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 BOUCHARD1

1Pennington Biomedical Research Center, Human Genomics Laboratory, Baton Rouge, Louisiana 70808; 2Division of Biostatistics, Washington University School of Medicine, St. Louis 63110; 3Physical Activity Sciences Laboratory, Laval University, Ste-Foy G1K 7P4; 4Laboratory of Molecular Endocrinology, Laval University, Ste-Foy, Quebec, Canada G1V 4G2; 5School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55455; 6Department of Kinesiology, Indiana University, Bloomington, Indiana 46405; 7Department of Health and Kinesiology, Texas A & M University, College Station, Texas 77843-4243; and 8Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, Missouri 63110-1093

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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 (VO2 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 VO2 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-
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, maximalheritabilities 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 correspondingheritability 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 (VO2max). 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% VO2max, subjects also exercised for 3 min at 80% VO2max. 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 CO2 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-VO2 relationship measured at baseline. During the first 2 wk, the subjects trained at a HR corresponding to 55% of the baseline VO2 max for 30 min per session. Duration and intensity of the training sessions were gradually increased to 50 min and 75% of the HR corresponding with baseline VO2 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 (Gp), and a residual nonfamilial component (ε). The effects of the trait locus and the pseudo-polygenic component on the phe-
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 for linkage of 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 understanding of the effects of exercise training on human cardiovascular function.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Blacks</th>
<th></th>
<th></th>
<th>Whites</th>
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<tr>
<td></td>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
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<td>SV50, ml/beat</td>
<td>Fathers</td>
<td>25</td>
<td>104.8</td>
<td>14.8</td>
<td>90</td>
<td>104.6</td>
<td>17.2</td>
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<tr>
<td></td>
<td>Mothers</td>
<td>51</td>
<td>84.9</td>
<td>13.2</td>
<td>91</td>
<td>84.2</td>
<td>12.9</td>
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<tr>
<td></td>
<td>Sons</td>
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<td>113.9</td>
<td>14.3</td>
<td>141</td>
<td>111.3</td>
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<tr>
<td></td>
<td>Daughters</td>
<td>131</td>
<td>86.9</td>
<td>14.3</td>
<td>153</td>
<td>86.7</td>
<td>14.8</td>
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<tr>
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<td>25</td>
<td>11.6</td>
<td>1.2</td>
<td>90</td>
<td>10.9</td>
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<td>Mothers</td>
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<td>1.4</td>
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<td>Daughters</td>
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<td>11.5</td>
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<td>ΔSV50, ml/beat</td>
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<td>25</td>
<td>+8.5</td>
<td>13.7</td>
<td>85</td>
<td>+2.4</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Sons</td>
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<td>11.4</td>
<td>131</td>
<td>+3.2</td>
<td>14.0</td>
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<tr>
<td></td>
<td>Daughters</td>
<td>117</td>
<td>+3.2</td>
<td>11.0</td>
<td>146</td>
<td>+3.0</td>
<td>10.2</td>
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<tr>
<td>ΔQ50, l/min</td>
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<td>-0.2</td>
<td>1.3</td>
<td>85</td>
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<tr>
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<td>Mothers</td>
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<td>1.0</td>
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<td>116</td>
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<td>1.1</td>
<td>146</td>
<td>-0.7</td>
<td>1.0</td>
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</table>

SV50, stroke volume at 50 W; Q50, cardiac output at 50 W.
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 genetically defined subgroup of Finnish dYZygotic 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-
and cardiac output phenotypes in the sedentary state and in response to endurance training in blacks.

Considering that integrin-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 (ESRBB) gene. The ESRBB 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.

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REFERENCES


