Quantitative genomics of voluntary exercise in mice: transcriptional analysis and mapping of expression QTL in muscle

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Kelly SA, Nehrenberg DL, Hua K, Garland T Jr, Pomp D. Quantitative genomics of voluntary exercise in mice: transcriptional analysis and mapping of expression QTL in muscle. Physiol Genomics 46: 593–601, 2014. First published June 17, 2014; doi:10.1152/physiolgenomics.00023.2014.—Motivation and ability to both undertake voluntary exercise, each with a potentially unique genetic architecture. Muscle structure and function are one of many morphological and physiological systems acting to simultaneously determine exercise ability. We generated a large (n = 815) advanced intercross line of mice (G4) derived from a line selectively bred for increased wheel running (high runner) and the C57BL/6J inbred strain. We previously mapped quantitative trait loci (QTL) contributing to voluntary exercise, body composition, and changes in body composition as a result of exercise. Using brain tissue in a subset of the G4 (n = 244), we have also previously reported expression QTL (eQTL) colocalizing with the QTL for the higher-level phenotypes. Here, we examined the transcriptional landscape of hind limb muscle tissue via global mRNA expression profiles. Correlations revealed an ~1.168% increase in significant relationships between muscle transcript expression levels and the same exercise and body composition phenotypes examined previously in the brain. The exercise trait most often significantly correlated with gene expression in the brain was running duration while in the muscle it was maximum running speed. This difference may indicate that time spent engaging in exercise behavior may be more influenced by central (neurobiological) mechanisms, while intensity of exercise may be largely controlled by peripheral mechanisms. Additionally, we used subsets of cis-acting eQTL, colocalizing with QTL, to identify candidate genes based on both positional and functional evidence. We discuss three plausible candidate genes (Insig2, Prcp, Sparc) and their potential regulatory role.

Adiposity; body weight; eQTL; experimental evolution; wheel running

THE PREDISPOSITION TO ENGAGE in voluntary activity is variable among humans and rodents and simultaneously influenced by genetics, the environment, and gene-by-environment interactions (27). Although voluntary exercise is exceedingly complex, it is hypothesized that some combination of both ability and motivation play an integral role in regulating the level of activity among individuals, with both of these components having a complex underlying genetic architecture (15).

Neurobiological investigations aimed at uncovering the motivational aspects of voluntary exercise have been discussed previously (see Ref. 26 and references therein). Here we focus on studies chronicling the variation in ability and trainability (broadly characterized as exercise sciences or exercise physiology). One major focus of exercise physiology is uncovering the mechanistic role of gene function and regulation in exercise performance (for a historical perspective see Ref. 5). For example, a total of 214 autosomal genes, seven loci on the X chromosome, and 18 mitochondrial genes were reported as influencing “physical performance” and “health-related fitness” phenotypes in humans (see Ref. 6, 2006–2007 update). This number has almost certainly increased in the subsequent years (e.g., Ref. 34). The performance phenotypes included in Ref. 6’s “human gene map” consist of “cardiorespiratory endurance,” “elite endurance athlete status,” “muscle strength,” “other muscle performance traits,” and “exercise intolerance of variable degrees.” The physical fitness traits are grouped into hemodynamic traits including exercise heart rate, blood pressure, and heart morphology; anthropometry and body composition; insulin and glucose metabolism; and blood lipid, lipoprotein, and hemostatic factors (6). Many, if not all, of the traits listed above would be hypothesized to affect the ability to engage in physical activity.

