Genetic Modifiers of Cardiorespiratory Fitness Response to Lifestyle Intervention

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ABSTRACT

PETER, I., G. D. PAPANDONATOS, L. M. BELALCAZAR, Y. YANG, B. ERAR, J. M. JAKICIC, J. L. UNICK, A. BALASUBRAMANYAM, E. W. LIPKIN, L. M. DELAHANTY, L. E. WAGENKNECHT, R. R. WING, J. M. MCCAFFERY, and G. S. HUGGINS. Genetic Modifiers of Cardiorespiratory Fitness Response to Lifestyle Intervention. *Med. Sci. Sports Exerc.*, Vol. 46, No. 2, pp. 302–311, 2014. **Purpose**: Numerous prospective studies indicate that improved cardiorespiratory fitness reduces type 2 diabetes risk and delays disease progression. We hypothesized that genetic variants modify fitness response to an intensive lifestyle intervention (ILI) in the Action for Health in Diabetes (Look AHEAD) randomized clinical trial, aimed to detect whether ILI will reduce cardiovascular events in overweight/obese subjects with type 2 diabetes compared with a standard of care. **Methods**: Polymorphisms in established fitness genes and in all loci assayed on the Illumina CARe iSelect chip were examined as predictors of change in MET level, estimated using a treadmill test, in response to a 1-yr intervention in 3899 participants. **Results**: We identified a significant signal in previously reported fitness-related gene *RUNX1* that was associated with 1-yr METs response in ILI (0.19 ± 0.04 MET less improvement per minor allele copy; $P = 1.9 \times 10^{-5}$) and genotype–intervention interaction ($P = 4.8 \times 10^{-3}$). In the chipwide analysis, *FKBP7* rs17225700 showed a significant association with ILI response among subjects not receiving beta-blocker medications (0.47 ± 0.09 METs less improvement; $P = 5.3 \times 10^{-7}$) and genotype–treatment interaction ($P = 5.3 \times 10^{-5}$). The Gene Relationships Among Implicated Loci pathway-based analysis identified connections between associated genes, including those influencing vascular tone, muscle contraction, cardiac energy substrate dynamics, and muscle protein synthesis. **Conclusions**: This is the first study to identify genetic variants associated with fitness responses to a randomized lifestyle intervention in overweight/obese diabetic individuals. *RUNX1* and *FKBP7*, involved in erythropoesis and muscle protein synthesis, respectively, are related to change in cardiorespiratory fitness in response to exercise. **Key Words:** METABOLIC EQUIVALENT, CLINICAL TRIAL, CARE ISELECT IBC CHIP, GENOTYPE–TREATMENT INTERACTION

EPIDEMIOLOGY

The prevalence of type 2 diabetes (T2D) is expected to rise sharply for the next 40 yr to a level where one in three U.S. adults could be affected (8). Numerous prospective epidemiological studies indicate that regular physical activity is related to a 15%–60% reduction in risk of T2D (reviewed in [31]) and that behavioral intervention that promotes physical fitness can reduce progression from prediabetes to T2D by up to 58% (22). Cardiorespiratory fitness has been inversely associated with incident T2D (25) and cardiovascular events (23). Exercise programs designed to increase physical fitness are recommended to patients with established T2D. The benefit of exercise can be seen with improved insulin sensitivity as well as reduced adiposity and adipose tissue inflammation (3).

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Studies in animal models demonstrate a significant role for genetic background in physical endurance (2). Similarly, cardiorespiratory fitness in humans was found to be heritable, with heritability estimates ranging between 25% and 65% (reviewed in [37]). Genome-wide association studies (GWAS) conducted in the Framingham Heart Study and HERITAGE Family Study using large arrays of single nucleotide polymorphisms (SNPs) identified no variants associated with pretraining levels or changes in heart rate or fitness in response to training at the genome-wide significance level (P value $< 5 \times 10^{-8}$) (7,39). Suggestive signals, however, were identified in the ryanodine receptor gene (*RYR2*) as well as in other genes that have a plausible role in fitness including *ACE, ADRB1, AGT, AGTR1*, *KCNH8*, and others. In another study, SNPs in three muscle-related genes (*CNTF*, *AMPD1*, and *NR3C1*) predicted whether a patient with coronary artery disease responded to a 3-month ambulatory supervised exercise training regimen (38). Finally, a study using a combination of transcriptomics and genomics demonstrated that about half of the variance of $\dot{V}O_{2max}$ trainability was accounted for either by the abundance of 29 muscle transcripts or by 11 SNPs (20). While these studies demonstrate that genetic predictors of fitness are starting to emerge, there is currently insufficient evidence to implicate specific genes responsible for the interindividual variation in fitness. Newer gene-centric array-based genotyping technologies that permit improved coverage of the candidate genes, and potentially deep re-sequencing approaches, that capture genetic diversity across populations may prove more effective in identifying fitness genes. Here, we analyzed data from the ITMAT-Broad-CARe (IBC) chip (19), primarily aimed at assaying SNPs in candidate genes and pathways for cardiovascular, inflammatory, and metabolic phenotypes to better define the complex and poorly characterized role of genetics in human fitness.

The Action for Health in Diabetes (Look AHEAD) randomized clinical trial demonstrated that an intensive lifestyle intervention (ILI), including both caloric restriction and physical activity, produced significantly greater weight loss and improved measures of glucose control in participants with established T2D after 1 yr, compared with a control intervention of diabetes support and education (DSE) (29). The ILI was also effective in increasing cardiorespiratory fitness in Look AHEAD subjects (16).

Here, we hypothesized that genetic variants modify the fitness response to ILI compared with DSE in the presence of established T2D. To test this hypothesis, we analyzed whether SNPs within genes already implicated in physical fitness and present on the IBC chip were associated with changes in fitness in response to 1 yr of intervention in Look AHEAD. A differential response to intervention by genotype would help identify biological pathways involved in fitness.

