Advances in Exercise, Fitness, and Performance Genomics in 2013

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Abstract

WOLFARTH, B., T. RANKINEN, J. M. HAGBERG, R. J. F. LOOS, L. PÉRUSSE, S. M. ROTH, M. A. SARZYNSKI, and C. BOUCHARD. Advances in Exercise, Fitness, and Performance Genomics in 2013. *Med. Sci. Sports Exerc.*, Vol. 46, No. 5, pp. 851–859, 2014. The most significant and scientifically sound articles in exercise genomics that were published in 2013 are reviewed in this report. No article on the genetic basis of sedentary behavior or physical activity level was identified. A calcineurin- and alpha actinin-2–based mechanism has been identified as the potential molecular basis for the observed lower muscular strength and power in alpha actinin-3–deficient individuals. Although baseline muscle transcriptomic signatures were found to be associated with strength training-induced muscle hypertrophy, no predictive genomic variants could be identified as of yet. One study found no clear evidence that the inverse relation between physical activity level and incident CHD events was influenced by 58 genomic variants clustered into four genetic scores. Lower physical activity level in North American populations may be driving the apparent risk of obesity in fat mass- and obesity-associated gene (*FTO*)-susceptible individuals compared with more active populations. Two large studies revealed that common genetic variants associated with baseline levels of plasma HDL cholesterol and triglycerides are not clear predictors of changes induced by interventions focused on weight loss, diet, and physical activity behavior. One large study from Japan reported that a higher fitness level attenuated the arterial stiffness-promoting effect of the Ala54 allele at the fatty acid binding protein 2 locus, which is a controversial finding because previous studies have suggested that Thr54 was the risk allele. Using transcriptomics to generate genomic targets in an unbiased manner for subsequent DNA sequence variants studies appears to be a growing trend. Moreover, exercise genomics is rapidly embracing gene and pathway analysis to better define the underlying biology and provide a foundation for the study of human variation. **Key Words:** GENETICS, EXERCISE TRAINING, CANDIDATE GENES, GENE-EXERCISE INTERACTION, SINGLE NUCLEOTIDE POLYMORPHISM, GENOME-WIDE ASSOCIATION STUDY, GENOMIC PREDICTORS

This publication is the fifth installment of an exercise genetics and genomics review. It summarizes the relevant literature published in the calendar year 2013. It is worth repeating that the review focuses on the strongest studies as defined by study design, sample size, novelty, and relevance of phenotypes considered in the study and potential implications for exercise science and sports medicine. The review is not a comprehensive summary of all published literature on genetics and genomics relative to exercise, fitness, and performance, as was explained in prior yearly installments of the publication.

The 2013 review is organized around the following topics: (a) muscular strength and power, (b) cardiorespiratory fitness and endurance performance, (c) body weight and adiposity, (d) insulin and glucose metabolism phenotypes, (e) lipid and lipoprotein metabolism, and (f) hemodynamic traits. The article ends with a discussion of the evidence and comments on issues that are fundamental to successful pursuits in exercise genomics research. Note that there were no articles published in the past year on the

genetics or genomics of physical activity behavior as such and exercise intolerance.

MUSCULAR STRENGTH AND POWER

In 2013, three articles made important contributions to our understanding of the genomics of muscular strength and power. In the first, Seto et al. (32) performed an extensive set of experiments examining the mechanisms behind the association of alpha actinin-3 (*ACTN3*) genotype and alterations in skeletal muscle performance and fiber-type phenotypes. This research group was the first to identify the R577X polymorphism in *ACTN3* as a potential contributor to strength and power performance (38) and later developed an *Actn3* knockout (KO) mouse model (19,20) to better understand the influence of ACTN3 deficiency on skeletal muscle traits observed in homozygous carriers of the nonsense polymorphism (X-allele) in *ACTN3*. In this most recent study, the group examined calcineurin activity in both mouse and human skeletal muscle with and without ACTN3. Calcineurin is an important signaling molecule in skeletal muscle, with its activation resulting in a transition to slow-twitch, oxidative phenotypes (7) and fatigue resistance (15) in skeletal muscle fibers. Consistent with their previous findings of enhanced oxidative metabolism in ACTN3-deficient muscle, Seto et al. (32) observed higher calcineurin activity in both *Actn3* KO mouse and *ACTN3* X/X human skeletal muscle compared with *ACTN3* positive muscle. The authors went on to demonstrate the role of alpha actinin-2 (ACTN2), which is upregulated in ACTN3 deficiency, in competing with calcineurin for binding with calsarcin-2, providing mechanistic insights into the signaling pathway. Thus, a mechanism for the reduction of strength and power characteristics observed in ACTN3-deficient skeletal muscle may have been identified. This is also one of very few studies to directly compare the *Actn3* KO mouse and *ACTN3* X/X human skeletal muscle; additional work is needed to clarify differences in the muscular traits noted in these two models.