Rodent studies have also demonstrated a genetic basis for individual variation in exercise ability. Importantly, the translational nature of rodent wheel running to human health has been extensively discussed elsewhere (see Refs. 15, 27, and references therein), and we believe that voluntary wheel running appropriately models voluntary exercise in human populations, a complex behavior simultaneously affected by central and peripheral mechanisms. Selective breeding for elevated endurance capacity during forced treadmill running in rats has resulted in greater skeletal muscle capillarity, muscle oxidative enzyme activities, VO2max, and peripheral oxygen transport and utilization (see Ref. 21 and references therein). Replicated artificial selection for increased voluntary wheel-running behavior has resulted in an approximate 2.5- to 3.0-fold increase in total revolutions/day (36). Mice bred for high wheel running [high runners (HR) lines] on days 5 and 6 of a 6-day test exhibit a number of constitutive traits (expressed in the absence of wheel access) that clearly or plausibly represent adaptations with respect to wheel-running ability: reduced body mass, less body fat, lower leptin levels, increased levels of adiponectin, resistance to high-fat diet-induced obesity, elevated maximal oxygen consumption during forced treadmill exercise (VO2max), greater treadmill endurance, mild cardiac hypertrophy, increased insulin-stimulated glucose uptake in the extensor digitorum longus muscle, a trend toward higher muscle aerobic capacities (via mitochondrial and glycolytic enzyme activities), lower anaerobic capacities, elevated muscle glycogen concentrations, greater muscle (plantaris) capillarity, and altered fiber types in gastrocnemius muscle (see Refs. 3, 16, 18, 37–41 and references therein; not an exhaustive list). The later three phenotypes are unique to selectively bred individuals expressing the minimuscle phenotype, characterized by an ~50% reduction in mass of the triceps surae muscle complex (gas-
trocnemius, plantaris, soleus) and in the mass of the entire hindlimb musculature (see Ref. 28 and references therein). This phenotype is caused by an autosomal recessive mutation representing a C-to-T transition located in a 709 bp intron between exons 11 and 12 of the myosin heavy polypeptide 4 (Myh4) skeletal muscle gene (28). It has been observed in two (lab designation line 3 and 6) of the four HR lines and one (lab designation line 5) of the four control lines (28).

In addition to differences observed between HR and control mice in the absence of wheel access (above), we have also observed enhanced plasticity (training effects) in some traits, such as the concentration of the glucose transporter GLUT4 in gastrocnemius muscle. This enhanced plasticity is not explainable by the greater running in HR lines but appears to reflect inherently greater plasticity in the HR lines (i.e., for a given amount of stimulus, such as wheel running/day, individuals in the HR lines show a greater response compared with individuals in the control lines). For complete context and discussion of these traits see Ref. (14).

Previously, we generated an advanced intercross line (AIL; G4) of mice through reciprocal crosses between a line selectively bred for high voluntary wheel running (lab designation line 8) and the inbred strain C57BL/6J (23). The inbred strain was chosen, rather than one of the control lines, in an attempt to maximize the number of fully informative genetic markers. The minimuscle phenotype, discussed above, has never been observed in the HR line utilized to create the AIL. The G4 population has formerly been utilized for investigation of the phenotypic relationships between and identification of QTL for voluntary exercise traits, body composition traits, food consumption, changes in body weight and composition in response to exercise, and skeletal architecture traits (13, 24, 25). Most recently, using whole-brain tissue (26), we reported on the transcriptional landscape relevant to motivational aspects of voluntary exercise, with the presumption that results would be more relevant to motivational aspects than to physical abilities for exercise. We identified genome-wide expression quantitative trait loci (eQTL) and, on the basis of both positional and functional evidence, discussed plausible candidate genes regulating voluntary activity, body composition, and their interactions.

Here, we build upon that initial model of underlying functional genomic architecture by use of hindlimb muscle tissue to capture the transcriptional landscape relevant to certain aspects of the ability to engage in voluntary exercise. Coupled with previous investigations (phenotypic, QTL, eQTL), this study continues to build upon a systems approach toward understanding the predisposition to engage in voluntary exercise. For a detailed discussion of systems approaches aimed at the dissection of complex traits see (see Figs. 1, 4 in Refs. 27 and 33, detailed discussion of systems approaches aimed at the dissection of complex traits see (see Figs. 1, 4 in Refs. 27 and 33).

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**Materials and Methods**

Population and phenotyping. An AIL (G4, n = 815) was created by reciprocally crossing mice selectively bred for high voluntary wheel running (HR line) and the inbred strain C57BL/6J (B6). Complete methods regarding the creation and phenotyping of the G4 population, single nucleotide polymorphisms utilized for QTL analyses (n = 530), and RNA isolation and microarray analysis procedures may be found elsewhere (23–26). Only a brief methodological description will be provided here. All procedures were approved by and were in accordance with guidelines set forth by the Institutional Animal Care and Use Committee at the University of North Carolina (UNC) at Chapel Hill. G4 mice (8 wk of age) were weighed, body composition assessed (% fat tissue and % lean tissue; EchoMRI-100, Echo Medical Systems, Houston, TX), and individually housed with access to running wheels (circumference = 1.1 m; model 80850; Lafayette Instruments, Lafayette, IN) for 6 days. Distance (total revolutions), time spent running (cumulative 1 min intervals in which at least one revolution was recorded), average speed (total revolutions/time spent running), and maximum speed were calculated daily, as were the mean values on days 5 and 6 (the criterion for which the HR line was selectively bred; see Ref. 36). Mice were removed from the wheels following the completion of the 6th day of wheel access (i.e., the morning of day 7) and killed the same day in the order in which they were given wheel access (which was randomly chosen across both sex and parent-of-origin types). Following postwheel access weight and body composition measures, mice were decapitated, and hindlimb (triceps surae complex, including lateral and medial heads of the gastrocnemius, plantaris, and soleus) muscles were harvested, flash-frozen in liquid nitrogen, and stored at −80°C.

