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RESEARCH ARTICLE

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An intron variant of the GLI family zinc finger 3 (GLI3) gene differentiates resistance training-induced muscle fiber hypertrophy in younger men

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Abstract

We examined the association between genotype and resistance training-induced changes (12 wk) in dual x-ray energy absorptiometry (DXA)-derived lean soft tissue mass (LSTM) as well as muscle fiber cross-sectional area (fCSA; vastus lateralis; n = 109; age = 22 ± 2 y, BMI = 24.7 ± 3.1 kg/m^2). Over 315 000 genetic polymorphisms were interrogated from muscle using DNA microarrays. First, a targeted investigation was performed where single nucleotide polymorphisms (SNP) identified from a systematic literature review were related to changes in LSTM and fCSA. Next, genome-wide association (GWA) studies were performed to reveal associations between novel SNP targets with pre- to post-training change scores in mean fCSA and LSTM. Our targeted investigation revealed no genotype-by-time interactions for 12 common polymorphisms regarding the change in mean fCSA or change in LSTM. Our first GWA study indicated no SNP were associated with the change in LSTM. However, the second GWA study indicated two SNP exceeded the significance level with the change in mean fCSA ($P = 6.9 \times 10^{-7}$ for rs4675569, 1.7×10^{-6} for rs10263647). While the former target is not annotated (chr2:205936846 (GRCh38.p12)), the latter target (chr7:41971865 (GRCh38.p12)) is an intron variant of the GLI Family Zinc Finger 3 (GLI3) gene. Follow-up analyses indicated fCSA increases were greater in the T/C and C/C GLI3 genotypes than the T/T GLI3 genotype

Abbreviations: ACE, angiotensin I converting enzyme; ACTN3, alpha actinin-3; ADRB2, beta2-adrenergic receptor; ANKRD6, ankyrin repeat domain 6; BDKRB2, bradykinin receptor B2; BMP2, bone morphogenic protein 2; DXA, dual x-ray energy absorptiometry; FAMuSS, Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength (FAMuSS) multicenter trial; fCSA, fiber cross-sectional area; FST, follistatin; FTO, fat mass and obesity-associated; GEO, Gene Expression Omnibus; GLI3, GLI family zinc finger 3; GWA, genome-wide association; IGF-1, insulin-like growth factor 1; IL15RA, IL-15 receptor-α; LEPR, leptin receptor; LSTM, lean soft tissue mass; MSTN, myostatin; NR3C1, glucocorticoid receptor; OPN, osteopontin; PPP3R1, calcineurin B; SNP, single nucleotide polymorphisms; VDR, vitamin D receptor.

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($P < .05$). Data from the Auburn cohort also revealed participants with the T/C and C/C genotypes exhibited increases in satellite cell number with training ($P < .05$), whereas T/T participants did not. Additionally, those with the T/C and C/C genotypes achieved myonuclear addition in response to training ($P < .05$), whereas the T/T participants did not. In summary, this is the first GWA study to examine how polymorphisms associate with the change in hypertrophy measures following resistance training. Future studies are needed to determine if the GLI3 variant differentiates hypertrophic responses to resistance training given the potential link between this gene and satellite cell physiology.

KEYWORDS
GLI3, hypertrophy, polymorphisms, skeletal muscle

1 | INTRODUCTION

In response to exercise training, phenotypic heterogeneity was elegantly demonstrated by data from the HERITAGE study,[1] which was a large multisite trial which sought to determine the genetic contributors to exercise adaptation. A follow-up analysis determined numerous single nucleotide polymorphisms (SNP) were associated with the heterogeneity response.[2] Specifically, 21 SNP accounted for 49% of the shared variance in VO_2max changes, and subjects who carried ≤9 of these alleles improved their absolute VO_2max by ~221 mL/min, whereas those who carried ≥19 of these alleles improved by ~604 mL/min. Genetics may also play a role in the hypertrophic response to resistance training given that numerous studies suggest low, moderate, and high responders exist.[3,4] The Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength (FAMuSS) multicenter trial was a targeted analysis that provided novel insight into polymorphisms that may affect the hypertrophic response to resistance training.[5] Other research groups have also determined that polymorphisms of the bradykinin type 2 receptor (BDKRB2), insulin-like growth factor 1 (IGF1), IL-15 receptor α (IL15RA),[6] and myostatin (MSTN)[7,8] genes may also influence skeletal muscle hypertrophy in response to resistance training.

Despite these key research findings, no study to date has utilized a genome-wide association (GWA) study to determine if polymorphisms, or novel gene variants, are associated with skeletal muscle hypertrophy in response to resistance training. Therefore, the current study had multiple aims. First, we determined if previously examined SNP, determined from a systematic search of published studies, were associated with hypertrophic outcomes. Importantly, we sought to determine if any of these SNP-differentiated changes in vastus lateralis mean fiber cross sectional area (fCSA) or whole-body (bone-free) lean/soft tissue mass (LSTM) assessed by dual x-ray absorptiometry (DXA) in 109 college-aged males that had undergone 12 wk of full-body resistance training. Additionally, we performed GWA studies to identify novel genetic markers associated with changes in these hypertrophic outcomes. We hypothesized that genes related to muscle growth (eg, MSTN, IGF1, etc.) would exhibit polymorphisms that were associated with hypertrophic outcomes.