Two articles examined skeletal muscle molecular networks in relation to hypertrophic responses to strength training. The first by Thalacker-Mercer et al. (34) explored the potential of predicting the hypertrophic response to strength training. In its previous work, the group identified skeletal muscle transcriptome profiles of men and women (*n* = 66) who were categorized as nonresponders, moderate responders, and extreme responders to strength training on the basis of vastus lateralis fiber size responses (2,25), with mechano growth factor and myogenin identified as differentially expressed among the groups. In this most recent study (34), the authors reported differential baseline (pretraining) transcriptional profiles that point to a propensity for strength training-induced hypertrophy in some individuals. In comparison to the nonresponders, responder groups demonstrated baseline gene expression profiles consistent with skeletal muscle development that were lower or absent in nonresponders (e.g., paxillin, 1.5-fold higher in extreme responders vs nonresponders; hepatocyte growth factor; paired box 7), possibly because of elevated proinflammatory signaling in the nonresponders. Moreover, the responder groups exhibited higher levels of some proteins at baseline (e.g., acetylated histone H3) and after (e.g., alpha tubulin; cyclin-dependent kinase inhibitor 1B) the first strength training bout compared with nonresponders. No attempt was made in this particular study to relate these differential expression levels to DNA sequence differences.

In the second molecular network article, Phillips et al. (26) similarly examined vastus lateralis genome-wide transcriptome profiles in 38 individuals before and after 20 wk of strength training. The subjects exhibited a wide range of hypertrophic responses to training, which correlated most closely with differences in baseline serine/threonine-protein kinase mTOR signaling. Unexpectedly, subjects with the lowest levels of expression (i.e., apparent inhibition of mTOR signaling) exhibited the greatest strength training-induced hypertrophy. Inhibition of myc proto-oncogene protein transcriptional activity was similarly correlated with lean mass gains. The authors confirmed that sex, age, and baseline lean mass did not account for the differences in hypertrophic response. In addition to these findings, Phillips et al. reported transcriptome profiles associated with decreases in skeletal muscle mass with aging (e.g., activation of progesterone receptor and retinoid X receptor and inhibition of v-myc avian myelocytomatosis viral oncogene homolog), observing considerable differences between the transcriptome profiles associated with strength training and those associated with age-related molecular signaling in skeletal muscle. Chromosomal loci on 1q12 and 13q21 contributed more than by chance to the age-related muscle variability gene list.

Comparison of the articles by Thalacker-Mercer et al. (34) and Phillips et al. (26) does not reveal a clear concordance of results; however, the groups used different microarrays for their baseline transcriptome profiling, and the age ranges of subjects somewhat differed. Thus, additional replication is needed to demonstrate predictive values. Nonetheless, these articles provide initial insights into the baseline transcriptomic signatures that may be predictive of hypertrophic responses and indicate the likelihood of a genetic contribution to strength training-induced skeletal muscle hypertrophy. They have revealed potential new targets for human variability in skeletal muscle mass and functions and their responses to exercise training and aging that should be further investigated for the contributions of DNA sequence differences.