MATERIALS AND METHODS

Study subjects. The design and methods of the Look AHEAD trial have been reported elsewhere (36), as have the baseline characteristics of the entire randomized cohort (9). Among 5145 ethnically diverse overweight and obese Look AHEAD subjects with T2D and age 45 to 76 yr at baseline, 4041 provided consent and DNA for genetic analysis. The Look AHEAD trial was approved by local institutional review boards, including genetic analyses.

Intervention. Subjects were randomly assigned to DSE or ILI. DSE received standard care plus 3 education sessions during the 1-yr period. ILI included individual and group contact throughout the year focusing on caloric restriction and increased physical activity, with the goal of achieving 10% or greater weight loss. ILI participants were instructed initially to increase their physical activity to at least 50 min·wk^{-1}, progressing to at least 175 min·wk^{-1} by week 26, with the intensity being moderate to vigorous (similar to brisk walking). Participants were also encouraged to increase lifestyle forms of physical activity (using stairs rather than elevators, walking rather than riding, and reducing use of labor saving devices).

Assessments. Subject characteristics, including age, sex, medication use, and race/ethnicity, were collected via questionnaire at baseline. Weight at baseline and 1 yr postrandomization was measured using the standardized methods as described previously (21).

Cardiorespiratory fitness was assessed using a graded exercise test (GXT) on a calibrated motor-driven treadmill as previously described using a standardized protocol (16). A self-selected walking speed of 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 mph was used with the speed held constant throughout the test. The grade of the treadmill was initiated at 0% and increased by 1% each minute until test termination. During the last 10 s of each minute and at the point of test termination, the heart rate was measured from a 12-lead ECG, and RPE was measured using the Borg 15-category scale (scale ranges from 6 to 20). Blood pressure was assessed during the last 45 s of each even minute and at test termination. A maximal graded exercise to the point of volitional fatigue was conducted at baseline. The baseline GXT was considered valid provided that that subject achieved either 85% of age-predicted maximal heart rate (defined as 220 − age) computed as if not taking a medication that would affect the heart rate response to exercise or RPE >18 if the subject was taking a medication that would affect the heart rate response to exercise (e.g., beta blocker). This baseline test was used to exclude individuals for whom exercise may have been contraindicated before study randomization. Because of cost constraints associated with the need for physician's presence for a maximal test regardless of health status, subjects completed a submaximal GXT at 1 yr using the same walking speed and grade increments as was used for the baseline test; however, the test was terminated at the point where the participant first exceeded 80% of age-predicted maximal heart rate if not on a beta-blocker at either baseline or year 1 or first exceeded RPE = 16 if on a beta-blocker at either baseline or year 1. The workload at test termination at 1 yr was compared with the workload from baseline where the same heart rate (80% age-predicted maximal heart rate) or RPE (RPE = 16) was

met on the baseline GXT. These workloads were converted to estimated METs using the American College of Sports Medicine's metabolic calculations for estimating energy expenditure (1), and the change in fitness was computed as the difference in METs at the same submaximal heart rate or RPE between the baseline and 1 yr GXT.

Genotyping and candidate gene selection. Genotyping was carried out on leukocyte DNA using the Illumina CARe iSelect chip (19), as previously described (28). Briefly, genomic DNA was extracted from whole blood (FlexiGene DNA Kit; Qiagen Inc., Valencia, CA) and genotyping was carried out at the Children's Hospital of Philadelphia. SNPs were clustered into genotypes using the Illumina BeadStudio software and subjected to quality control filters. Samples were excluded for individual call rates <90%, sex mismatch, and duplicate discordance. SNPs were removed for call rates <95%. Because of the low power for capturing genetic effects of the many low-frequency variants included in the design, we filtered out SNPs of minor allele frequency (MAF) <5%. This left 32,561 SNPs on the IBC chip with MAF ≥5%, whose mean genotyping success rate was 99.8%. After excluding individuals that failed the IBC chip genotyping, had low call rate, or had discrepancy between self-reported and X-chromosome-determined sex, the study cohort consisted of 3899 individuals. We performed a detailed literature review of all available fitness genetic association studies. From our review, we selected studies with a substantial sample size (>470 participants) that identified a total of 158 candidate genes previously reported by candidate gene, genetic linkage and GWAS, and gene expression studies to be associated with fitness traits based on standardized exercise treadmill test traits (Table 1, Supplementary Digital Content, http://links.lww.com/MSS/A343); 63 of these genes were represented on the IBC. We then performed gene-level replication by prioritizing the analysis of 1317 SNPs within the 63 genes included on IBC.

Statistical analysis. We conducted a joint analysis of baseline and 1-yr METs measurements, using an unstructured covariance matrix. Longitudinal models evaluating the effects of time (baseline vs 1 yr), study arm (ILI vs DSE), and individual SNPs markers (0/1/2 minor allele copies) and their interactions on fitness outcomes were estimated with Splus 8.2 (Tibco Software, Inc., 2010) using restricted maximum likelihood. An additive genetic model was assumed for all genetic markers, with regression coefficients interpreted as the effect on METs of each additional copy of the corresponding minor allele.

After excluding SNPs in linkage disequilibrium (LD, $r^2 > 0.3$), EIGENSTRAT was used to compute principal components for use as covariates to control for population admixture in the regression analyses (26).

Our models additionally adjusted for study site, sex, age, weight, use of beta-blockers, and the first two principal components to adjust for population admixture and controlled for the effect on fitness of both baseline values and of change in time-varying covariates allowing these effects to differ by study arm.

