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A Genome Scan for Loci Associated with Aerobic Running Capacity in Rats

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Aerobic capacity is a complex trait that defines the efficiency to use atmospheric oxygen as an electron acceptor in energy transfer. Copenhagen (COP) and DA inbred rat strains show a wide difference in a test for aerobic treadmill running and serve as contrasting genetic models for aerobic capacity. A genome scan was carried out on an F_2(COP × DA) segregating population (n = 224) to detect quantitative trait loci (QTLs) associated with aerobic running capacity. Linkage analysis revealed a significant QTL on chromosome 16 (lod score, 4.0). A suggestive linkage was found near the p-terminus of chromosome 3 (lod score, 2.2) with evidence of an interaction with another QTL on chromosome 16 (lod score, 2.9). All three QTLs showed a dominant mode of inheritance in which the presence of at least one DA allele was associated with a greater distance run. These results represent the first aerobic capacity QTLs identified in genetic models.

Key Words: inbred, models, gene, linkage analysis, QTL, oxygen

INTRODUCTION

Biochemical and physiological systems that use atmospheric oxygen were favored by natural selection in the process of evolution [1]. Oxidation reactions that use molecular oxygen as the final electron acceptor obtain a greater yield of ATP relative to substrate-level phosphorylation; thus, axiomatically the efficiency of aerobic energy transfer is pivotal to cellular metabolism and defines a large part of mammalian biology. Aerobic capacity is a quantitative trait influenced by the interaction of multiple genetic and environmental factors. Studies on the heritability of aerobic endurance capacity in humans suggest that between 70% and 90% of the total phenotypic variance can be attributed to the genetic component [2,3].

Fitness tests of aerobic capacity are used clinically to assess cardiorespiratory function and as a general test of overall physical health status [4]. It is a known tenet that levels of physical fitness are strong predictors of cardiovascular morbidity and all-cause mortality [5]. Exercise has been shown to ameliorate numerous diseases such as cardiovascular disease, hypertension, diabetes mellitus, obesity, and lipid abnormalities [6]. Despite evidence and evolutionary support that aerobic exercise capacity is a heritable trait of primary importance, the underlying genes remain undefined.

Most genomic research on aerobic capacity has focused on exercise as an environmental intervention affecting quantitative measures such as body composition, muscle mass, blood pressure, or disease outcomes such as hypertension or diabetes [6]. A genomic scan for maximal oxygen consumption (VO_2 max) in humans (HERITAGE Family Study) [7] revealed that chromosomal regions linked to VO_2 max in the untrained (sedentary) state were different from those linked to VO_2 max in response to exercise training. This implies that there is a complement of genes that determine levels of intrinsic aerobic capacity in the untrained state and apparently another set of genes that dictate the response to aerobic exercise training. Therefore, genetic substrates for both intrinsic aerobic capacity and the adaptational response to aerobic exercise will have to be resolved in order to understand the role that aerobic capacity plays in defining the continuum between health and disease.

Model organisms, typically inbred, in which genetic and environmental variance approach minimums have been of value in detecting quantitative trait loci (QTLs) for many complex phenotypes [8]. In previous work we tested maximal treadmill running capacity in a panel of 11 different inbred rat strains to evaluate the genetic variance that exists for intrinsic (untrained) aerobic capacity [9]. We found a continuum in running capacity among the strains. At the extremes, male DA rats showed the highest capacity and ran 969 meters to exhaustion, whereas male Copenhagen (COP) rats were the lowest performers and became exhausted by 262 meters. This wide difference (3.69-fold) in phenotypic value suggests that the male COP and DA rats provide divergent genetic substrate for a segregating population to test for an association between allelic variation and aerobic running capacity.

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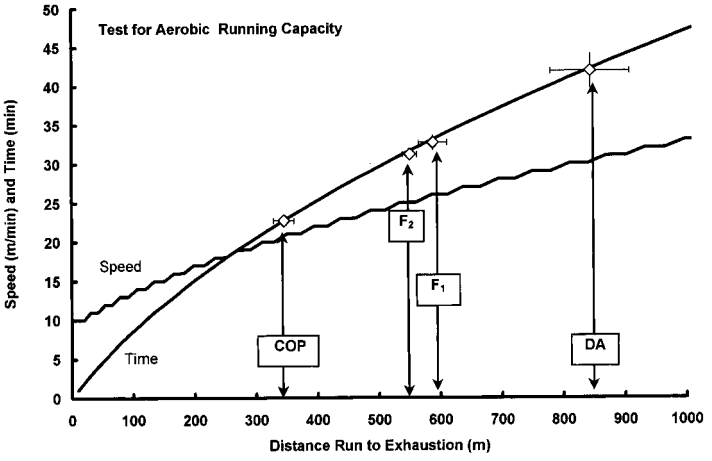

FIG. 1. Aerobic running capacity was measured as total distance run on a ramped treadmill running test, to exhaustion. The starting speed was 10 m/min and was increased by 1 m/min every 2 minutes. The graph relates speed and time to distance run. Mean distance and time for the parental, F_1 and F_2 male populations ± SEM are shown.

