Major gene effects on exercise ventilatory threshold: the HERITAGE Family Study

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1Division of Biostatistics, and 4Departments of Genetics and Psychiatry, Washington University School of Medicine, Saint Louis, Missouri 63110; 2Department of Health and Human Performance, Human Performance Laboratory, University of Montana, Missoula, Montana 59812; 3Pennington Biomedical Research Center, Baton Rouge, Louisiana 70808; 5Department of Health and Kinesiology, Texas A&M University, College Station, Texas 77843; 6Department of Kinesiology, Indiana University, Bloomington, Indiana 46405; 7School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55455

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Feitosa, Mary F., Steven E. Gaskill, Treva Rice, Tuomo Rankinen, Claude Bouchard, D. C. Rao, Jack H. Wilmore, James S. Skinner, and Arthur S. Leon. Major gene effects on exercise ventilatory threshold: the HERITAGE Family Study. J Appl Physiol 93: 1000–1006, 2002.—This study investigates whether there are major gene effects on oxygen uptake at the ventilatory threshold (VO2VT) and the VO2max (V̇O2 max) at baseline and in response to 20 wk of exercise training by using data on 336 whites and 160 blacks. Segregation analysis was performed on the residuals of VO2VT and VT%V̇O2 max. In whites, there was strong evidence of a major gene, with 3 and 2% of the sample in the upper distribution, that accounted for 52 and 43% of the variance in baseline VO2VT and VT%V̇O2 max, respectively. There were no genotype-specific covariate effects (sex, age, weight, fat mass, and fat-free mass). The segregation results were inconclusive for the training response in whites, and for the baseline and training response in blacks, probably due to insufficient power because of reduced sample sizes or smaller gene effect or both. The strength of the genetic evidence for VO2VT and VT%V̇O2 max suggests that these traits should be further investigated for potential relations with specific candidate genes, if they can be identified, and explored through a genome-wide scan.

segregation analysis; heritability; familial aggregation; oxygen uptake at ventilatory threshold; maximal oxygen uptake

LOW CARDIORESPIRATORY FITNESS and low levels of physical activity have been associated with a higher risk of death, mainly due to cardiovascular disease (7, 18, 26–28, 34) but also, to some extent, to various cancers (7). In fact, low cardiorespiratory fitness is as strong a predictor of mortality as other conventional risk factors like hypercholesterolemia, cigarette smoking, and hypertension (6, 32, 40). Cardiorespiratory fitness can best be measured by maximal oxygen uptake (VO2 max, ml/min and ml·kg−1·min−1). However, most daily activities are executed at submaximal exercise intensities. Ventilatory threshold (VT) is a point reached during progressively increasing workload at which carbon dioxide output (V̇CO2) begins to increase more rapidly than oxygen uptake (VO2). It is also characterized by an increase in rates of the pulmonary ventilation (V̇E)-to-V̇O2 ratio without concurrent increases in the V̇E-to-V̇CO2 ratio (V̇E/V̇CO2), VT, which generally correlates well with lactate threshold (16, 43), is the result of complex interactions between oxygen transport and utilization, muscle fiber type and enzyme levels, and substrate availability and other complex physiological processes (4, 15, 24). In addition to the physiological processes, VT has also been shown to be an indicator of the sustainable aerobic exercise intensity and a marker of the capacity to sustain prolonged aerobic physical activity (21, 38). VO2 at VT (VO2VT) relative to VO2max (VT%VO2 max) indicates the percentage of maximal aerobic power utilized while performing work at VT.