For candidate gene analyses, involving 1317 SNPs within 63 candidate genes previously reported to be associated with exercise treadmill test traits that were present on IBC (Table 1, Supplementary Digital Content, http://links.lww.com/MSS/A343), we determined the number of uncorrelated markers to be 687, after accounting for LD using the Li and Ji approach (24). Therefore, after adjustment for multiple hypothesis testing, a P value threshold for statistical significance was set at 7.4×10^{-5} when testing for 1-yr change in either the ILI or DSE arms. However, because these analyses attempt to replicate the associations with genetic markers previously implicated in METs and/or related treadmill-test traits, we also point out at least nominal (P value < 0.05) associations.

For chipwide analyses, we also calculated the effective number of uncorrelated markers among the 32,561 IBC SNPs under investigation and found it to equal 17,669 after LD correction (24). After controlling for multiple comparisons, this resulted in a chipwide significance threshold of $P = 2.9 \times 10^{-6}$. We used a false discovery rate (FDR) approach to guide our reporting of suggestive (FDR<20%) associations, operationalized via a rank ordering of the genetic markers according to their q-values. FDR controls the expected proportion of false-negative results among those deemed significant. The q-values are marker-specific quantities that recalibrate the rank ordering of P values by the probability that they represent a false discovery, calculated using the q-value package of Dabney et al. (10).

Given higher power needed to detect interaction effects, we did not explicitly test for ILI-DSE differences in genetic effects on METs change across the entire marker set. However, we do report these interactions for the subset of markers showing associations with METs change in either study arm, reducing the number of multiple comparisons. However, it may also cause us to miss interactions caused by genetic effects on both ILI and DSE change that are modest in size and of opposite sign.

In addition to the full-sample analyses, we conducted a sensitivity analysis excluding individuals receiving beta-blocker medications because their METs phenotype was calculated using different methodology than for the remaining study participants.

To identify biological associations between the top genes involved in cardiorespiratory fitness, the Gene Relationships Among Implicated Loci (GRAIL) was used (33). GRAIL scores association signals by evaluating whether observed genomic regions are nonrandomly linked to the other genes through word-similarity metrics in PubMed abstracts (33). We used the list of SNPs that showed at least nominal associations in the candidate genes and possible associations from the chipwide analyses (q-value < 0.30) with 1-yr response to ILI to assess the degree of connectivity between the genes.

RESULTS

Look AHEAD genetic study. At baseline, 3899 Look AHEAD subjects who participated in this genetic study were

evenly distributed between the ILI and DSE intervention arms with regard to age, sex, ethnicity, weight, and baseline fitness (Table 1). At 1 yr, METs levels of study participants that did not use beta-blockers at baseline increased, on average, by 1.02 U in individuals in ILI versus 0.23 U in DSE (Table 1). Comparable intervention effects were observed among individuals receiving beta-blockers at baseline, with METs levels increasing by 0.91 U in ILI versus 0.21 U in DSE. Beta-blocker use itself was stable across time, with only 5.3% of subjects switching regimens from baseline to follow-up.

Candidate gene analysis of treatment response. We analyzed the association of 1-yr change in METs for 1317 SNPs (Table 1, Supplementary Digital Content, http://links.lww.com/MSS/A343). After the adjustment for multiple hypothesis testing, one significant association was identified between *RUNX1* rs9976623 (MAF = 0.24) and 1-yr change in METs in the ILI group ($P = 1.9 \times 10^{-5}$; Table 2; Figure 1A, Supplementary Digital Content, http://links.lww.com/MSS/A344. Carriers of the *RUNX1* rs9976623 minor allele in the ILI group gained 0.19 ± 0.04 less METs per copy than noncarriers. Carriers in the DSE group showed no significant difference in 1-yr METs ($P = 0.82$). These ILI-DSE differences resulted in a nominally significant interaction ($P = 4.8 \times 10^{-3}$; Table 2) between rs9976623 minor allele status and treatment response. Two other SNP in moderate LD with rs9976623 showed nominal significance as indicated on the regional plot (see Figure 1A, Supplementary Digital Content, http://links.lww.com/MSS/A344). Minor alleles of multiple common *COL4A1* SNP, in LD with each other, showed at least nominally significant associations with 0.15 ± 0.04 more METs gain per copy than noncarriers in the ILI arm ($P < 2.6 \times 10^{-4}$; Table 2; Figure 1B, Supplementary Digital Content, http://links.lww.com/MSS/A344. No difference was detected in the DSE group ($P < 0.18$), resulting in at least nominally significant treatment–genotype interactions ($P < 1.1 \times 10^{-3}$, Table 2). In addition, at least nominally significant associations for within-arm change were detected for *ACE* and *AGT* in the DSE group and for *PRKAG2* in the ILI group ($P < 5 \times 10^{-4}$, Table 2).

Of the remaining 58 candidate genes on the IBC chip, at least nominally significant associations were observed between 1-yr change in response to ILI and DSE and multiple SNP within 29 genes, including *RYR2*, *CASR*, and *ACE* (see Table 1, Supplementary Digital Content, http://links.lww.com/MSS/A343). Moreover, the majority of these genes also showed at least nominal genotype–intervention interactions, indicating effect modification

TABLE 1. Characteristics of the Look AHEAD participants available for genetic study at baseline and after 1 yr of intervention.

