Family Study. *J Appl Physiol* 88: 551–559, 2000.
9. **Cazorla O, Freiburg A, Helmes M, Centner T, McNabb M, Wu Y, Trombitas K, Labeit S, and Granzier H.** Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circ Res* 86: 59–67, 2000.
10. **Chagnon YC, Borecki IB, Perusse L, Roy S, Lacaille M, Chagnon M, Ho-Kim MA, Rice T, Province MA, Rao DC, and Bouchard C.** Genome-wide search for genes related to the fat-free body mass in the Quebec family study. *Metabolism* 49: 203–207, 2000.
11. **Collier CR.** Determination of mixed venous CO_2 tensions by rebreathing. *J Appl Physiol* 9: 25–29, 1956.
12. **Elston RC, Buxbaum S, Jacobs KB, and Olson JM.** Haseman and Elston revisited. *Genet Epidemiol* 19: 1–17, 2000.
13. **Freiburg A, Trombitas K, Hell W, Cazorla O, Fougerousse F, Centner T, Kolmerer B, Witt C, Beckmann JS, Gregorio CC, Granzier H, and Labeit S.** Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity. *Circ Res* 86: 1114–1121, 2000.
14. **Haseman JK and Elston RC.** The investigation of linkage between a quantitative trait and a marker locus. *Behav Genet* 2: 3–19, 1972.
15. **Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, and Bell GI.** Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26: 163–175, 2000.
16. **Hsueh WC, Mitchell BD, Schneider JL, Wagner MJ, Bell CJ, Nanthakumar E, and Shuldiner AR.** QTL influencing blood pressure maps to the region of PPH1 on chromosome 2q31–34 in Old Order Amish. *Circulation* 101: 2810–2816, 2000.
17. **Kotchen TA, Kotchen JM, Grim CE, George V, Kaldunski ML, Cowley AW, Hamet P, and Chelius TH.** Genetic determinants of hypertension: identification of candidate phenotypes. *Hypertension* 36: 7–13, 2000.
18. **Kruglyak L and Lander ES.** Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57: 439–454, 1995.
19. **Lahat H, Eldar M, Levy-Nissenbaum E, Bahan T, Friedman E, Khoury A, Lorber A, Kastner DL, Goldman B, and Pras E.** Autosomal recessive catecholamine- or exercise-induced polymorphic ventricular tachycardia: clinical features and assignment of the disease gene to chromosome 1p13–21. *Circulation* 103: 2822–2827, 2001.
20. **Li D, Ahmad F, Gardner MJ, Weilbaecher D, Hill R, Karibe A, Gonzalez O, Tapscott T, Sharratt GP, Bachinski LL, and Roberts R.** The locus of a novel gene responsible for arrhythmogenic right-ventricular dysplasia characterized by early onset and high penetrance maps to chromosome 10p12-p14. *Am J Hum Genet* 66: 148–156, 2000.
21. **Perola M, Kainulainen K, Pajukanta P, Terwilliger JD, Hiekkalinna T, Ellonen P, Kaprio J, Koskenvuo M, Kontula K, and Peltonen L.** Genome-wide scan of predisposing loci for increased diastolic blood pressure in Finnish siblings. *J Hypertens* 18: 1579–1585, 2000.
22. **Province MA, Rice T, Borecki IB, Gu C, and Rao DC.** A multivariate and multilocus variance components approach using structural relationships to assess quantitative trait linkage via SEGPATH. *Genet Epidemiol* In press.
23. **Rankinen T, An P, Rice T, Sun G, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, and Bouchard C.** Genomic scan for exercise blood pressure in the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. *Hypertension* 38: 30–37, 2001.
24. **Rao DC and Province MA.** The future of path analysis, segregation analysis, and combined models for genetic dissection of complex traits. *Hum Hered* 50: 34–42, 2000.
25. **Rice T, Borecki IB, Bouchard C, and Rao DC.** Commingling analysis of regional fat distribution measures: the Quebec family study. *Int J Obes Relat Metab Disord* 16: 831–844, 1992.
26. **Rice T, Rankinen T, Province MA, Chagnon YC, Perusse L, Borecki IB, Bouchard C, and Rao DC.** Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. *Circulation* 102: 1956–1963, 2000.
27. **Ross RS and Borg TK.** Integrins and the myocardium. *Circ Res* 88: 1112–1119, 2001.
28. **SAGE.** *Statistical Analysis for Genetic Epidemiology* (computer program package). Cleveland, OH: Dept. of Epidemiology and Biostatistics, Case Western Reserve University, 2001.
29. **Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, and Kimura A.** Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. *Biochem Biophys Res Commun* 262: 411–417, 1999.
30. **Self GA and Liang KY.** Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J Am Stat Assoc* 82: 605–610, 1987.
31. **Siu BL, Niimura H, Osborne JA, Fatkin D, MacRae C, Solomon S, Benson DW, Seidman JG, and Seidman CE.** Familial dilated cardiomyopathy locus maps to chromosome 2q31. *Circulation* 99: 1022–1026, 1999.
32. **Skinner JS, Wilmore KM, Krasnoff JB, Jaskolski A, Jaskolska A, Gagnon J, Province MA, Leon AS, Rao DC, Wilmore JH, and Bouchard C.** Adaptation to a standardized training program and changes in fitness in a large, heterogeneous population: the HERITAGE Family Study. *Med Sci Sports Exerc* 32: 157–161, 2000.
33. **Wilmore JH, Farrell PA, Norton AC, Cote RW III, Coyle EF, Ewy GA, Temkin LP, and Billing JE.** An automated, indirect assessment of cardiac output during rest and exercise. *J Appl Physiol* 52: 1493–1497, 1982.
34. **Wilmore JH, Stanforth PR, Gagnon J, Rice T, Mandel S, Leon AS, Rao DC, Skinner JS, and Bouchard C.** Cardiac output and stroke volume changes with endurance training: the HERITAGE Family Study. *Med Sci Sports Exerc* 33: 99–106, 2001.
35. **Wilmore JH, Stanforth PR, Turley KR, Gagnon J, Daw EW, Leon AS, Rao DC, Skinner JS, and Bouchard C.** Reproducibility of cardiovascular, respiratory, and metabolic responses to submaximal exercise: the HERITAGE Family Study. *Med Sci Sports Exerc* 30: 259–265, 1998.