We report here a genome scan for intrinsic aerobic exercise capacity QTLs in rats based on 210 polymorphic microsatellite markers and an F_2 intercross population (n = 224) derived from COP and DA strains. Our long-term goal is to use these inbred model systems to identify genes, proteins, and intermediate phenotypes that collectively are causative of the differences between low and high aerobic capacity.

RESULTS

Aerobic Running Capacity Phenotype

Figure 1 is a nomogram displaying the ramped exercise protocol used for measuring the phenotype of aerobic capacity. It shows the mean values for distance run for male rats of the parental strains (COP and DA), the F_1(COP × DA), and F_2(COP × DA) populations. The COP males ran to exhaustion upon reaching 345 ± 17 meters, the equivalent of 22.7 ± 1 minutes of running time, whereas DA males ran on average 844 ± 64 meters and were exhausted after 41.9 ± 2 minutes. The value of distance run for male F_1(COP × DA) rats (n = 42) was approximately

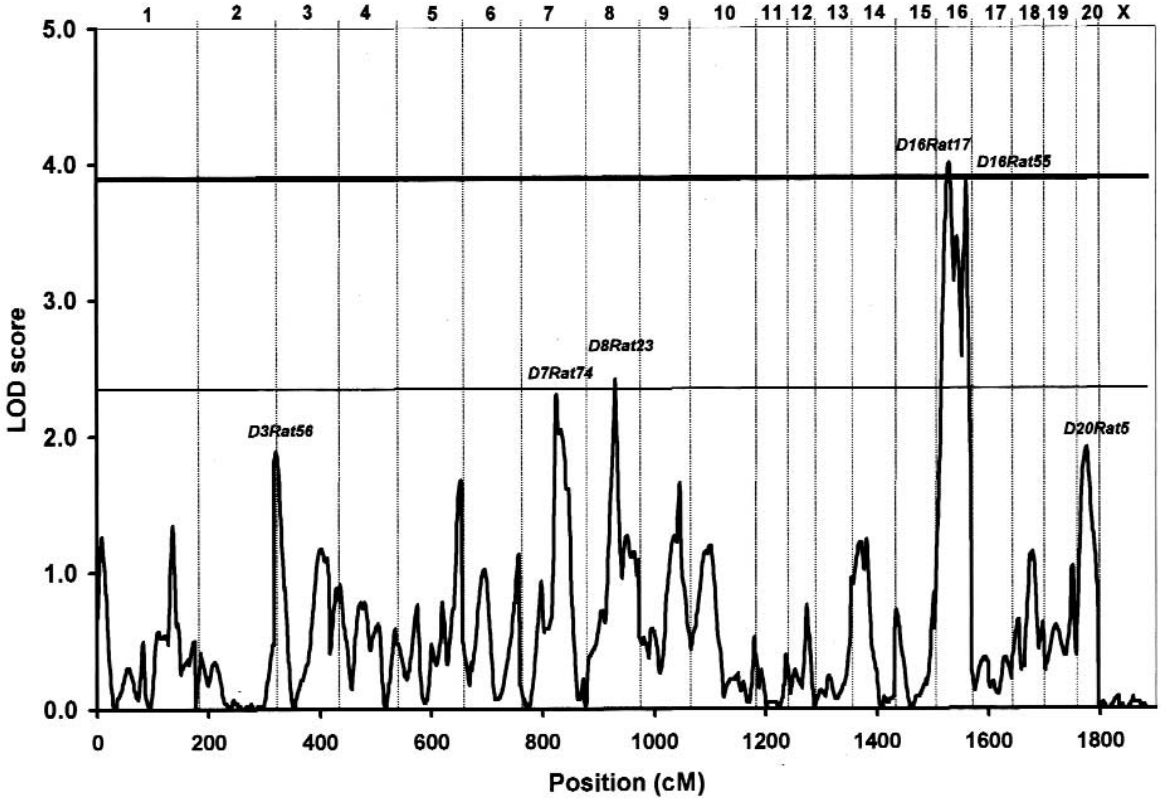

FIG. 2. Lod plot of the genome-wide scan for aerobic running capacity QTLs. Extremes of the F_2 (COP × DA) population were selectively genotyped with 210 polymorphic microsatellite markers. Lod scores are plotted as a function of marker location in centimorgans (cM) for free model of regression. Chromosome numbers are represented at the top of the plot and the dashed lines separate each chromosome. The bold line indicates lod score threshold for statistically significant linkage for QTLs and normal line indicates suggestive linkage.