CARDIORESPIRATORY FITNESS AND ENDURANCE PERFORMANCE

Three provocative articles published in 2013 have been retained for this review. The study of Seto et al. (32) from the laboratory of Kathryn North of the Murdoch Childrens Research Institute, briefly discussed already in the Muscular Strength and Power section, also reported data of interest to

human adaptation to endurance training. In one experiment, six *Actn3* wild-type and nine *Actn3* KO mice were trained for 4 wk, 5 d·wk^{-1}, with training intensity increasing to a maximum of 10% grade and 20 m·min^{-1} running speed. It was found that calcineurin activity was increased in ACTN3-deficient muscles (*Actn3* KO mice), which apparently potentiated the response to endurance training. Comparing the quadriceps muscle of sedentary and trained *Actn3* wild-type and KO mice, they showed that *Actn3* KO muscles exhibited higher oxidative enzyme activities and activation of a slow myogenic program leading to altered fiber-type distribution and cross-sectional area in response to endurance training. To confirm that these observations were relevant to humans, they examined muscle biopsies from 11 humans with *ACTN3* 577RR (wild type, $n = 5$) and 577XX (*ACTN3* deficient, $n = 6$) genotypes for differences in calcineurin activity. Consistent with the animal results, they found increased expression of the regulator of calcineurin (*RCAN1-4*) in *ACTN3* 577XX humans compared with humans with the *ACTN3* wild-type version (2.4-fold, $P = 0.004$).

In summary, the authors provided highly suggestive evidence that an ACTN3-deficient muscle has an enhanced adaptive response to endurance training because of its increased calcineurin activity. Despite all the previous reports on the role of *ACTN3* genotypes on skeletal muscle physiology and association with muscle strength and power, little had been available up to now on the potential mechanisms involved and on the role that the mutation may play in adaptation to endurance training.

In another study that we believe is worth highlighting, Chomistek et al. (8) investigated the interaction between panels of DNA variants of the so-called "fitness genes" to test whether they influenced the association between physical activity and CHD. They relied on the data of a prospective cohort study of 23,016 initially healthy women from the Women's Genome Health study. The median observation time was 14.4 yr during which 320 incident CHD events occurred. They found the well-known reverse association between physical activity level and CHD events ($P < 0.001$). To identify whether specific gene variants were influencing this relation, they used the 2009 version of "The Human Gene Map for Performance and Health-Related Fitness Phenotypes: The 2006–2007 Update" (6) and retained 58 single nucleotide polymorphisms (SNP) from which they created four separate genetic fitness scores that they labeled endurance, muscle strength, $\dot{V}O_{2max}$, and overall fitness. Using statistical modeling, they found only a weak inverse association between the muscle strength SNP score and CHD events ($P = 0.05$). There was no evidence that the inverse relation between physical activity and CHD was modified by any of the other fitness genetic scores. Although this is the first study to address the issue of whether genetic variations can differentially modify the associations between health outcomes and activity level (and perhaps sedentary behavior), this is clearly a research track that should receive more attention in the exercise genomics community.

One case-control study attempted to overcome the common problem of small sample size so endemic in exercise genomics reports of this type. Eynon et al. (9) pooled subjects from Spain, Poland, and Russia, resulting in a cohort comprising 551 athletes (266 endurance athletes and 285 sprint/power athletes) and 1416 ethnically matched controls. They asked whether the fat mass and obesity-associated (*FTO*) A/T polymorphism (rs9939609) was related to athletic status in endurance and sprint/power events. They concluded that there was no association between athletic status and the *FTO* A/T polymorphism. Such results must always be interpreted with caution because an endurance athlete (or power athlete, etc.) in one sample may not be exactly the same as an endurance athlete in another sample. The assumption being made in such studies (which are generally based on samples collected for different studies) is that if the effect size from a DNA variant is large enough, it should be picked up despite the limitations associated with pooling heterogeneous samples.

BODY WEIGHT AND ADIPOSITY

In the past year, large-scale genome-wide association studies (GWAS) identified 18 additional loci that show robust associations with obesity-related traits. Three of these novel loci are associated with body mass index (BMI) in African ancestry populations (22), 11 loci were identified in a GWAS that compared the extremes of the distributions of BMI and waist-to-hip ratio (4), and four new loci were reported for association with severe early-onset obesity (37). These 18 novel loci, together with the 59 previously established loci, bring the total number of obesity-susceptibility loci to 77. Because these loci, in particular the most recent ones, have been identified in GWAS reports that comprised tens of thousands of individuals, studies that aim to follow up on the identified loci need to be large as well to ensure unequivocal inferences. But so far, few large-scale follow-up studies have examined the role of physical activity as either a mediating or interacting factor in the associations between these loci and obesity risk.