Considerable interindividual differences in the trainability of cardiorespiratory endurance traits have been observed after exposure to identical training programs (8, 30, 36). These differences are described as a normal biological phenomenon largely reflecting genetic diversity (8, 13). VO2 max and submaximal VO2 are complex traits that are influenced by several genetic and environmental factors, as demonstrated by twin and familial studies. Some twin investigations have shown that monozygotic pairs are more alike than dizygotic pairs for VO2max (12, 19, 25, 31), with heritability estimates ranging from 25 to 66%. Moreover, familial aggregation has been demonstrated for maxi-
mal (10, 9, 30) and submaximal (20, 33) aerobic performances, both in a sedentary state and in response to exercise training. In the HERITAGE Family Study, a previous investigation suggested heritabilities of 58 and 54% for baseline \( \dot{V}O_2 \) and 22 and 51% for the training response in white and black families, respectively (20). Despite these suggestions of genetic factors acting on the familial resemblance of submaximal \( \dot{V}O_2 \) as well as \( \dot{V}O_2 \max \), a major gene hypothesis has never been investigated. Thus, the aim of this study was to determine whether \( \dot{V}O_2 \) and \( \dot{V}O_2 \max \) are influenced by major genes with/without genotype-specific effects of covariates by using complex segregation analysis.

**METHODS**

**Sample.** The HERITAGE Family Study is a large multicenter investigation of the role of genetic factors on cardiovascular and diabetes risk-factor responses to endurance exercise training. The specific aims, design, and measurements of the study have been described elsewhere (11).

The present report is limited to only subjects from whom valid VT data were available and is based on a total of 336 white and 160 black subjects from 99 and 111 families, respectively. Several criteria were used to select the subjects for participation. In brief, family units were recruited at four clinical centers and were required to be sedentary at baseline, which was defined as not having engaged in regular vigorous physical activity over the previous 6 mo. Subjects were required to be between 17 and 65 yr of age, in good health, and with a body mass index of <40 kg/m\(^2\), unless certified by a physician that the subject was capable of undertaking the testing and training program. Subjects with a blood pressure >159 mmHg for systolic and/or >99 mmHg for diastolic or were on lipid, diabetic, or hypertensive medications were excluded. The study was approved by each of the Institutional Review Boards, and written, informed consent was obtained from each subject.

**Endurance training program.** Subjects trained under supervision on a cycle ergometer three times a week for 20 wk by using the same standardized training protocol at each of the four clinical centers (37). The intensity and duration of the training program was adjusted every 2 wk, beginning at a heart rate (HR) corresponding to 55% of their baseline \( \dot{V}O_2 \max \) for 30 min/session and increasing gradually to a training HR associated with 75% of an individual’s \( \dot{V}O_2 \max \) for 50 min during the last 6 wk. The power output of the cycle ergometer was adjusted automatically to provide the appropriate HR response during all training sessions by a built-in computer program.

**Measurement.** Two maximal exercise tests were conducted at baseline (i.e., sedentary state), separated by at least 48 h, and two were conducted after 20 wk of training on SensorMedics ErgoMetrics 800S cycle ergometers (Yorba Linda, CA). HR was monitored by an electrocardiogram. Gas exchange, which included \( \dot{V}O_2 \), \( \dot{V}CO_2 \), \( \dot{V}e \), and respiratory exchange ratio, were obtained by using a SensorMedics 2900 metabolic measurement cart throughout each exercise test as a rolling average of the last three 20-s intervals of each exercise stage. The criteria for \( \dot{V}O_2 \max \) were a respiratory exchange ratio of >1.1, a plateau in \( \dot{V}O_2 \) (changes of <100ml/ min in the last three consecutive 20-s averages), and an HR within 10 beats/min of the maximal level predicted by age. All subjects reached \( \dot{V}O_2 \max \) by one of these criteria in a least one of the two tests. VT was concurrently determined by three validated methods: 1) ventilatory equivalent method (35); 2) excess carbon dioxide method (3, 39); and 3) modified V-slope method using 20-s averaged data (5). Visual evaluation to determine VT was carried out independently by two experienced investigators using these three methods. Additionally, a computer algorithm was developed to establish VT from the V-slope method (21). Details of these measurements and exercise procedures have been described elsewhere (10, 21, 33, 37, 42). Fat mass (FM) and fat-free mass (FFM) were measured by underwater weighing (41).