Characteristic	Pooled	ILI	DSE
N	3899	1935 (50)	1964 (50)
Women (%)	2192 (56)	1096 (57)	1096 (56)
Ethnicity			
African American (%)	618 (16)	313 (16)	305 (16)
American Indian/Alaskan Native (%)	20 (0.5)	11 (0.6)	9 (0.5)
Asian/Pacific Islander (%)	41 (1)	22 (1)	19 (1)
Hispanic/Latino (%)	307 (8)	148 (8)	159 (8)
Non-Hispanic White (%)	2835 (73)	1405 (73)	1430 (73)
Other (multiple) (%)	78 (2)	36 (2)	42 (2)
Beta-blocker use at baseline (%)	893 (23)	470 (24)	423 (22)
Beta-blocker use at 1 yr (%)	877 (25)	466 (26)	411 (23)
Age (yr)	59.1 ± 6.8	59.0 ± 6.9	59.2 ± 6.8
Weight (kg) at baseline			
Women	96.7 ± 17.5	96.8 ± 17.7	96.6 ± 17.4
Men	109.6 ± 18.5	109.8 ± 19.2	109.4 ± 17.8
Weight (kg) at 1 yr			
Women	92.1 ± 17.8	88.7 ± 17.3	95.6 ± 17.5
Men	104.1 ± 18.9	99.4 ± 18.8	108.7 ± 17.9
Fitness (submaximal, METs) at baseline[a]			
Women	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.4
Men	5.7 ± 1.6	5.8 ± 1.6	5.6 ± 1.6
Subjects on beta-blockers			
Women	5.1 ± 1.2	5.1 ± 1.2	5.2 ± 1.2
Men	5.9 ± 1.5	5.9 ± 1.5	5.9 ± 1.6
Subjects not on beta-blockers			
Women	4.6 ± 1.4	4.6 ± 1.4	4.6 ± 1.4
Men	5.6 ± 1.7	5.7 ± 1.7	5.5 ± 1.6
Fitness (submaximal, METs) at 1 yr[a]			
Women	5.3 ± 1.6	5.6 ± 1.7	4.9 ± 1.4
Men	6.4 ± 2.0	6.9 ± 2.1	5.9 ± 1.7
Subjects on beta-blockers			
Women	5.6 ± 1.6	5.8 ± 1.6	5.3 ± 1.4
Men	6.6 ± 1.9	7.0 ± 1.9	6.2 ± 1.7
Subjects not on beta-blockers			
Women	5.2 ± 1.6	5.5 ± 1.7	4.8 ± 1.4
Men	6.3 ± 2.0	6.9 ± 2.1	5.8 ± 1.7

Values are presented as *n* (%) and mean ± SD.
[a]Estimated MET level based on the treadmill workload at 80% of HR_{max} in participants not using beta-blockers or at an RPE of 16 in those participants using beta-blockers.

TABLE 2. Top SNPs in previously reported candidate genes associated with 1-yr change in METs in either the ILI or DSE arms ($N = 3889$).[a]

SNP	Gene	Chr	Position	Marker Allele[b]	MAF	Beta ILI (SE)[c]	*P*-Value ILI	Beta DSE (SE)[c]	*P*-Value DSE	*P*-Value ILI-DSE
rs9976623	*RUNX1*	21	35191378	A/G	0.24	−0.19 (0.04)	1.90E−05	−0.01 (0.05)	0.82	4.78E−03
rs648705	*COL4A1*	13	109654154	C/A	0.40	0.15 (0.04)	1.76E−04	−0.05 (0.04)	0.23	5.11E−04
rs645098	*COL4A1*	13	109654272	A/G	0.33	0.15 (0.04)	2.38E−04	−0.04 (0.04)	0.33	1.09E−03
rs598893	*COL4A1*	13	109657744	A/G	0.40	0.15 (0.04)	2.58E−04	−0.06 (0.04)	0.18	4.73E−04
rs1860743	*PRKAG2*	7	151050874	G/A	0.10	−0.24 (0.07)	3.53E−04	0.03 (0.07)	0.62	3.78E−03
rs1800764	*ACE*	17	58904261	G/A	0.48	−0.01 (0.04)	0.73	−0.15 (0.04)	2.45E−04	1.95E−02
rs2148582	*AGT*	1	228916422	G/A	0.47	−0.01 (0.04)	0.88	0.14 (0.04)	4.16E−04	9.16E−03

[a]Ranking based on significance levels for within-arm change ($P < 5.0E-04$).
[b]Marker alleles are presented in major/minor allele order, as calculated from the full sample.
[c]Effect per minor allele (additive genetic model).
ILI, intensive lifestyle intervention; DSE, diabetes support and education.

in response to ILI and DSE based on the genotype status (data not shown).

Chipwide association analysis of response to lifestyle intervention. No variants were associated with 1-yr METs change in response to ILI or DSE at a chip-wide level of significance. However, in addition to *RUNX1*, 13 SNP representing 11 independent loci showed genetic associations with ILI treatment response at q-value < 0.20 after the adjustment for covariates (Table 3). The majority also showed nominal genotype–treatment interaction ($P < 0.05$). These findings included 2 SNPs each at *TBC1D1* and *MTMR15*, and one SNP each at *GNAI2*, *PROP1*, *FKBP7, SMURF1*, *NRG3*, *PLA2G4B*, *C20orf75*, and *THBD*. An intergenic SNP on chromosome 11p15.5 was not mapped to any known gene (Table 3).

Sensitivity analysis. Twenty-three percent of the Look AHEAD genetic sample ($N = 893$) received beta-blockers at the time of the treadmill test. As METs were estimated differently among participants receiving beta-blockers (see Materials and Methods section), we performed a sensitivity analysis excluding such subjects. In candidate gene analysis, multiple *COL4A1* SNP were at least nominally associated with fitness response to intervention with rs11069830 passing the threshold for suggestive association (Table 4). In chipwide analyses, the elimination of subjects taking beta-blockers changed the order of some of the top hits in the ILI group, with *FKBP7* rs17225700 now showing a stronger effect that passed the chipwide significance threshold (1-yr gain of 0.47 ± 0.09 less METs per allele copy; $P = 5.3 \times 10^{-7}$; Table 4). Specifically, Figure 1 demonstrates a significant effect of ILI on fitness change in *FKBP7* rs17225700-A allele carriers that was significantly diminished in GG homozygotes for both men and women. Substantial genotype–treatment interaction was also detected ($P = 5.3 \times 10^{-5}$). The new signals with q-value < 0.20 that were not detected in the pooled analysis included *DDN*, *RGS2*, *ALCAM*, *VAX2*, and *LOC652968* (Table 4).