RNA isolation and microarray analysis. Isolation and purification of total RNA with TRIzol (Invitrogen, Carlsbad, CA) was performed from a homogenate of the right triceps surae complex. A subset (n = 243, 4 individuals were removed from the final analyses because of a lack of genotype information) of the total G4 population (n = 815) was utilized and represented the population-wide variation in running distance, each of two parent-of-origin types [whether a G4 individual was descended from a progenitor (F0) cross of HR♂ × B6♀ or B6♂ × HR♀], and both sexes. These 243 individuals overlapped with those previously used in Ref. 26. We used the MouseWG-6 v2.0 Beadchip (Illumina, San Diego, CA) to profile 45,281 transcripts and processed them with the Illumina Microarray Services at Expression Analysis, (Durham, NC). Profiles were normalized by Loess-Quantile normalization methods with R v. 2.8.1 statistical software (R Development Core Team; http://www.r-project.org, lumi package), and detection scores &gt; 0.95 were utilized for correlation and eQTL analyses (8, 19, 32).

Correlation analysis. Genes significantly expressed above background (detection scores ≥ 0.95) were tested for correlation with exercise (n = 36) and body composition (n = 17) phenotypes previously measured in the G4 population by the PROC CORR procedure in SAS® (version 9.1, SAS Institute, Cary, NC). Correlations were adjusted for sex and parent-of-origin type, factors with known phenotypic effects (23). P values were adjusted for multiple comparisons in SAS (PROC MULTTEST procedure) by the false discovery rate (FDR) procedure controlling the overall type I error rate at 5% (10).

eQTL analysis. We identified eQTL by the multiple imputation method within R/qtl for the R environment (7, 35). Statistical models included sex and parent-of-origin type. Following Ref. 26, a significance threshold [logarithm of odds (LOD) &gt; 3.8] was calculated via permutation tests (n = 1,000) of 100 randomly selected transcripts (an approach also similar to Ref. 43). cis-acting (or local) eQTL were defined as being 10 Mb or less away from the midpoint of the physical location of the gene each represented, while trans-acting eQTL were &gt;10 Mb away (following Ref. 12).
RESULTS

Correlation analysis. Transcripts were normalized (Loess-Quantile normalization), and 12,794 (of 45,281) were identified with a detection score (calculated across all 243 mice) ≥ 0.95. After adjustment for multiple testing, 4,413 (0.66% of total possible) partial correlations were found to be statistically significant (P < 0.05), indicating potential functional relevance (Fig. 1). Relationships between exercise-related traits and transcript levels accounted for the largest proportion (92.9%) of observed significant correlations (Fig. 1). Among the exercise traits, maximum running speed represented the largest percentage of significant relationships with transcript levels (39.0%). Collectively, body weight and composition-related traits accounted for 5.6% of significant correlations (Fig. 1). Among the exercise traits, changes in body weight and composition, as a result of a 6 days of exercise, represented 0.7% of significant correlations with transcript levels. Correlations with the greatest magnitude between exercise/body composition traits and transcript levels are presented in supporting information (Supplemental Table S1).1

eQTL analysis. In total, 1,186 cis-acting and 1,330 trans-acting statistically significant eQTL were observed (Fig. 2, A and B). The average LOD score for cis-acting eQTL was 14.9 (range = 4.3–84.0), while for trans-acting eQTL the mean LOD score was 5.5 with a range of 4.3–95.9. Among cis-acting eQTL, the median distance of the mapped location to the midpoint of the physical location of the gene was 2.00 Mb, and the distance was generally negatively correlated with the significance level (Fig. 2C). For comparison, our prior work using brain tissue in the same population yielded a median distance of 1.94 Mb (26). Moreover, in a recombinant inbred mouse strain panel, the pre-Collaborative Cross, the median liver eQTL-gene distance was 0.92 Mb (4). Trans-acting eQTL were identified on all chromosomes, with a potential master regulatory region observed on the proximal end Chr. 1 at ~16.7–20.1 Mb harboring 126 eQTL (Fig. 2B). An additional potential master regulatory region was observed on Chr. 18 at ~56.7–59.8 Mb with 106 eQTL (Fig. 2B). A potential master regulatory region on Chr. 1 was also previously identified from brain expression data. However, this region was found distally at ~170–180 Mb and contained 332 trans-acting eQTL.