TABLE 1: Coverage of polymorphic microsatellite markers for phase I of the genome scan

Chromosome	cM Length (Kosambi)	% Length	No. of markers	Max. distance between adjacent markers (cM)	Average distance between adjacent markers (cM)	% Chromosome within 15 cM of a marker
1	177	9.31	18	37.0	10.4	96
2	142	7.47	15	18.7	10.1	100
3	113	5.96	12	20.4	10.3	100
4	108	5.70	12	16.3	9.8	100
5	120	6.33	12	17.3	10.9	100
6	103	5.43	10	29.0	11.4	100
7	116	6.12	14	16.6	8.9	100
8	99	5.22	10	19.0	11.0	100
9	92	4.85	12	18.7	8.4	100
10	120	6.33	15	20.6	8.6	100
11	54	2.84	8	19.4	7.7	100
12	50	2.64	6	13.4	10.1	100
13	67	3.53	6	19.8	13.4	100
14	78	4.11	10	15.7	8.7	100
15	73	3.85	7	19.9	12.2	100
16	67	3.53	7	14.7	11.2	100
17	74	3.90	9	17.0	9.3	100
18	55	2.90	8	13.5	7.9	100
19	58	3.04	6	33.5	11.5	94
20	44	2.32	7	15.5	7.4	100
X	88	4.61	6	28.7	17.5	100
Totals	1896	100	210	20.2	10.3	99.5

equidistant (588 ± 23 m, 32.8 ± 1 minutes) from those observed for COP and DA rats and did not differ significantly from the calculated midparental value of 594.5 meters. The distance run by males of the F_2 intercross population was 550 ± 11 meters (31.2 ± 0.5 minutes) and was also intermediate between the values for male COP and DA rats. The minimum and maximum distances recorded for male rats from the F_2 (COP × DA) population were 218 and 1097 meters, respectively.

No significant differences in body weight were observed among the male parental COP (228 ± 7 g) and DA (231 ± 10 g), F_1(COP × DA) (240 ± 12 g), and F_2(COP × DA) (235 ± 24 g) populations. Regression analysis showed a small but significant negative association between body weight and distance run within males from the F_2 (COP × DA) population ($y = -0.025x + 248.23$; $P = 0.002$). Approximately 4% ($r = 0.2$) of the variance in body weight in intercross rats could account for differences in distance run.

Genome Scan

Table 1 gives the genomic coverage of polymorphic markers for each chromosome (1–20, X). The average distance between adjacent markers was 10.3 cM and the maximum distance averaged 20.2 cM. Overall, 99.5% of the genome was within 15 cM of a marker. The total length of the chromosome map was 1896 cM, which compares well with other estimates of the size of the rat genome.

Identification of Aerobic Running Capacity QTLs

The information for the linkage maps is based on genotype data from male rats selected from the phenotypic extremes of aerobic running capacity in the F_2(COP × DA) distribution ($n = 90$). The 45 rats constituting the upper 20% of the F_2(COP × DA) population had an average aerobic running capacity of 830 ± 16 m, whereas the 45 rats constituting the lower 20% ran 342 ± 6 m. Figure 2 summarizes the results from the selective genome-wide scan for aerobic running capacity QTLs (phase I). QTLs were identified using the Map Manager QTX computer program with an unconstrained regression model that makes no assumption regarding the dominance properties of the genes underlying a particular QTL. The most significant associations of chromosomal regions with aerobic running capacity were found for two loci, *D16Rat17* and *D16Rat55*, located approximately 30 cM apart on chromosome 16 (RNO16), which had lod scores of 4.0 and 3.9, respectively. Other chromosomal regions on rat chromosome 3 (RNO3), rat chromosome 7 (RNO7), rat chromosome 8 (RNO8), and a broad region on rat

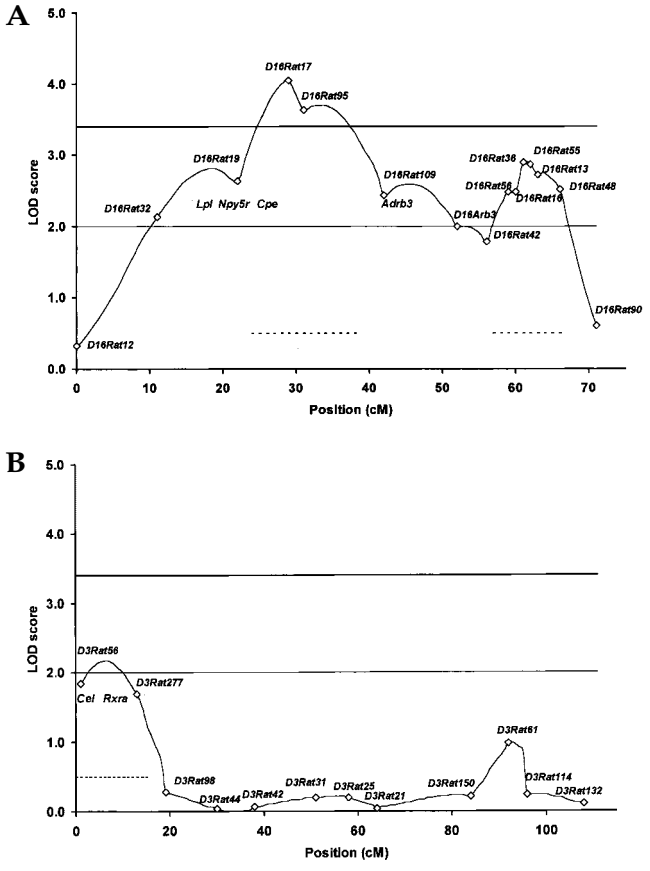

FIG. 3. Lod plots for aerobic running capacity QTLs on rat chromosomes 16 (A) and 3 (B). The bold line in each plot represents the lod threshold for significant linkage and the normal line shows the threshold for suggestive linkage for dominant models of inheritance. Heavy dashed lines below each plot represent 1-lod confidence intervals. Candidate genes in the QTL regions are listed.

chromosome 20 (RNO20) had lod scores that approached the suggestive threshold for a QTL (LOD > 1.85).