In our 2010 review (12), we described the findings of a British population-based study of 20,430 individuals of European ancestry in which the influence of physical activity on the genetic susceptibility to obesity was examined (18). The genetic susceptibility was assessed using a genetic risk score (GRS) that counted the number of BMI-increasing alleles at 12 established BMI-associated loci that each study participant had inherited. The study showed that a higher GRS was associated with an increased risk of obesity. Most importantly, in physically active individuals, the effect of this GRS on obesity risk was approximately 40% less pronounced than that in those who lived a sedentary lifestyle (18).

In 2013, a meta-analysis of 11 studies including 111,421 individuals of European descent from across Europe (eight studies, 71,611 individuals) and North America (three studies, 39,810 individuals) aimed to replicate the previously observed

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interaction between genetic susceptibility to obesity and physical activity and to further explore how study-specific characteristics influenced their observations (1). Using the same GRS based on 12 BMI-associated loci, the meta-analysis showed that each additional risk allele increased BMI by 0.16 kg·m^{-2} ($P = 2.1 \times 10^{-176}$) (equivalent to 465 g per allele for a 1.70-m-tall person) (1), which is similar to the overall effect reported in the previous study (18). Ahmad et al. (1) subsequently replicated the observation that physical activity attenuated the association between the GRS and BMI ($P_{interaction} = 0.015$). More specifically, in physically inactive individuals (approximately 20% of the population, assessed with the Cambridge Physical Activity Index), each additional risk allele in the GRS increased the BMI by 0.186 kg·m^{-2} (or 538 g for a 1.70-m-tall person), whereas in physically active individuals (80% of the population), the increase in BMI was 0.150 kg·m^{-2} per risk allele (or 434 g for a 1.70-m-tall person). Although the effect attenuation is directionally consistent with the previous study (18), the difference in the effect of the GRS on BMI between active and inactive individuals is only half of that seen before (i.e., approximately 19% vs approximately 40%). Furthermore, even though the original study was in British individuals, when the current meta-analysis was stratified by continent, the interaction between the GRS and physical activity reached significance only in studies from North America ($P_{interaction} = 0.014$), not in studies from Europe ($P_{interaction} = 0.28$). But the interaction effect estimates were directionally consistent in studies of both continents and did not differ significantly from each other.

In subsequent meta-analyses that tested the influence of physical activity on the BMI association of each of the 12 loci separately, only the FTO ($P_{interaction} = 0.003$) locus showed evidence of a significant interaction after accounting for multiple testing. Consistent with the observations for the overall GRS, the interaction effect between the FTO locus and physical activity was more pronounced in studies from North America than from Europe. Furthermore, when the FTO locus was removed from the GRS, no significant interaction between this new GRS and physical activity on BMI was observed in all ($P_{interaction} = 0.25$), in North American ($P_{interaction} = 0.39$), or in European studies ($P_{interaction} = 0.44$). Together, these findings suggest that the observed interaction between the overall GRS and physical activity on BMI in the 111,421 individuals may have been driven by the interactions seen for FTO, in particular, in the studies from North America (1). This is consistent with the results from the meta-analysis on the interaction between the FTO locus and physical activity in 218,166 individuals from 45 studies (17), which we described in our 2011 review (31). In this meta-analysis, the interaction was significant in studies from both continents but more pronounced in those from North America than in those from Europe (17). Of note is that 56% of the North American individuals in the previous meta-analysis (17) overlap with those studied in the current meta-analysis (1); thus, results do not represent independent observations. We speculated in our

previous review that the reason for the difference between continents might be the generally lower physical activity levels and higher obesity prevalence among North Americans than Europeans, but also that differences in physical activity measurement might underlie the divide (31).

In summary, the recent large-scale meta-analysis (1) replicates observations made 3 yr ago to the effect that a physically active lifestyle attenuates the genetic susceptibility to obesity (18). However, the attenuation is only half as large as originally reported. We can posit that it will take very large studies to confirm whether interactions with physical activity are indeed observed only for FTO or whether other obesity-associated loci, individually or combined, are also sensitive to physical activity levels.