Pathway-based analysis. To identify pathway connections between genes associated with fitness, we performed a GRAIL analysis. A strong connectivity between the genes by their enrichment in overlapping pathways was identified among 24 of the 33 previously reported fitness genes that were found to be at least nominally associated with change in METs in Look AHEAD and were in the GRAIL database, including those that influence vascular tone, muscle protein synthesis, and contraction as well as cardiac energy substrate dynamics (Figure 2, Supplementary Digital Content, http://links.lww.com/MSS/A345). Genes with the largest number of connections included *AGT*, *CAV1*, *NOS3*, *ADRA1A*, *ADRA1B*, *COL4A1*, *COL4A2*, and *ACE* (GRAIL $P < 2 \times 10^{-6}$, data not shown). Of the 35 genes associated with METs response to ILI in the chipwide analysis at q-value < 0.30, eight genes, *GHSR*, *HCRTR2*, *PROP1*,

TABLE 3. Top chip-wide associations for 1-yr change in METs in either the ILI or DSE arm.[a]

SNP	Gene	Chr	Position	Marker Allele[b]	MAF	Beta ILI (SE)[c]	*P*-Value ILI	Beta DSE (SE)[c]	*P*-Value DSE	*P*-Value ILI-DSE
rs17497074	*TBC1D1*	4	37614636	A/G	0.10	0.30 (0.06)	4.02E−06	0.00 (0.06)	0.97	1.25E−03
rs2735469	Intergenic	11	1979380	G/A	0.13	−0.26 (0.06)	7.24E−06	−0.08 (0.06)	0.19	2.39E−02
rs17225700	*FKBP7*	2	179044846	A/G	0.06	−0.35 (0.08)	1.54E−05	0.03 (0.08)	0.66	6.05E−04
rs2282751	*GNAI2*	3	50266789	G/A	0.25	0.22 (0.05)	2.57E−05	0.00 (0.05)	0.97	3.01E−03
rs2395018	*SMURF1*	7	98551822	G/A	0.22	0.23 (0.06)	4.04E−05	−0.03 (0.06)	0.57	1.23E−03
rs17579011	*TBC1D1*	4	37613244	G/A	0.28	0.18 (0.04)	4.42E−05	0.06 (0.04)	0.18	4.85E−02
rs565	*MTMR15*	15	29018482	G/A	0.18	−0.21 (0.05)	4.52E−05	−0.06 (0.05)	0.21	4.68E−02
rs6493352	*MTMR15*	15	29021356	G/A	0.18	−0.20 (0.05)	4.84E−05	−0.07 (0.05)	0.15	0.07
rs1197687	*PLA2G4B*	15	39905943	G/A	0.15	−0.22 (0.06)	7.11E−05	−0.02 (0.05)	0.77	8.64E−03
rs2233788	*PROP1*	5	177352193	A/G	0.08	0.28 (0.07)	7.12E−05	−0.01 (0.07)	0.88	3.49E−03
rs1040585	*THBD*	20	22988066	C/A	0.13	0.24 (0.06)	7.17E−05	0.12 (0.06)	0.04	0.16
rs3862551	*NRG3*	10	84088823	C/A	0.10	−0.28 (0.07)	8.79E−05	−0.08 (0.07)	0.26	4.17E−02
rs6038334	*C20orf75*	20	5965259	G/C	0.32	−0.16 (0.04)	8.80E−05	−0.05 (0.04)	0.28	4.85E−02

[a]Ranking based on chip wide FDR for within-arm change ($q < 0.20$).
[b]Marker alleles are presented in major/minor allele order, as calculated from the full sample.
[c]Effect per minor allele (additive genetic model).
ILI, Intensive Lifestyle Intervention; DSE, Diabetes Support and Education.

TABLE 4. Top SNP in previously reported candidate genes and chipwide associated with 1-yr change in METs in either the ILI or DSE arms in individuals not receiving beta-blockers (N = 3006).[a]