Using the G4 population we previously identified 39 significant and 18 suggestive QTL representing various exercise traits (21). Here, as in Ref. 26 we compared the locations of cis-acting eQTL within the confidence intervals (CI, defined by 1 LOD drop) of QTL observed for subsets of the mean exercise traits (distance, duration, average speed, and maximum speed; Fig. 3).

Cis-acting eQTL localizing with running distance QTL (mean on days 5 and 6) revealed 19 positional candidate genes on Chr. 7 (Fig. 3A). Among these 19 candidate eQTL, cytochrome c oxidase assembly factor 4 (Coa4; Chchd8, old name) was significantly (FDR, P = 0.0248, r = 0.2737) correlated with running distance. For running distance on days 1 or 2, 18 potential candidate genes were identified under 2 QTL on Chr.

1 The online version of this article contains supplemental material.
1 (Fig. 3B). For mean running duration on days 5 and 6, we identified 22 candidate genes on Chr. 7 (Fig. 3C). The candidate genes unique to running duration resulted from an expansion of the CI for running duration loci (91–129 Mb) compared with the CI for the running distance QTL (99–124 Mb). A statistically significant partial correlation (FDR, $P = 0.0014$, $r = 0.3439$) was observed between running duration and HtrA serine peptidase 1 (Htral) (Fig. 3C). We observed 28 significant cis-acting candidate eQTL that mapped under the previously identified QTL (Chr. 2) for running speed (average and maximum) on days 5 and 6 of the 6-day wheel exposure (Fig. 3D). We identified 10 cis-acting candidate eQTL for average running speed on Chr. 14 (Fig. 3E). Of these, partial correlations indicated that N (alpha)-acetyl transferase 16 ($Naa16$; $Narg1l$, old name) and retinoblastoma 1 ($Rb1$) were statistically significantly correlated with average running speed on days 5 and 6 (FDR, $P = 0.0328$, $r = -0.2652$; $P = 0.0455$, $r = 0.2536$, respectively). In addition to those identified on Chr. 2, 31 cis-acting candidate eQTL were identified on Chr. 11 for maximum running speed (Fig. 3F). Of these correlation analysis revealed that secreted acidic cysteine rich glycoprotein ($Sparc$) was significantly correlated with maximal running speed (FDR, $P = 0.0033$, $r = -0.3253$).

We also examined colocalizing cis-acting candidate eQTL and loci previously identified for change in body weight and body composition in response to 6 days of exercise. Comparisons between cis-acting eQTL and loci observed for percentage change in body mass, as a result of 6 days of exercise,
In addition, we observed 15 candidate genes on Chr. 5 for maximum running speed (Chr. 2) on D14Ertd581e test. Note: eQTL 5930424F13Rik away from their physical gene midpoints. 20.0 Mb (Farp2, 9330132O05Rik) correction for multiple comparisons (FDR, discovery rate (FDR), names in boldface were significantly correlated (partial, controlling for parent and sex) with running distance after correction for multiple comparisons false positive rate $0.05$, $r_{0.25}$. Gene names in italics were suggestively correlated (partial, controlling for parent and sex) with running distance after correction for multiple comparisons $0.20$).

In comparison with previously mapped candidate eQTL in brain tissue (see Ref. 26), muscle tissue revealed fewer colocalizing cis-acting candidate eQTL was significantly correlated with any change in body weight or body composition variable in response to 6 days of exercise.

In comparison with previously mapped candidate eQTL in brain tissue (see Ref. 26), muscle tissue revealed fewer cis-
transcript eQTL (LOD / -log P)
or artificial selection acting on the complex phenotype of voluntary exercise.