**Data adjustments.** Baseline \( \dot{V}O_2 \max \) (ml/min) was transformed by using natural logarithm to correct for nonnormality. All adjustments before genetic analysis were carried out separately in each of eight sex-by-generation-by-race groups by using stepwise multiple regression analysis and retaining terms that were significant at the 5% level. Baseline \( \dot{V}O_2 \max \) was adjusted for the effects of a polynomial in age (age, age\(^2\), age\(^3\)) and weight (kg), as well as for these covariates, FM, and FFM. The training response of \( \dot{V}O_2 \max \) (posttraining minus baseline) was adjusted for the effects of a polynomial in age, weight, and baseline \( \dot{V}O_2 \max \) values, whereas VT%\( \dot{V}O_2 \max \) (ml/min) was adjusted for a polynomial in age. The adjusted phenotypes were finally standardized to a mean of 0 and a standard deviation of 1.

**Segregation model.** Segregation analysis was performed using the Pedigree Analysis Package, version 4.0 (23). This is a mixed model, in which each phenotype is assumed to be influenced by the independent and additive contributions from a major gene locus, a polygenic/multifactorial background, and a nontransmitted environmental residual component. The major gene effect results from segregation at a single locus having two alleles (\( A, a \)), for which the upper-case allele is associated with lower values and the allele frequency is noted by \( p \). The other parameters in the model are 1) the mean values for the three genotypes (\( \mu_{AA}, \mu_{Aa}, \mu_{aa} \)), where the order of the means are constrained to be \( \mu_{AA} \leq \mu_{Aa} \leq \mu_{aa} \); 2) the common standard deviation within major locus genotypes; 3) the multifactorial component (\( H \)) representing the proportion of the residual familial variance (after adjusting for the major gene effect) that is attributable to polygenes and/or cultural inheritance; and 4) parent-to-offspring transmission probabilities for the three genotypes (\( \tau_{AA}, \tau_{Aa}, \tau_{aa} \)). For a single diallelic locus, the three \( \tau \) genotypes denote the probabilities of transmitting allele \( A \) for the genotypes \( AA, Aa, \) and \( aa \), with Mendelian expectations of 1, 1/2, and 0, respectively; while under an environmental (nontransmitted) model, \( p = \tau_{AA} = \tau_{Aa} = \tau_{aa} \). Recessive (\( \mu_{AA} = \mu_{aa} \)) and dominant (\( \mu_{Aa} = \mu_{aa} \)) modes of transmission were tested. In addition, complete segregation analyses with genotype-dependent covariate effects (\( \beta_{AA}, \beta_{Aa}, \beta_{aa} \)) were also carried out, in which sex, age, weight, FM, and FFM covariates were modeled separately. All analyses were conducted by using maximum likelihood methods, and the most parsimonious models were determined by using likelihood ratio tests and Akaike’s Information Criterion (AIC), which is computed as minus twice the log likelihood of the model plus twice the number of estimated parameters (1). The model with the lowest AIC indicates the best fit to the observed data.

**RESULTS**

Descriptive data for \( \dot{V}O_2 \max \) and VT%\( \dot{V}O_2 \max \) have already been reported by Gaskill et al. (20). In summary, there were significant sex and generation mean
differences for most of these phenotypes in both races and between race groups.

Weight and age together accounted for 29, 14, 9, and 15% of the VO2\textsubscript{VT} phenotypic variability in white fathers, mothers, sons, and daughters, respectively. When FM and FFM were also included in the adjustment of VO2\textsubscript{VT}, only FFM entered as a significant covariate in white families, accounting for 33, 25, 15, and 24% of the phenotypic variability in fathers, mothers, sons, and daughters, respectively. In black families, weight (77%), age and FFM (54%), and FFM (38%) accounted for the phenotypic variability in mothers, sons, and daughters, respectively. For the training response of VO2\textsubscript{VT}, baseline VO2\textsubscript{VT}, weight, and age together accounted for 8, 20, 14, and 21% of the phenotypic variability in fathers, mothers, sons, and daughters, respectively. For the VT\%VO2\textsubscript{max}, age accounted for 13, 29, and 3% of the phenotypic variability in white fathers, mothers, and sons, respectively, and between race groups.