No epistatic interactions for aerobic running capacity were found with pair-wise comparisons between marker loci, nor were there significant associations with inheritance of either the Y or mitochondrial chromosomes. All of the chromosomal regions mentioned above were further evaluated in phase II of the genome scan.

In phase II, the genotypes for the remaining male rats (n = 134) in the F_2(COP × DA) population that phenotypically lie in the middle of the distribution were added to the analysis for markers in the chromosomal regions that showed aerobic running capacity QTLs (RNO3, RNO7, RNO8, RNO16, RNO20). Interval mapping of these chromosomes was conducted using dominant, additive, and recessive models of inheritance. Table 2 shows the marker loci located nearest to possible aerobic running capacity QTLs, the mean distance run value for each genotype, the genetic model that best fits the data, and the highest lod score found in the chromosomal region. Significance is noted for lod scores that show statistical evidence for suggestive or significant linkage for aerobic running capacity QTLs as defined by the guidelines of Lander and Kruglyak [10].

Following completion of phase II of the genome scan, RNO16 (Fig. 3A) still yielded the highest lod score (LOD = 4.0) for an aerobic running capacity QTL near *D16Rat17*. The one-lod support interval for this QTL spans 16 cM between markers *D16Rat19* and *D16Rat109*. Inheritance of alleles at *D16Rat17* best fits the dominant model of inheritance, in which the presence of at least one DA allele was associated with a 24% higher mean aerobic running capacity, compared with rats homozygous for the COP allele. The second peak on the RNO16 lod plot, located near *D16Rat55*, was attenuated by one lod score (LOD = 2.9) in phase II of the genome scan. Only the QTL on RNO3 (Fig. 3), among those chromosomal regions approaching suggestive linkage detected in phase I, had a lod score (LOD = 2.2) that exceeded the suggestive threshold for linkage to aerobic running capacity in the genome scan of the entire F_2(COP × DA) male population.

All markers located near lod-plot peaks in phase II (Table 2) were tested for interactions using two-way ANOVA. A significant interaction affecting aerobic running capacity was observed only between markers *D3Rat56* and *D16Rat55* (P = 0.027). Selective genotyping in phase I identified a significant running capacity QTL near *D16Rat55* (LOD = 3.9), but a weak suggestion for a running capacity QTL on RNO3 (LOD = 1.9). However, when all male rats in the entire F_2(COP × DA) population were genotyped and analyzed, an interaction between the *D3Rat56* and *D16Rat55* loci became apparent. The mean distance run was 21% higher in rats carrying at least one DA allele at both the *D3Rat56* and *D16Rat55* loci, compared with rats that were homozygous for the COP allele at either or both loci (Fig. 4). When rats were homozygous for the DA allele at both marker loci, the running distances increased to 42%

TABLE 2: Phase II of the genome scan on the entire F_2(COP × DA) population

		Genotype				
Chromosome	Locus	COP/COP	COP/DA	DA/DA	Lod score	Model
3	*D3Rat56*	486 ± 18	560 ± 13	592 ± 19	2.2[a]	Dominant
7	*D7Rat74*	493 ± 20	562 ± 14	572 ± 20	1.6	Dominant
8	*D8Rat23*	588 ± 24	549 ± 18	505 ± 25	1.5	Additive
16	*D16Rat17*	466 ± 19	568 ± 14	601 ± 22	4.0[b]	Dominant
16	*D16Rat55*	488 ± 19	567 ± 15	597 ± 23	2.9[a]	Dominant
20	*D20Rat5*	570 ± 29	565 ± 18	494 ± 28	1.6	Recessive

[a]Lod scores above the statistical threshold for suggestive linkage.
[b]Lod scores above the statistical threshold for significant linkage.

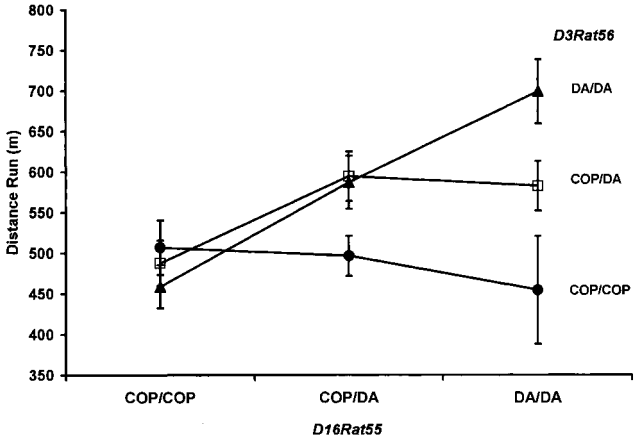

FIG. 4. Graphic representation of aerobic running capacity as the effect of an interaction ($P = 0.027$) linked to markers *D3Rat56* and *D16Rat55*. Each point represents the mean running distance ± SEM for each genotype combination for both markers.