INSULIN AND GLUCOSE METABOLISM PHENOTYPES

Two studies providing evidence of gene–exercise or gene–physical activity interactions for insulin and glucose metabolism phenotypes or diabetes risk were retained for inclusion in this year's review. One study was based on data from the Diabetes Prevention Program, a large multicenter trial in which 3234 subjects with elevated fasting glucose and impaired glucose tolerance were randomized to placebo, metformin, or a lifestyle program aimed at producing 7% weight loss through healthy eating and physical activity (150 min of moderate-intensity physical activity per week). In that study, 20 SNPs in the melanocortin 4 receptor gene (MC4R) were tested for association with short-term (6 months) and long-term (2 yr) weight changes and type 2 diabetes incidence (23). The MC4R SNP rs17066829 showed nominal evidence of lifestyle program interaction ($P < 0.05$) with risk of diabetes, each copy of the minor allele at SNP rs17066829 being associated with a reduced risk of diabetes. Despite the large number of subjects, the nominal evidence of interaction reported did not withstand adjustment for multiple testing.

A total of 211 nondiabetic overweight subjects were enrolled into a 6-wk lifestyle intervention program combining diet (step 1 National Cholesterol Education Program—Adult Treatment Panel III low-caloric diet) and exercise (30-min brisk walk). The ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) K121Q polymorphism (rs1044498), a nonsynonymous polymorphism with the less common Q121 variant being a stronger inhibitor of insulin receptor signaling, was found to modulate the effect of weight loss on fasting glucose (21). The relation between BMI changes and changes in plasma glucose was significant ($P = 0.00008$) only in the Q121 subjects, who showed a greater reduction of glucose levels per unit of BMI loss compared with KK subjects.

LIPID AND LIPOPROTEIN METABOLISM

The year 2013 did not generate a large number of publications examining gene–exercise or gene–physical activity interactions on lipid and lipoprotein phenotypes. Of the

articles that were published, the majority had small sample sizes and included three or fewer genetic variants. However, one study that examined whether genetic factors modify lipid and lipoprotein responses to a randomized lifestyle behavior intervention in overweight/obese diabetic individuals stood out because of its quality and large sample size. Specifically, Huggins et al. (13) examined whether 82 SNPs from 31 loci, identified in previous GWAS reports to be associated with HDL cholesterol (HDL-C) and/or triglycerides (TG), modified the responses of HDL-C and TG to a 1-yr intensive lifestyle intervention (that included physical activity) relative to usual care in over 3500 participants of the Look AHEAD study.

The Look AHEAD study is a multicenter trial that randomly assigned participants with type 2 diabetes who were overweight or obese to an Intensive Lifestyle Intervention (ILI), with the goal of producing 7% weight loss through calorie restriction and physical activity, or to Diabetes Support and Education (DSE), with no weight loss or physical activity goals (35). The physical activity program prescribed in the ILI relied heavily on home-based exercise with gradual progression toward a goal of 175 min of moderate-intensity physical activity per week. Both groups improved HDL-C and TG levels at year 1, with the ILI group showing significantly greater improvements compared with the DSE group (27). The authors found that 12 and 6 SNPs showed nominal interactions ($P < 0.05$) with response to behavioral treatment (i.e., SNP–treatment interaction) for HDL-C and TG, respectively. In stratified analyses, half of the 12 SNPs were associated with HDL-C change and two of the six SNPs with TG change in response to ILI.

Two hepatic lipase (*LIPC*) SNPs showed evidence of behavioral treatment effect modification at year 1 for both HDL-C and TG in the full cohort, whereas a third *LIPC* SNP was associated in non-Hispanic whites only. Cholesteryl ester transfer protein, plasma (*CETP*) SNP rs3764261 was the only SNP significantly associated with baseline HDL-C ($P = 2.5 \times 10^{-24}$) that was also associated with behavioral treatment response. Specifically, *CETP* rs3764261 was associated with HDL-C change in response to ILI ($P = 0.0038$) and showed a nominal treatment interaction at year 1 ($P = 0.047$). In response to ILI, *CETP* rs3764261 minor allele carriers exhibited a greater increase in HDL-C than noncarriers (0.81 mg·dL^{-1} per minor allele copy; 95% CI, 0.26–1.36) in both men and women, with no difference by minor allele status observed in the DSE group. Conversely, minor alleles within the glucokinase regulator (*GCKR*), apolipoprotein B (*APOB*), and zinc finger protein 259 (*ZNF259*) predicted resistance to HDL-C improvement to ILI (i.e., decreases in HDL-C after ILI). The strongest SNP–treatment interaction was found with phosphatidylglycerophosphate synthase-1 (*PGS1*) rs4082919 on TG in the total sample ($P = 0.005$) and in non-Hispanic whites only ($P = 0.0009$), as each copy of the minor allele was associated with a 3% reduction in TG within the ILI group and a 4% increase in the DSE group (13).