SNP	Gene	Chr	Position	Marker Allele[b]	MAF	Beta ILI (SE)[c]	*P*-Value ILI	Beta DSE (SE)[c]	*P*-Value DSE	*P*-Value ILI-DSE
Candidate gene analysis										
rs11069830	*COL4A1*	13	109619133	C/A	0.33	−0.17 (0.05)	3.84E−04	0.08 (0.05)	0.09	2.01E−04
Chipwide analysis										
rs17225700	*FKBP7*	2	179044846	A/G	0.06	−0.47 (0.09)	5.26E−07	0.05 (0.09)	0.58	5.25E−05
rs6038334	*C20orf75*	20	5965259	G/C	0.32	−0.22 (0.05)	6.88E−06	−0.06 (0.05)	0.19	2.63E−02
rs2735469	Intergenic	11	1979380	G/A	0.13	−0.30 (0.07)	1.65E−05	−0.03 (0.07)	0.67	4.79E−03
rs2811239	*HCRTR2*	6	55229913	G/A	0.17	0.25 (0.06)	1.78E−05	0.04 (0.06)	0.48	1.48E−02
rs7311091	***DDN***	12	47669474	G/A	0.06	−0.37 (0.09)	2.09E−05	0.09 (0.09)	0.32	2.49E−04
rs2746073	***RGS2***	1	191045850	T/A	0.24	0.22 (0.05)	2.14E−05	0.08 (0.05)	0.14	0.05
rs13418962	*STRN*	2	36990142	G/A	0.48	−0.19 (0.05)	3.52E−05	−0.02 (0.03)	0.64	9.68E−02
rs3122169	*HCRTR2*	6	55221370	A/C	0.18	0.23 (0.06)	4.16E−05	0.03 (0.06)	0.62	1.41E−02
rs12214549	*CDKAL1*	6	20670059	A/G	0.06	0.37 (0.09)	4.81E−05	0.16 (0.09)	0.07	0.11
rs572169	*GHSR*	3	173648421	G/A	0.27	−0.21 (0.05)	5.02E−05	−0.03 (0.05)	0.50	1.45E−02
rs978436	***ALCAM***	3	106712239	G/A	0.14	0.25 (0.06)	5.23E−05	0.03 (0.06)	0.59	1.45E−02
rs17497197	*STRN*	2	37614636	A/G	0.21	0.22 (0.06)	5.63E−05	0.01 (0.06)	0.88	7.01E−03
rs532625	*COL4A1*	13	109662226	A/T	0.45	−0.18 (0.05)	6.93E−05	0.05 (0.05)	0.32	4.41E−04
rs2233788	*PROP1*	5	177352193	A/G	0.08	0.33 (0.08)	7.09E−05	−0.01 (0.08)	0.89	3.32E−03
rs877549	***LOC652968***	22	29002420	G/A	0.11	0.27 (0.07)	9.38E−05	−0.04 (0.07)	0.55	1.65E−03
rs1981719	***VAX2***	2	71012756	A/G	0.38	0.18 (0.05)	1.03E−04	−0.04 (0.05)	0.35	6.47E−04
rs6576551	Intergenic	15	24027268	G/A	0.25	0.24 (0.06)	1.11E−04	0.01 (0.06)	0.87	7.21E−03
rs17497074	*TBC1D1*	4	37614636	A/G	0.10	0.29 (0.08)	1.12E−04	0.01 (0.07)	0.86	8.33E−03
rs2395018	*SMURF1*	7	98551822	G/A	0.22	0.25 (0.06)	1.15E−04	0.01 (0.07)	0.89	9.78E−03

[a]Ranking based on significance levels for within-arm change ($P < 5E-04$). Boldface indicates genes that were only detected in the analysis of individuals not receiving beta-blocker treatment.
[b]Marker alleles are presented in major/minor allele order, as calculated from the full sample.
[c]Effect per minor allele (additive genetic model).
ILI, intensive lifestyle intervention; DSE, diabetes support and education.

VWF, *THBD*, *TGFB3*, *CORIN*, and *TIPM1*, demonstrated significant connections, with GRAIL $P < 0.05$ (Fig. 2).

DISCUSSION

This is the largest study to date in a cohort of well-characterized overweight and obese individuals with T2D determining whether genetic variants located at or near genes previously associated with fitness traits or within ~2100 genes associated with cardiovascular, inflammatory, and metabolic traits help explain variation in fitness response to a 1-yr lifestyle intervention.

The candidate gene analysis identified a significant signal in *RUNX1*, previously identified as a fitness gene from the aerobic training-responsive transcriptome (20) that in the present study was associated with 1-yr METs response in the ILI group and genotype–intervention interaction. *RUNX1* is the runt-related transcription factor gene involved in erythropoesis. Thus, there is a plausible contribution to fitness via its effects on the red blood cell pool and the delivery of oxygen to tissues, including muscle, due to improved oxygenation during times of physical stress. Carriers of the *RUNX1* rs9976623 minor allele increased their fitness by 0.19 U less per copy in response to ILI; there was no association observed

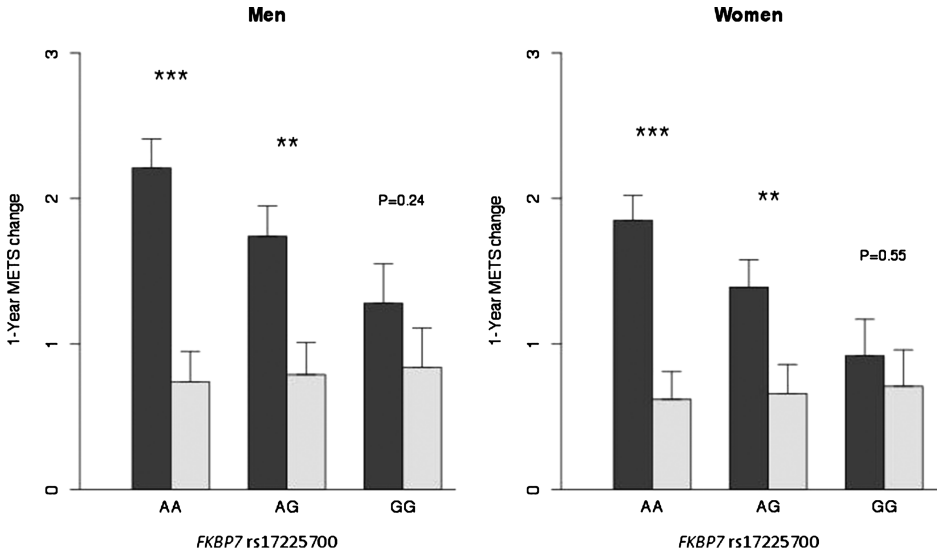

FIGURE 1—One-year change in METs in subjects not receiving beta-blockers by *FKBP7* rs17225700 and sex. ***$P < 0.001$, **$P < 0.01$. P value for interaction = 5.25E−05.