above rats homozygous for the COP allele at both markers. Furthermore, rats homozygous for the COP allele at either the *D16Rat55* or *D3Rat56* loci measured the lowest running capacity regardless of the genotype at the alternate locus. Collectively, these results indicate that at least two running capacity QTLs are present on RNO16. The QTL near *D16Rat17* had effects on running capacity that were independent of other putative running capacity QTLs detected by the genome scan, whereas the other running capacity QTL on RNO16, located near *D16Rat55*, interacted with a running capacity QTL located near *D3Rat56* (Fig. 4). Comparative gene mapping in humans, mice, and rats suggests that the candidate region for aerobic capacity QTL on RNO16 (16p14–16q11) is orthologous to segments of mouse chromosome 8 (http://www.informatics.jax.org) and human chromosomes 4q and 8p (http://www.ncbi. nlm.nih.gov).

Identification of Heart Weight and Body Weight QTLs

Additional interval mapping studies in the F_2(COP × DA) male population were carried out to determine whether heart weight, body weight, or relative heart weight (that is, heart weight-to-body weight ratio) QTLs colocalized with chromosomal regions containing putative aerobic running capacity QTLs (Table 3). A suggestive (LOD = 2.6) relative heart weight QTL was observed on RNO7, between *D7Rat74* and *D7Rat100* (15 cM region). In phase I, a running capacity QTL was observed near *D7Rat74*, although its effect was decreased in phase II of the genome scan (LOD = 1.6; Table 2). Male F_2(COP × DA) rats homozygous for the DA allele at *D7Rat74* had a 6% greater relative heart weight compared with male rats that were homozygous for the COP allele (2.69 ± 0.06 versus 2.54 ± 0.32 g/kg; $P = 0.004$). Similarly, suggestive heart weight (LOD = 2.7) and body weight (LOD = 2.2) QTLs were found on RNO8 in a 28-cM central region near *D8Rat23*. In phase I of the genome scan, a suggestive aerobic running capacity QTL near *D8Rat23* yielded a lod score of 2.4, but was attenuated to LOD = 1.5 in phase II. Hearts from the male F_2 (COP × DA) rats carrying at least one DA allele at marker *D8Rat23* were on average 6% heavier compared with those of male rats that were homozygous for the COP allele (0.71 g versus 0.67 g; $P = 0.002$). Furthermore, body weights in the male rats homozygous for the DA allele at *D8Rat23* were 5% heavier than those homozygous for the COP allele (228 g versus 239 g, respectively; $P = 0.008$). These data are consistent with the idea that differences in aerobic running capacity may be associated, in general, with differences in cardiac function and/or body composition.

DISCUSSION

Rat models have proven to be a useful tool for dissecting genetic loci that contribute to the variance of physiologically complex traits [11,12]. Typically, two inbred strains divergent for a trait of interest can be bred to produce a segregating population from which an association of phenotype with genotypes can be evaluated for statistical significance for QTLs. However, each inbred strain represents only a fraction of the genetic variation found in a heterogeneous population, and thus does not represent the total number of QTLs contributing to the phenotype. Of greater merit for genetic analysis are models in which animal lines derived from outbred stock are selectively bred for a given trait such that many alleles determining the trait variance are concentrated at the extremes for the trait. We are currently in the long-term process of developing rat models for low- and high-capacity aerobic endurance running by artificial selection from the widely heterogeneous outbred N:NIH stock [13] for subsequent genetic dissection. As a parallel strategy, a segregating population bred from two commercially available inbred strains used in this study (COP and DA) provided a suitable genetic substrate for the first detection of chromosomal

TABLE 3: Genomic scan for heart weight and body weight QTLs in the F_2(COP × DA) segregating population

Genotype							
Trait	Chromosome	Locus	COP/COP	COP/DA	DA/DA	Lod score	Model
Relative heart weight (g/kg)	7	*D7Rat74*	2.55 ± 0.03	2.54 ± 0.02	2.69 ± 0.06	2.6*	Recessive
Total heart weight (g)	8	*D8Rat23*	0.67 ± 0.01	0.71 ± 0.01	0.71 ± 0.01	2.7*	Dominant
Body weight (g)	8	*D8Rat23*	228 ± 2.6	236 ± 2.0	239 ± 3.0	2.2*	Additive

*Lod scores above the statistical threshold for suggestive linkage.

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regions that contain alleles causative of differences in the intrinsic ability for aerobic treadmill running.

The most significant linkage for an aerobic running capacity QTL was found on RNO16 (Table 2 and Fig. 3). Although the location of the peak in the lod plot gives the best estimate of the map position for the QTL, confidence intervals constructed on either a one-lod support interval [14] or an interval showing a two-lod difference have a high probability of containing a QTL [15]. Almost 90% of the lod plot for RNO16 was above the suggestive threshold of linkage for a QTL. This broad plateau in the lod plot on RNO16 between *D16Rat32* and *D16Arb3* (Fig. 3A) may be the result of multiple aerobic running capacity QTLs that are in relatively close proximity [16].