The study by Huggins et al. represents one of the largest studies to date to examine how the interaction of genetic factors with a behavioral intervention affects changes in lipid and lipoprotein traits. The obvious strengths of the study are its large sample size, randomized design, controlling for various confounding factors such as medications and hormone replacement therapy, and the inclusion of multiple SNPs from 31 loci identified by previous lipid GWAS reports. The weaknesses of the study include the fact that the authors reported on nominally significant interactions that did not meet their experiment-wide multiple testing-corrected threshold of $P < 0.0009$. The generalizability of this study is limited, because all subjects were overweight/obese and diabetic middle-aged adults. Also, the physical activity intervention was less strenuous and standardized compared with clinic-based exercise training studies. However, given the fact that the physical activity portion of the ILI took place at home, the improvements in fitness and the lipid profile found in Look AHEAD are likely to be applicable to the general population of people with diabetes.

In summary, the Look AHEAD–based study indicates that genetic variants associated with HDL-C and TG levels cross-sectionally do not necessarily predict behavioral treatment response. These observations are concordant with those previously reported for baseline level versus response traits in several experimental situations. Similarly, in the Diabetes Prevention Program, a GRS based on 32 GWAS-based lipid SNPs was significantly associated with baseline HDL-C and TG levels but did not modify the effect of a 1-yr lifestyle intervention on either trait (28). These studies highlight the need for unbiased, systematic genome-wide analyses of gene–exercise or gene–physical activity intervention interactions on lipid and lipoprotein levels.

HEMODYNAMIC TRAITS

A search of the literature indicates that three articles with reasonable sample sizes were published in 2013 that addressed the association between exercise or physical activity, cardiovascular parameters, and genotypes (10,30,33).

One of the retained articles was that of Fujie et al. (10) in which, in a cross-sectional study in 837 Japanese men and women, the independent and interactive effects of cardiovascular fitness, measured during a progressive cycle ergometer test, and fatty acid binding protein 2, intestinal (*FABP2*) Ala54Thr genotype on cardiovascular, metabolic, and body composition parameters were assessed. One concern with this study is why this gene was selected as the primary candidate locus, especially because the gene is primarily expressed in the small intestine. It is generally considered a "metabolic" gene, and the authors provided virtually no biological plausibility for it as a candidate locus to affect cardiovascular parameters. Moreover, one could wonder whether several genetic variants were actually typed on these individuals, but perhaps the only one demonstrating statistically significant genotype-dependent effects was *FABP2*.

In fact, this metabolic gene variant did not have a significant effect, either independently or interactively with cardiovascular fitness levels, on any of the metabolic parameters assessed, including plasma lipoprotein lipid and blood glucose levels. *FABP2* genotype also had no independent effect on the cardiovascular parameters that were measured. However, the investigators did find significant interactive effects of *FABP2* genotype and cardiovascular fitness on arterial β-stiffness and systolic and diastolic blood pressure. For all three of these measures, *FABP2* Ala carriers or homozygotes had the highest values in the low-cardiovascular-fitness group, exhibiting approximately 20% higher β-stiffness and 3–5 mm Hg higher systolic and diastolic blood pressure. But in the high-fitness individuals, *FABP2* genotype had no effect whatsoever on these cardiovascular phenotypes, and all genotype groups exhibited values similar to the low-risk, low-cardiovascular-fitness Thr homozygotes.

Thus, on the basis of these results, it could be argued that back when humans had a much less sedentary lifestyle, there would have been no effect of the common *FABP2* Ala54Thr because everyone would have been in the high-fitness group. However, a somewhat inconsistent finding in this study is that the high-risk allele at this locus was the Ala54 (greater arterial stiffness, higher blood pressure in low-fitness individuals), whereas in virtually all previous studies, either the Thr54 allele was found to be the high-risk allele or no effect of the variant was evident (36). It is also possible that this discrepant finding is the result of different ethnic backgrounds, because the individuals in the present study were Japanese, whereas most of the previous studies were in Caucasian populations.