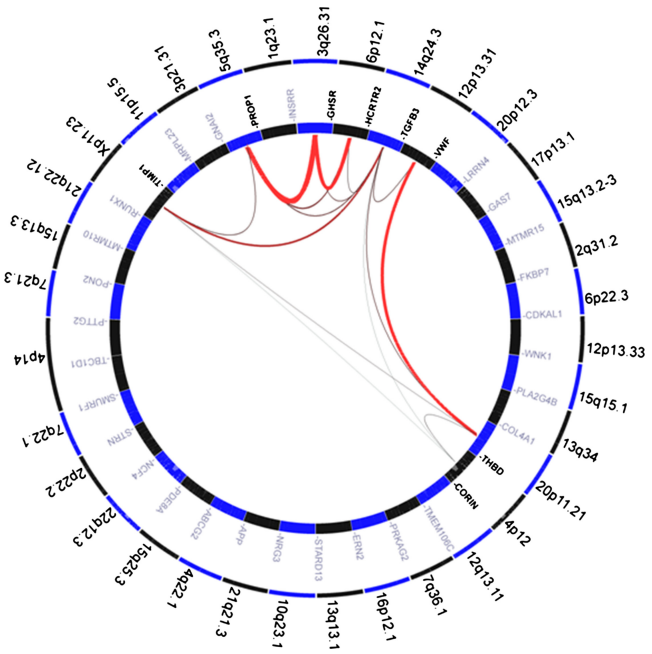

FIGURE 2—Pathway-based analysis of the top genes detected in chip-wide screening for 1-yr change in METs in the ILI group. Thirty five independent genes associated with METs at *q*-value < 0.30 are arranged along the inner circle using VIZ-GRAIL (32); bold indicates gene with GRAIL connections. The redness and thickness of lines connecting pairs of genes represent the strength of the connections with the thickness of the lines being inversely proportional to the probability that a literature-based connection would be seen by chance. Pathway-related links among 8 of the 35 genes that scored GRAIL *P* < 0.05.

in the DSE group. We also detected an at least nominally significant association with *COL4A1* and *PRKAG2* with METs change in ILI. *COL4A1* is the major type IV alpha collagen chain of basement membranes that has been observed to be differentially expressed in human muscle of high responders when compared with low responders after 6 wk of aerobic exercise training (20). Variants in *PRKAG2*, an energy sensor that modulates glucose uptake and glycolysis leading to enhanced glycolytic capacity and protection against hypoxic injury in tissues such as the heart (35), were also identified by the GWAS of treadmill exercise responses in the Framingham Heart Study to be associated with heart rate during the recovery period after exercise (39).

Variants in angiotensinogen (*AGT*) and angiotensin converting enzyme (*ACE*) were suggestively associated with change in fitness, but only in the DSE group. The *ACE* gene insertion/deletion (I/D), known to affect serum enzyme levels (34), has been associated with exercise response (17) and muscle endurance by some studies, but not others (14). The closest associated *coding* SNP rs1800764 was located only 140 base pairs away from the I/D variant, and has been reportedly associated with diabetic kidney disease (13).

We also observed nominally significant associations of multiple *RYR2* gene variants with fitness response. *RYR2* SNPs were found to be associated with heart rate during treadmill test in the Framingham Heart Study (39) and implicated in $\dot{V}O_{2max}$ training response to a standardized 20-wk exercise program in the HERITAGE study GWAS (7). The SNPs identified in our study were found to not be in LD with the *RYR2* SNP previously reported. *RYR2* is a cardiac-type ryanodine receptor that plays a key role in triggering cardiac muscle contraction (4). Defects in *RYR2* are the cause of familial arrhythmogenic right ventricular cardiomyopathy 2 and of exercise-induced polymorphic ventricular tachyarrhythmias (30).

Next, in an analyses of all SNPs across the IBC chip, no chipwide significant associations were identified in treatment response that passed the correction for multiple hypothesis testing. The strongest association with regard to ILI response was detected for *TBC1D1*, with carriers of the minor allele being more likely to gain METs. *TBC1D1* is an insulin-sensitive regulator of GLUT4 function in skeletal muscle, suggesting that variation in *TBC1D1* may alter glucose uptake, which could have effects on physical fitness. Importantly, a nonsynonymous polymorphism in the *TBC1D1* gene (*R125W*, rs35859249), located ~34 kb upstream of our top *TBC1D1* hit, has been associated with severe familial obesity (27). Interestingly, *TBC1D1* variation was also found to be associated with 1-yr weight loss in the ILI group (26).

Secondary analysis of data after exclusion of subjects receiving beta blockers revealed a variant in *FKBP7* that showed a significant association with ILI response and treatment interaction. Carriers of the minor allele randomized to ILI showed a 0.47 ± 0.09 less MET increase per copy, whereas no difference between the genotypes was detected in the DSE group. Kavanagh et al. (18) reported that each 0.3 MET increase in peak $\dot{V}O_2$ above a threshold of 3.7 METs associated with a marked benefit in prognosis of cardiovascular and all-cause mortality and conferred a 10% reduction in cardiac mortality in women with known coronary artery disease, indicating that the effect size observed in our study may have clinical implications. *FKBP7* is a member of the FKBP-type peptidyl-prolyl *cis*/*trans* isomerase family that interacts with FK-506, which is the drug target of rapamycin known to influence muscle protein synthesis in response to exercise (12). The role for the molecular chaperone FKBP7 in cellular signaling is not defined; however, our studies raise the possibility that FKBP7 may modulate cellular signaling processes related to fitness.

We sought to identify connections between all significant genes and those with the association *q*-value < 0.20 that influence fitness by applying GRAIL, a program that uses abstracts from the entirety of the published scientific literature to look for relatedness among genes within associated regions that may represent key pathways (33). *COL4A1*, whose variants we found to be associated with behavioral treatment interaction and fitness response in the ILI group, was linked with integrin beta 1 (*ITGB1*), along with caveolins (*CAV1* and *CAV2*). These genes were expressed differentially in human muscle from high and low responders to 6 wk of aerobic exercise training (20). GRAIL also identified

connectivity with nitric oxide synthase 3 (*NOS3*), or endothelial NOS, which regulates vascular smooth muscle relaxation, as well as *ADRA1A* and *ADRA1B*, which are expressed in the heart and play a major role in smooth muscle contraction (Figure 2, Supplementary Digital Content, http://links.lww.com/MSS/A345. *ADRA1B* has been involved in the control of vascular tone linked to cardiomyopathy and heart failure (5). Pathway-based analysis also suggested the involvement of endogenous hormones in cardiorespiratory response to ILI through association signals in *GHSR*, a growth hormone secretagogue receptor, and *PROP1*, responsible for pituitary development and hormone expression (Fig. 2). Interestingly, these genes were also biologically related to the hypocretin receptor type 2 (*HCRTR2*) gene, which encodes a G-protein-coupled receptor involved in the regulation of feeding behavior.