Results of segregation analysis for baseline VO2\textsubscript{VT} [ml/min] (Table 1).
In blacks, inconclusive results were generally obtained, probably due to the small sample sizes. However, a few reduced models were derived, in particular one in which there was only a multifactorial effect (model 1), another with only a major effect (model 2), and a third sporadic model for no familial resemblance (model 3). For \( \dot{V}O_2 \text{VT} \), similar AIC values were obtained across all three of these hypotheses (model 1: AIC = 458.27; model 2: AIC = 458.92; model 3: AIC = 459.10), and the hypothesis estimating only the multifactorial component (\( H = 0.362 \pm 0.219 \)) was the parsimonious hypothesis. Similar results were found for VT%\( \dot{V}O_2 \text{max} \) in blacks. In addition, the same problems were obtained for the training responses for both phenotypes in both races, and only a few reduced models could be estimated. For \( \dot{V}O_2 \text{VT} \) response in white families, the major gene model (AIC = 895.19) was more parsimonious than one for only a multifactorial effect (AIC = 895.24) or the sporadic model (AIC = 895.97). This major gene accounted for 28% of the phenotypic variance. Similar results were obtained for training response \( \dot{V}O_2 \text{VT} \) in black families with the major gene accounting for 35% of the phenotypic variance. However, we emphasize that the baseline results in black families and the training responses in both races are inconclusive since we could not estimate the full model and thus provide a test of the significance of the effects. This is probably due to insufficient power because of reduced sample sizes, smaller gene effects, or both.

**DISCUSSION**

Previous studies have reported evidence for a genetic influence on \( \dot{V}O_2 \text{max} \) and submaximal \( \dot{V}O_2 \) on the basis of twin (2, 9, 10, 12, 19, 25, 31) and family data (20, 30, 33). Important contributions regarding the genetic/familial influences on cardiorespiratory fitness have been provided by the HERITAGE Family Study, in which significant familial resemblance was reported for both maximal and submaximal aerobic performances. Heritabilities reached 50 (10) and 47% (9) for \( \dot{V}O_2 \text{max} \) in the sedentary state and for its response to training, respectively. At a 50 W submaximal workload, heritabilities reached 41 and 42% for baseline stroke volume and cardiac output, respectively, and were 29 and 38% for their respective responses to training (2). The heritabilities for submaximal \( \dot{V}O_2 \) at three power outputs ranged from 48 to 74% and from 23 to 57% at baseline and for their responses to training, respectively (33). Moreover, recently Gaskill et al. (20) reported familial aggregation with heritabilities of 58 and 54% for \( \dot{V}O_2 \text{VT} \) and 22 and 51% for their responses to regular exercise in white and black families, respectively. Whereas these familial aggregation studies indicate whether there are familial genetic and/or environmental effects, segregation analysis as used in the current study can suggest the presence of major genes (genes with large effects). To the best of our knowledge, major gene evidence for VT phenotypes has never been reported.
The segregation results of the current study showed strong evidence of a major gene in white families. About 3 \( (\overline{V}O_{2\text{VT}}) \) and 2% \( (\text{VT}\%\overline{V}O_{2\text{max}}) \) of the individuals were in the upper distribution, i.e., higher oxygen intake, and the putative major genes accounted for 52 and 43% of the variance, respectively, in white families. As shown in Fig. 1, comparisons of distributions with the curves representing the major gene effect seem to fit the observed data very well. Further adjustment of \( V_{O2VT} \) data for FM and FFM did not modify the results. Moreover, genotype-specific covariate effects were not significant, i.e., the effect of the major gene did not depend on any of the covariates considered, namely sex, age, weight, FM, and FFM.

Our results for \( V_{O2VT} \) and \( \text{VT}\%\overline{V}O_{2\text{max}} \) phenotypes in the sedentary state were consistent with those reported earlier (20) that used a different methodology. That is, both complex segregation analysis and familial correlation analysis suggested 1) significant familial aggregation, 2) approximately similar familial variances for \( V_{O2VT} \) (52 and 58%) and \( \text{VT}\%\overline{V}O_{2\text{max}} \) (43 and 38%), and 3) familial factors accounting for more variance in \( V_{O2VT} \) than in \( \text{VT}\%\overline{V}O_{2\text{max}} \). The somewhat smaller familial component for \( \text{VT}\%\overline{V}O_{2\text{max}} \) could reflect the relative independence of \( V_{O2VT} \) from \( \text{VT}\%\overline{V}O_{2\text{max}} \). For instance, both \( V_{O2max} \) and \( V_{O2VT} \) decrease during the aging process. However, \( \text{VT}\%\overline{V}O_{2\text{max}} \) increases with age as a result of the more rapid decrease in \( V_{O2max} \) than in \( V_{O2VT} \) (which is relatively stable after 30 yr), and consequently individuals work closer to their maximal aerobic power while performing sustained work at their \( V_{O2VT} \). As \( V_{O2VT} \) approaches \( \overline{V}O_{2\text{max}} \), the reserve capacity diminishes until individuals no longer have the ability to exceed \( V_{O2VT} \) (Gaskell SE, personal communication).