The remaining two studies, with sample sizes of 131 and 143, addressed different clinically relevant cardiovascular phenotypes (30,33). Silva et al. (33) reported that an endothelial nitric oxide synthase (*NOS3*) haplotype consisting of the C-786T and G894T variants impacted the vascular reactivity after a maximal exercise test in sedentary healthy men and women. In the final study, Rokamp et al. (30) reported that the G16R β2-adrenergic receptor (*ADRB2*) polymorphism was associated with a 0.4- to 0.5-L·min^{-1} difference in cardiac output at rest and during submaximal exercise.

COMMENTS AND SUMMARY

As a group of collaborators, we have been reviewing the exercise genetics and genomics scientific literature for 14 yr. It is obvious to us, and it should be to those who have been following the yearly installments of the reviews, that the field has grown in sophistication and that the science has become stronger. The crop of 2013 articles is typical in this regard. What is becoming increasingly clear is that exercise scientists who have an interest in exercise genomics questions are adopting new emerging technologies at a faster pace compared with prior decades. This bodes well, as science is dramatically influenced and often driven by new technologies. Consistent with the defined aims of this series

of special reports, we have again this year relied on our collective wisdom in the search for the strongest and most significant articles. We search for innovation and excellence in study design, phenotype measurements, and exercise or physical activity exposure, but we are also strongly influenced by sample size, standards for statistical evidence, quality of genotyping, and other methodological issues.

Human genetics has led the way in advocating for high standards before statistical significance is recognized. One of the most vivid examples is the worldwide recognition among geneticists that genome-wide explorations for association with a trait should use an alpha level of 5×10^{-8} as the threshold for declaring that an association is statistically significant. Unfortunately, such high standards are the exception. Most articles still rely on an alpha level of 0.05 to conclude about the significance of an observation. Considering that most articles report multiple and often many tests of significance, there is a high likelihood that many of the findings are false positive. This has led some to provocatively affirm that a good number of published research findings from human studies are false (14). One suggestion has been made recently to change the old way and use more rigorous standards such as a minimum *P* level of 0.005 for significance and 0.001 for high significance (16). This is an area where exercise genomics should take the lead. Implementing higher standards for evidence would have a salutary effect on the credibility of our science and published research.

We have seen this year (a trend that began a few years ago) several articles genotyping multiple genetic markers, or using GRS, or relying on microarray analyses to identify genomic targets, or performing pathway and network analyses on the basis of evidence for genomics or transcriptomics. These are encouraging advances, but more rigorous standards must be adhered to if these advances are to translate into true findings. We review here a few examples of such cases.

Ben-Zaken et al. (3) compared genetic scores (on the basis of a very small number of loci) between 82 power-speed athletes, 87 endurance athletes, and 119 nonathlete controls. Four genetic scores were created: power-based genetic scores based on two (*ACE* and *ACTN3*) and five (*ACE*, *ACTN3*, *IL6*, *NOS3*, and *AGT*) SNPs and endurance-based genetic scores based on two (*ACE* and *ACTN3*) and five (*PPARGC1A*, *PPARA*, *PPARD*, *NRF2*, and *HIF*) SNPs. They found significant differences in mean genetic score values between the three groups, and the differences between groups decreased in the models for the larger genetic scores. Before a combination of SNPs can be used to create a genetic score, it should be a prerequisite to establish that the individual SNPs are valid (i.e., nominally associated with the trait of interest) and that the study is adequately powered to detect differences at the single and multiple SNP levels. Unfortunately, the information on the associations of the individual SNPs with the respective athlete status was not reported in the article. Given the small sample sizes in each of the three

groups and the number of statistical tests performed (a P level of 0.05 was used), the results should be considered exploratory and taken with great caution.

Rampersaud et al. (29) performed microarray analyses on mRNA from whole blood from 60 sedentary women who completed 12 wk of combined aerobic and resistance training to identify gene subsets that were differentially expressed (not baseline expression but changes in gene expression) between individuals who experienced the greatest and least improvements in fitness. This is not a genetic study as such, but it could have the potential to generate genes to be targeted in subsequent candidate gene investigations. An overall fitness composite score was created by summing the responsiveness status (i.e., high or low responder) across 20 traits from the following four categories: anthropomorphic, blood biomarkers (e.g., lipids, insulin), blood pressure, and cardiorespiratory and muscular strength. The authors identified 43 transcripts in 39 unique genes (false discovery rate <10%, fold change >1.5) whose expression increased the most in "high" versus "low" premenopausal female responders, which were enriched in six biological pathways including oxidative phosphorylation.