There are numerous candidate genes that can be relevant to a trait as complex as aerobic capacity. One plausible unifying pathway apparent between candidate genes near the lod peak on RNO16 and those in other chromosomal regions with suggestive aerobic capacity QTLs is that of lipid metabolism. Fatty acid molecules are the most plentiful source of potential energy in cells, particularly for those in high oxidative muscle fibers. Therefore, a difference in the contribution of fatty acid substrates to the conversion of aerobic energy between the COP and DA strains represents one hypothesis of cause and effect. A gene of particular interest in the aerobic capacity QTL on RNO16 encodes lipoprotein lipase (LPL), an enzyme that has a central role in lipid catabolism. LPL is localized on the surface of capillary endothelium in active tissues and facilitates energy release from fatty acids by hydrolysis of the triacylglycerol component of plasma lipoproteins. *Lpl* and its variants have been investigated for constituent roles as candidate susceptibility genes for cardiovascular disease [17]. A recent report from the HERITAGE Family Study, one of the few human studies that have looked at gene–exercise interactions, showed that a polymorphism in *LPL* (S447X) influences the changes in body fat induced by exercise training [18]. In rat models, *LPL* has been investigated as a gene linked to both hypertension and lipid metabolism, and has been mapped to RNO16 [19,20].

Two other possible candidate genes within the running capacity QTL near *D16Rat17* are known to encode molecular targets for pathways regulating energy balance [21]. The β-3 adrenergic receptor (*Adrb3*), expressed in white and brown adipose tissue, is known to mediate triglyceride breakdown and thermogenesis. Neuropeptide Y5 receptor (*Npy5r*), found primarily in the central nervous system including the hypothalamus, is involved with the integration of energy homeostasis and the expression of enzymes involved in lipogenesis. Lipid metabolism may be regulated during exercise through the activation of the sympathetic nervous system and these potential regulatory pathways.

It is an interesting observation that differences in aerobic running capacity may be related to mechanisms investigated in other metabolic disease models, such as obesity and diabetes, for which exercise has been shown to be beneficial. The carboxypeptidase E gene (*Cpe*), located near the lod plot peak on RNO16, encodes an enzyme that coordinates the processing of several peptide prohormones such as proinsulin and proglucagon. The obesity and hyperglycemia elicited in obese fat/fat mice are due to prohormone processing defects associated with a Ser202Pro mutation in a *Cpe* allele [22]. The putative aerobic capacity QTLs on RNO3, RNO8, and RNO20 (phase I of genome scan) encode several lipolytic enzymes such as carboxyl ester lipase (*Cel*, near *D3Ucsf4*), hepatic lipase (*Lipc*, near D8Rat32), and pancreatic colipase (*Clps*, near *D20Rat41*), which have been investigated for their influence on hypertension, dyslipidemia, and atherosclerosis [23].

Another possible set of candidate genes within putative aerobic capacity QTLs is the recently investigated gene network represented by nuclear receptors for fatty acids, called peroxisome proliferator-activated receptors (PPARs). PPARs bind as a heterodimer with retinoid X receptors (RXR) to function as lipid-activated transcription factors for expression of genes crucial to the genetic regulation of fatty acid oxidation [24,25]. Of the three PPAR isotopes identified (α, δ, and γ), PPARα targets genes responsible for fatty acid uptake and binding in the cells, fatty oxidation in cellular organelles, and lipoprotein assembly and transport. *Ppara* has been mapped in the rat to RNO7 and lies within the putative aerobic capacity QTL. *Ppard* (also called β) can be found on RNO20. *Rxra* is located at the p-terminus of RNO3 near *D3Rat56* and thus may be implicated in the interaction detected between RNO3 and RNO16.

A substantial amount of physiological evidence stemming from previous work [26] supports the idea that the central physiological component of heart function can also be a limiting factor to the transport of oxygen during aerobic exercise. Studies in the rat [27,28] have verified that oxygen consumption increases linearly as a function of ramped speed during treadmill running; in turn, cardiac output, heart rate, stroke volume, and oxygen extraction increase linearly as a function of oxygen consumption. We recently investigated several cardiovascular-related phenotypes intermediate to aerobic running capacity in the COP and DA strains. We found that isolated hearts from DA rats have on average a 50% greater cardiac output compared with hearts from COP rats in a Langendorff-Neely working heart model [9]. In addition, we reported that DA hearts were significantly greater than the COP for maximal developed tension in isolated papillary muscles, and for fractional shortening and amplitude of calcium transients as measured in individual dispersed myocytes [29]. Finally, at the systemic level, DA rats showed a wider range for sympathetic and parasympathetic control of heart rate and more sympathetic support of blood pressure compared with the COP rats [30].