On the other hand, despite the presence of significant familial effects on baseline \( V_{O2VT} \) in black families reported by Gaskell et al. (20) in these same HERITAGE families, the present segregation results were inconclusive with respect to major gene effects. Taking into account that segregation analysis is a more complex approach involving the estimation of more parameters, the lack of a complete segregation picture is probably due to insufficient power caused by reduced sample sizes and/or smaller gene effects. In regard to the lack of segregation results for the training response in \( V_{O2VT} \) in both races, the same explanation may be true. However, in the Gaskell et al. study (20), the spouse correlations in white and black families (0.35 and 0.63, respectively) were about two to three times higher than the average of the other familial correlations, suggesting primarily shared environmental effects rather than genetic effects for these phenotypes. In any case, whether the familial variability, characterized by a high trainability pattern in some families and by low responsiveness in others, is controlled by a major gene remains unresolved for \( V_{O2VT} \) and \( \text{VT}\%\overline{V}O_{2\text{max}} \).

The noteworthy finding from the current study is that these cardiorespiratory phenotypes, frequently
used to quantify the level of fitness, showed evidence of a putative major gene with a large effect at baseline in white families. There is no common agreement regarding the units in which VT should be expressed. In the literature, one finds VT defined in terms of \( V_{O2} \) or power output (watts). The subjects of the present study were exposed to 60 training sessions. Although this was not a mild-intensity exercise program, it was not of the type that is likely to generate significant increases in skeletal muscle type I fibers. As is well documented in prior publications (11), the subjects of HERITAGE were all sedentary. The mean increase in \( V_{O2}\text{max} \) was on the order of 18%. The concordance of the evidence for VT \( V_{O2} \) and VT \%\( V_{O2}\text{max} \) suggests that there were no important biases caused by the fact that we elected to use VT in ml \( O_2/\text{min} \) as the key indicator of VT. VT is a complex phenotype and is correlated with lactate threshold in some (16, 43) but not all studies (17, 29). VT probably includes interactions between oxygen transport, muscle fiber type, substrate utilization, thermoregulation, and other complex physiological processes (4, 15, 24). However, despite this complex determination of VT, evidence of a single major gene was found by segregation analysis. It is possible that this underlying genetic component represents oligogenic effects (i.e., several major genes working in similar ways) instead of a single gene controlling the variability of VT. To identify these putative genes, further genetic studies should be carried out by using linkage and association analyses. As shown recently by a genomic scan of the \( V_{O2}\text{max} \) phenotype (14), there are some indications of linkages in the sedentary state (chromosomes 4q, 8q, 11p, and 14q). Because \( V_{O2}\text{max} \) and \( V_{O2}\text{max} \) phenotypes, although highly correlated \((r = 0.76)\), are not identical, it is important to verify whether the same chromosomal regions provide evidence of linkage also with \( V_{O2}\text{max} \) and VT \%\( V_{O2}\text{max} \) variability.

In summary, the results of the present study imply that individual differences in VT in a sedentary state are influenced by a gene or a few genes that have low-frequency alleles with large effects. These alleles appear to be present in the sample of whites from the HERITAGE cohort because their effects were not detected in blacks. Moreover, these alleles contribute to VT in the sedentary state but do not appear to influence the trainability of VT. There are a number of physical and biochemical candidates through which putative major genes could exert their effects on VT. It is a very complex undertaking to resolve these major effects in terms of specific genes and DNA sequence variants. We intend to begin this effort by relying first on a genome-wide scan because no candidate gene comes readily to mind.

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