The main limitations of the study by Rampersaud et al. (29) are the use of whole blood for gene expression analyses, the timing of the expression profile used, and the reliance on a complex and very heterogeneous fitness composite score as the dependent variable. Many cells are present in peripheral blood, some enucleated, some young, but others much older; in other words, they represent a highly heterogeneous set of cells and mRNAs. Moreover, it is not clear what the rationale is for using mRNAs from blood cells when there is little evidence that adaptation to endurance or resistance exercise is linked to the changes occurring in these cells. The same analyses performed on mRNAs isolated from skeletal muscle would be more logical and likely have more internal validity. Furthermore, the timing of the blood draw (e.g., time from last meal or time from last exercise session) may affect gene expression levels. This may have been standardized, but it is not clear from the report. More importantly, the analysis plan reported is not on the associations between the baseline expression profile and the changes in the fitness score, but rather on the changes in expression levels (something which would be the reflection of the correlations between deltas as opposed to a baseline predictive expression profile). The article does not provide a rationale for the selection of the 20 variables that make up the fitness score. Several of the variables are highly correlated, and only 10 of the 20 variables changed significantly in response to exercise training in the total sample. The underlying pathways influencing the training responses of each or subsets of these 20 traits are undoubtedly numerous and heterogeneous. In summary, despite all the merits of the study, given the study design and analytical structure, one cannot be certain what the gene expression data actually represent for our understanding of human variation in trainability.

As the technical capabilities to generate and analyze genetic data have improved dramatically over the past two decades, our understanding of the genetic architecture of complex human traits has also evolved. One clear lesson we have learned is that such traits are much more polygenic in nature than was anticipated even a decade ago. For example, on the basis of the latest GWAS meta-analyses, the current estimate of the number of genes and sequence variants contributing to variation in adult height reaches several hundreds (24).

Statistical geneticists have devoted considerable efforts to develop new methods to model and quantify these polygenic effects, and bioinformatics specialists have proposed innovative ways to evaluate and interpret their biological significance. One approach is to apply systems biology tools on genome-wide association data to identify pathways and networks where significantly associated sequence variants are enriched more frequently than would be expected by chance alone. The advantage of such an approach is that it is not as restricted in terms of statistical significance as a traditional GWAS is, because the focus is on enrichment of variants in pathways and networks rather than on individual SNPs. This approach adds another dimension to the hypothesis-generating characteristics of unbiased genome-wide exploration technologies.

A report applying pathway and network analyses to exercise-related traits was published in 2013. Ghosh et al. (11) relied on such a strategy in their exploration of the GWAS results for $\dot{V}O_{2max}$ training responses using over 2 million genotyped and imputed SNPs from the HERITAGE Family Study. The gene-level analyses confirmed the findings of the original single-SNP GWAS report (5). However, pathway-level analyses provided evidence of several biological mechanisms that were involved in $\dot{V}O_{2max}$ trainability in previously sedentary individuals. Some of these pathways were already established (fatty acid metabolism, Ca^{2+} signaling, and muscle function), whereas others were considered to be novel (e.g., cell–cell communications and innate immunity-related pathways).

Although the pathways underlying human variability in $\dot{V}O_{2max}$ training response identified by Ghosh et al. must still be considered as hypotheses that need to be tested in future studies, the report provides a proof of concept that a systems biology approach is applicable also to exercise physiology traits. Given the potential of these methods to advance our knowledge in exercise biology, it is likely that pathway analyses and other systems biology approaches will become increasingly popular among exercise scientists, especially when genomics and other omics data (transcriptomics, epigenomics, metabolomics, etc.) are incorporated in the models.

Pathway, network, and systems biology studies are tools that exercise genomics colleagues should become acquainted with to be in a better position to interrogate data regarding the biological significance of their findings and their putative biological and behavioral implications. An increased reliance on these new resources has the potential to foster

renewed dialogue and favor closing the well-known divide between reductionist science and integrative physiology.

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