Although we previously observed greater transcript expression levels and diversity in brain tissue (26), we detected far fewer significant relationships between gene expression and phenotypes compared with muscle tissue (Fig. 1). We observed an increase of ~1,168% in statistically significant relationships between muscle transcript expression levels and the same exercise and body composition phenotypes examined by Ref. 26. This increase is most notably reflected in the elevated number of significant partial correlations (controlling for sex and parent-of-origin type) between muscle transcript expression and exercise-related traits. Of note, the exercise trait most often significantly correlated with gene expression in the brain was running duration, while in the muscle it was maximum running speed. The differences in the distribution of proportions of correlations potentially indicate that total time spent engaging in exercise behavior may be more influenced by central (neurobiological) mechanisms, while intensity of exercise may be largely controlled by peripheral mechanisms (in the current case muscle morphology or physiology). Lending support to the above hypothesis, Ref. 31 observed that noninvasive brain stimulation over the temporal cortex in trained cyclists regulates activity of autonomic nervous system and the perception of effort during exercise.

We do acknowledge that a limitation to the current approach is the choice of a nonspecific brain region as our measure of comparison. This choice may have produced a significant signal dilution relative to skeletal muscle and may contribute, or account for, the large differences in the number of statistically significant relationships between muscle and brain transcript levels and relevant phenotypes. As we stated in Ref. 26, in our opinion, there is no one particular brain (or muscle) region sufficient to account for the diversity of behavioral and physiological traits measured in the G4 population (e.g., running distance, weight regulation, food consumption). Our compromise was to bisect the hemispheres, run our initial expression assays on one hemisphere, and reserve the remaining hemisphere for potential follow-up studies in a more focused/targeted fashion.

Identification of potential candidate genes. As was done previously (see Ref. 26), we will only discuss a fraction of the potential candidate genes depicted in Figs. 3 and 4. We have chosen the genes discussed below based on some combination of their cis-acting nature, colocalization with previously identified phenotypic eQTL, and significant correlation with phenotypes of interest.

Insulin induced gene 2 (Insig2) was found to be a highly significant cis-acting eQTL (LOD = 58.6), colocalizing with loci previously identified for exercise and body composition-related traits on Chr. 1. Insig2 has been previously associated with human obesity, total plasma cholesterol levels, cholesterol biosynthesis, and lipid and cholesterol metabolism (2, 9, 11, 20, 30). Similar findings for Insig2 were previously reported in brain tissue (LOD = 100.0 in Ref. 26), potentially indicating a dual role in underlying both motivation and ability for exercise behavior. Perhaps more importantly, these findings taken together reinforce the implication of Insig2 in the regulation of the relationship between exercise and body weight.

Prolyl carboxypeptidase (angiotensinase C) (Prcep; 2510048K03Rik, old name) was found to be a highly significant (LOD = 62.9) cis-acting eQTL colocalizing with previously identified QTL for running distance and duration on Chr. 7. Prcep-null mice showed elevated levels of brain α-MSH and reduced food intake, were leaner and shorter than wild-type controls, and were resistant to high-fat diet-induced obesity (22, 43). Similar phenotypes have been observed in the HR strain of mice utilized here (37, 41). Prcep was also a focal candidate gene in brain tissue (LOD = 99.5, in Ref. 26), colocalizing with the same phenotypes and as described may play a pivotal nontissue specific role in the regulation of the relationship between exercise and body weight.

Secreted acidic cysteine rich glycoprotein (Sparc) was found to be a highly significant (LOD = 37.5) cis-acting eQTL mapped to Chr. 11, a region contained within the confidence intervals of previously identified QTL for running maximum running on days 5 and 6. Sparc muscle secretion has been previously reported to increase following exercise and has been shown to inhibit colon tumorigenesis by increasing apoptosis (1). Partial correlations also revealed that Sparc was significantly correlated with maximal running speed, further increasing the likelihood of its functional relevance. Although not specifically highlighted in our previous work on brain tissue, Sparc was a significant cis-acting eQTL (LOD = 63.1) colocalizing with same phenotypes described above, however, not significantly correlating with any focal phenotype.

The validation of the results presented above will necessitate future studies and additional lines of evidence as described elsewhere (27, 29). A high priority should be to validate the functional role of these candidate genes, in the context of both motivation and ability to engage in voluntary exercise behavior. Regardless, these results coupled with those of Ref. 26 further develop an initial model of the underlying functional genomic architecture of predisposition to engage in voluntary exercise and its effects on body weight and composition in the context of a neurobiological and muscular physiological framework.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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