The strengths of this study include its randomized assignment of a lifestyle intervention of documented health relevance and the objective measurement of fitness by treadmill testing in the largest sample size to date. Further, the randomized intervention reduces the effect of confounding factors in association studies based on cross-sectional and observational data. Finally, the use of GRAIL to identify subsets of genes involved in similar biological processes related to cardiorespiratory fitness increases the confidence in the biological plausibility of our findings, beyond that provided by statistical estimates of FDR.

Although the IBC chip is a strength of our study, permitting analysis of more than 32,000 SNP in relation to fitness response, we note that this genotyping array is focused on ~2100 candidate genes previously associated with cardiovascular, inflammatory and metabolic phenotypes. Indeed, of 158 genes selected from the prior literature (Table 1, Supplementary Digital Content, http://links.lww.com/MSS/A343), we were able to represent at least one SNP in 63 of these genes. For example, no SNPs were available in *PAPSS2*, the region identified in a prior GWAS of physical activity participation (11). In several cases, the SNP available was not in close proximity or co-inherited with the previously identified marker. Therefore, although many new regions were queried in the present analysis, this array provided a limited window through which prior candidate gene and GWAS studies associated with fitness could be replicated, leaving the possibility that more direct replication attempts may prove fruitful.

Our intervention is also a strength, given its basis on the intervention deployed in the Diabetes Prevention Program that was successful in reducing diabetes incidence for 4 yr among individuals with impaired glucose tolerance (22). The Look AHEAD intervention also successfully increased cardiorespiratory fitness across more than 2500 overweight individuals with T2D. The Look AHEAD intervention, providing physical activity goals and behavioral strategies and counseling to support physical activity uptake and maintenance, nonetheless differs from many prior genetic studies of exercise involving directly supervised exercise. In particular, individuals exposed to the intervention may not have taken up exercise in the same frequency, intensity, and duration as would likely occur under supervised exercise training. We believe these approaches are complimentary, as genetic factors that influence uptake of exercise could be equally important as genetic factors that influence physiologic response to a standardize exercise training. Fitness changes resulting from flexible supervised moderate physical activity may be applicable to a larger segment of the population in the community and may elicit different physiologic changes when compared with those observed with standardized supervised exercise training.

We acknowledge additional limitations in our study, including the use of a submaximal test at year 1 and lack of replication cohort with comparable intervention and outcome measurements. While restricting our analysis to 1-yr follow-up may mitigate detectable genetic effects associated with the ability to improve cardiorespiratory fitness, we note that the largest change in weight and fitness in the Look AHEAD cohort occurred during the first year of ILI (40). Similar to other genetic association studies based on randomized clinical trials, our results may not be generalizable to the general population and our power to detect modest effects is limited. Because of the strong overlap between body size and fitness, weight change may influence change in physical fitness. To control for the effect of weight on fitness, we incorporated weight at baseline and 1-yr postintervention in our models. Finally, data on physical activity were not included in this study. Self-reported physical activity was available only in a subset of Look AHEAD participants, which would have considerably reduced our active sample size and power. Furthermore, our group has found that self-reported physical activity appears to overestimate exercise behaviors when compared with objectively-measured physical activity (6).

In summary, using a gene-centric genotype chip with ~2100 genes implicated in cardiovascular, inflammatory, and metabolic traits, we identified genetic associations of *RUNX1* and *FKBP7*, involved in erythropoesis and muscle protein synthesis, respectively, with change in cardiorespiratory fitness in response to lifestyle intervention. Despite the substantial sample size and state-of-the-art statistical approaches, we were able to detect a small number of significant associations. Our findings speak to the complex nature of the fitness phenotype, with multiple genes likely to be involved, each of modest effect size. We may have failed to identify important fitness genes or gene variants that were not included on the IBC chip. Future genome-wide association studies or genetic analyses that include rare variants (MAF<5%) may identify fitness gene variants not described here.

Looking forward, the replication of our findings in independent cohorts would allow for a targeted validation of the novel variants in the context of less stringent *P* values, such as those used in our study, due to a focused hypothesis testing approach. Similar to the analysis of other common traits, meta-analysis of fitness data from GWASs with larger

sample sizes can identify novel variants with smaller effects. However, it is important to acknowledge that fitness tests are very labor intensive and may not be readily performed on large epidemiologic cohorts. Nonetheless, standardized measures of fitness and physical activity could be adopted by large studies to allow for more powerful joint analyses. For example, PhenX is a tool designed to build consensus for standard measures of phenotypes and exposures used in genetic studies (15) that could help standardize fitness measures suitable for large genetic studies. In addition, next generation sequencing of DNA from participants with "phenotypic extremes" (i.e., highly physically trained individuals or persons resistant to training) may identify genetic variants responsible for extreme fitness responses. Mendelian randomization studies aimed at determining whether the contribution of the variants in the candidate fitness genes is causal for the development of cardiovascular and metabolic outcomes are warranted. Lastly, it will ultimately be important to integrate multiple genetic variations in the DNA code (e.g., SNPs, copy number variants, and methylation patterns) and gene expression to further explore the role of the genome in fitness.

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