We have shown here that other cardiac trait differences may also contribute to the genetic differences in running capacity. Heart weight and relative heart weight are significantly greater in the DA strain compared with the COP strain [30]. From genetic analysis in the F_2(COP × DA) population, a heart weight QTL was identified on RNO8 and a relative heart weight QTL on RNO7 (Table 3). Both of these QTL regions colocalized to markers *D7Rat74* and *D8Rat23* (Fig. 2) near putative aerobic running capacity QTLs identified in the first phase of our genome scan using selective genotyping. A report by Tsujita *et al.* [31] identified a QTL for left ventricular mass near our rela-

tive heart weight QTL on RNO7. Other loci on RNO7 have been also shown to influence heart weight in association with blood pressure in rat genetic models of hypertension [32,33].

The males from COP and DA strains were not significantly different in body weight; our data, however, showed a small but significant negative correlation between body weight and running capacity in the F_2 (COP × DA) male population; that is, rats with lower body weights tended to run further. We also identified a body weight QTL (Table 3) located in the same region of RNO8 that contained aerobic capacity and heart weight QTLs whose effects approached the threshold for suggestive linkage. Body weight was recently demonstrated to have a negative correlation with running capacity in rats selectively bred for low and high aerobic running capacity [34]. Male rats selected for high running capacity were 16% lighter in body weight and ran 142% further on the treadmill than their low-selected counterparts after six generations of selection. The genetic component of this correlation is likely to stem from either a gene that has a pleiotropic effect on both running capacity and body weight, or a gene that represents linkage disequilibrium between distinct loci that affect both traits.

Chromosomal intervals showing statistical evidence for suggestive or significant linkage to aerobic running capacity are suitable candidates for the construction of congenic strains and substrains. This is accomplished by systematically introgressing chromosomal intervals from donor strains containing either high or low aerobic capacity QTL alleles into recipient strains having the reciprocal genetic backgrounds from the contrasting strain. Congenic strains can be used to confirm the presence of aerobic capacity QTLs and to delimit the chromosomal regions containing alleles responsible for the QTL [12]. Construction of "double" congenic strains is another strategy, where the chromosomal regions in the donor strain containing two QTLs on separate chromosomes are introgressed into the same recipient strain to test whether there is such an interaction [35,36]. Indeed such an approach may be necessary to study the effects of the aerobic running capacity QTLs located near *D16Rat55* and *D3Rat56* if these two loci interact in a congenic strain derived from COP and DA rats as they did in the F_2(COP × DA) segregating population.

MATERIALS AND METHODS

Animals. Inbred Copenhagen (COP/Hsd) and DA (DA/OlaHsd) rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and the genetic crosses were carried out in the animal care facility at the Medical College of Ohio. Reciprocal crosses between COP females with DA males and DA females with COP males produced F_1 progeny. The F_1 progeny were then reciprocally intercrossed to produce an F_2 generation containing 224 males. The COP strain was originated by Curtis [37] at Columbia University Institute for Cancer (USA) and was initially bred for resistance to induction of mammary tumors. Inbreeding for the DA strain was initiated by Odell at the Oak Ridge National Laboratory (USA) and completed at the Wistar Institute (USA) in 1965 [38]. The strain DA was so designated because it expressed the d blood group allele and had the agouti coat color. These two rat strains exhibit a polymorphism frequency of 46% for microsatellite genetic markers (http://www.genome.wi.mit.edu).

Offspring were weaned at 28 days of age and housed two per cage. Rats were fed standard rat chow (Ralston Purina, diet 5001) and water *ad libitum* and placed on a 12-hour light-dark cycle with the light cycle coinciding with daytime. The capacity to use oxygen to perform work has been evaluated primarily by tests of endurance performance [4]. The protocol used here for estimation of aerobic running capacity required 2 weeks and was started when the rats were 10 weeks old. Rats phenotyped for aerobic capacity were subsequently euthanized with sodium pentobarbital (150 mg/kg, I.P.) at 15 weeks of age and liver tissue (20 g) was collected for genetic analysis. Body weights and heart weights were measured. All procedures were carried out with approval by our Institutional Animal Care and Use Committee and complied with the *Guiding Principles in the Care and Use of Animals* (NIH publication, No. 86-23, revised 1985).

Phenotype. A ramped test for maximal treadmill running ability was used to measure the aerobic running capacity phenotype as described [13,39]. In brief, each rat was first oriented to run on the treadmill for 5 minutes at a speed of 10 m/min and a constant slope of 15° (Model Exer-4, Columbus Instruments, Columbus, OH) over a 5-day period. During the second week, each rat was tested for maximal endurance running capacity on five consecutive days. Each daily trial began at a starting velocity of 10 m/min and a constant slope of 15°. Treadmill velocity was then increased by 1 m/min every 2 minutes and each rat was run until exhausted. Exhaustion was operationally defined as the third time a rat could no longer keep pace with the speed of the treadmill and remained for longer than 3 seconds on an activated shock grid (1.2 mAMP at 3 Hz) located off the back of the moving belt. Rats were then removed from the treadmill and body weight was measured.

The total distance run (m) for each trial was derived as a function of total running time at each speed increment. The single furthest distance run by each rat, out of the five daily trials, was considered the best indicator of the genetic component of intrinsic aerobic capacity and was recorded as the value for the trait of aerobic running capacity [39]. This tenet is based upon the idea that the environmental component can have an infinite negative influence upon a phenotype on any given day (reduce the measure to zero), but a finite positive influence for a given genotype.

Genome scan. DNA samples from the male DA, COP, and F_2(COP × DA) rats were extracted from liver tissue using DNeasy 96 tissue kits (Qiagen, Valencia, CA). PCR-based genotyping was done using microsatellite markers. Markers polymorphic between COP and DA strains were selected from the following published listings: The Whitehead Institute for Biomedical Research (http://www-genome.wi.mit.edu); The Laboratory for Genetic Research at The Medical College of Wisconsin (http://rgd.mcw.edu) [40]; and The Wellcome Trust Centre for Human Genetics (http://www.well.ox.ac.uk/rat_mapping_resources) [41]. The 210 microsatellite markers chosen for the genome scan were purchased from Research Genetics (Huntsville, AL).

The PCR amplification of microsatellite markers was performed in 10 ml reactions containing 200 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 units *Taq* polymerase, and 3.6 pmol of each oligonucleotide primer using a PTC-100-96 AgV thermocycler (MJ Research, Watertown, MA) with the following cycle profile: 94°C for 5 minutes; followed by 35–40 cycles of 94°C for 40 seconds, 50–60°C (depending on the primer set) for 45 seconds, and 1.5 minutes at 72°C, and a final incubation for 5 minutes at 72°C. PCR products were fractionated on 4% agarose gels (3:1 or Metaphor (FMC, Rockland, ME)) and visualized using ethidium bromide staining and ultraviolet illumination. When PCR-products were too similar in size to be distinguished on agarose gels, product bands were resolved using 8% polyacrylamide gels. Primers were end-labeled with ^{32}P (30 μCi) and the PCR-products were visualized by autoradiography.

The genome-wide scan was carried out in two phases. In phase I, the F_2(COP × DA) population was selectively genotyped [42] from the 20% of the population (n = 45) having the highest aerobic capacity and the 20% (n = 45) having the lowest aerobic capacity [42]. Microsatellite markers polymorphic (n = 210) between COP and DA were chosen to be spaced 10–20 cM apart throughout the genome. Genotypic data were ordered into linkage groups using the Map Manager QTX version b10 computer program [43].

In phase II of the genome scan, the entire male F_2 (COP × DA) population (n = 224) was genotyped with markers (n = 22) within chromosomal regions near or above the thresholds for suggestive statistical evidence for linkage to QTLs (as defined in [10]) in phase I. Therefore, we added genotype data for the remaining rats in the F_2(COP × DA) population (n = 134) with

moderate phenotypes to those extreme phenotypic rats used in phase I. Additional microsatellite markers ($n = 8$) were selected in these chromosomal regions at intervals, spaced 5–10 cM apart, to further characterize the putative QTL-containing regions.

Statistical analysis. Phenotype data were analyzed using the SPSS computer program (Chicago, IL). A one-way analysis of variance (ANOVA) was done to compare values for distance run between parental, F_1(COP × DA), and F_2(COP × DA) populations. The Kolmogorov–Smirnov test indicated that the values for distance run in the F_2(COP × DA) population departed from a normal distribution. To correct for this, the distance run values were transformed by taking the $\log\text{arithm}_{10}$ of the values. The Kolmogorov-Smirnov test indicated that the log (distance run) values were distributed normally. Accordingly, we used log (distance run) as the phenotypic measure of aerobic exercise capacity in interval mapping.

In phase I of the genome scan (selective genotyping), the Map Manager QTX program was used to detect and localize QTLs using an unconstrained genetic model (free) for regression analysis. The likelihood ratio statistic (LRS) generated within Map Manager QTX as a measure of the significance of a QTL [44] was converted into lod scores (LOD = LRS/4.6) for reporting purposes. Permutation tests [45] were used to calculate critical values for the LRS indicating suggestive (LOD = 2.3) and significant (LOD = 3.9) statistical threshold values for linkage which follow described definitions [10]. Pair-wise interactions between all marker loci and distance run values were examined throughout the genome using a computer program written by Gary Churchill (http://www.jax.org/research/churchill) that uses MATLAB programming software (The Mathworks, Inc., Natick, MA). Chromosomal regions showing an association with aerobic running capacity (that is, yielding lod scores ≥ 1.85, at least approaching the suggestive threshold for linkage) in phase I were analyzed further in phase II of the scan.

In phase II, the entire male F_2 (COP 3 DA) population was genotyped with markers within chromosomal regions containing the putative aerobic running capacity QTLs identified in phase I. In addition, these regions were analyzed for body weight, heart weight, and heart weight-to-body weight ratio QTLs. Interval mapping for each chromosome was performed using constrained genetic models (dominant, additive, or recessive) to indicate the dominance properties of each QTL. Both simple and composite interval mapping techniques were applied. Associations between genotype and phenotype were assessed for selected loci using ANOVA within the SPSS program. The 5% confidence level was chosen for determining the level of significance and phenotype data are presented as means ± 1 standard error of the mean (SEM).

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