2 | METHODS

2.1 | Participants and training regimens

Participants were involved in studies approved by each respective Institutional Review Board (Auburn University; Hamilton Integrated Research Ethics Board). All participants provided verbal and written consent to participate, and this study conformed to the standards set by the latest revision of the Declaration of Helsinki. In the Auburn University study, screening forms ensured all eligible participants were healthy and recreationally active but: (a) had not engaged in structured resistance training for at least 6 months before study initiation (<2 resistance training exercise or high-intensity aerobic exercise sessions/week), (b) were not currently consuming a high-protein diet (>2.0 g/kg/d), (c) were not using anabolic agents (eg, anabolic steroids, supplemental protein, creatine monohydrate, or prohormones). In the McMaster University study, participants self-reported being recreationally trained for at least two years (performing at least 1 d/wk of lower-body training) and were not using anabolic agents.

In both studies, the training regimens involved full-body workouts (3-4 d/wk), and participants were instructed to refrain from any additional exercise outside of the studies. Participants in the Auburn University study trained using a daily undulating periodization training model (3 d/wk).

Free-weight barbell back squats, bench press, deadlifts, and supinated-grip bent-over rows were performed for 4 sets of 10 repetitions (Monday or Tuesday), 6 sets of 4 repetitions (Wednesday or Thursday), and 5 sets of 6 repetitions (Friday or Sunday). Laboratory staff members supervised all training, and progressive overload was implemented daily in response to participants' ratings of perceived exertion. Participants in the McMaster University study performed resistance training 4 d/wk (Monday, Tuesday, Thursday, and Friday). Each day included five exercises, consisting of two separate supersets and one additional exercise. Exercises were performed for three sets, with each set executed until volitional failure. Each workout was repeated twice per week [Monday/Thursday: inclined leg press with seated row (superset 1), barbell bench press with cable hamstring curl (superset 2), and front planks (exercise 5). Tuesday/Friday: machine-guided shoulder press with bicep curls (superset 1), triceps extension with wide-grip pull downs (superset 2), and machine-guided knee extension (exercise 5)]. Laboratory staff members supervised all training to ensure that each set was performed to volitional failure with correct technique. Participants' load was increased with subsequent training sessions when they could perform more repetitions than their designated repetition range.

Auburn University participants consumed one of five dietary supplements during the 12-week intervention including a maltodextrin placebo (89 g/d), leucine (6 g/d) + maltodextrin (86 g/d), whey protein concentrate (52 g/d), whey protein hydrolysate (51 g/d), or soy protein concentrate (78 g/d) group. Pre- to post-training changes in DXA LSTM and mean fCSA between supplement groups were similar (two-way ANCOVA P-values $> .100$). More details about the Auburn University study can be found in Mobley et al.[9] In the McMaster University study, participants engaged in either a high repetition or low repetition full body training paradigm and all participants were administered 60 g of whey protein to consume per day. Notably, both training paradigms were shown to similarly increase DXA LSTM and mean fCSA (two-way ANOVA P values $> .05$). For more details about the McMaster University, readers are referred to Morton et al.[10]

2.2 | Testing sessions

Pre- and post-training testing sessions in both studies occurred following an overnight fast. Additionally, these sessions occurred prior to the 12-week training intervention (Pre), and 72 hours following the last training bout (Post). Other testing procedures occurred specific to each study, but only the DXA, muscle biopsy procedures, and relevant downstream assays are discussed.

2.2.1 | Dual x-ray absorptiometry (DXA) for body composition assessment

Body composition was assessed in both studies using a GE Lunar iDXA total body scanner (GE Medical Systems Lunar, Madison, WI). Briefly, participants wore athletic clothing and were placed in a supine position within the machines' scanning frame. Scans typically lasted between 7-12 minutes, and LSTM was analyzed with associated software (Lunar enCORE version 14.1; GE Medical Systems Lunar). The Auburn University iDXA scanner has been reported to produce an intraclass correlation coefficient of 0.998 for LSTM during a calibrate/scan/re-calibrate/re-scan on 10 human participants.[11] Using a whole-body phantom, the McMaster University iDXA scanner produced intrascan (without repositioning) and interscan (on different occasions separated by 10-12 wk) variability coefficients of <1.6% for LSTM.[10]

2.2.2 | Muscle tissue collection and immunohistochemistry for mean fCSA

Vastus lateralis muscle biopsies from both studies were collected using a 5-gauge needle under local anesthesia. Immediately following tissue procurement, tissue was teased of excess blood and fat, and embedded in cryomolds containing optimal cutting temperature (OCT) media (Tissue-Tek, Sakura Finetek Inc; Torrence, CA, USA). Cryomolds were then frozen using liquid nitrogen-cooled isopentane and subsequently stored at −80°C until histology. Methods related to sectioning, histology, image capture details, and fiber typing as well as satellite cell quantification are explained in greater detail elsewhere.[9,10]

2.3 | DNA isolation and analyses

2.3.1 | DNA isolation and SNP array

DNA isolation for all samples occurred at Auburn University using skeletal muscle embedded in OCT. Briefly, samples were allowed to equilibrate to room temperature, and muscle (~8-20 mg) was removed and rinsed in 1x phosphate-buffered saline. Subsequently, a column-based DNA isolation kit and reagents (DNeasy Blood & Tissue Kit; Qiagen, Venlo, Netherlands) were used to isolate high fidelity DNA per the manufacturer's recommendations including RNase treatment. Following precipitation and pelleting, DNA was eluted with 100 μL of elution buffer from the kit, and DNA concentrations were determined in duplicate at an absorbance of 260 nm by using a NanoDrop Lite (Thermo Fisher Scientific, Waltham, MA, USA). DNA was then stored at −80°C until

TABLE 1 Interrogated polymorphisms selected from the systematic literature review

Gene	SNP ID	Alleles	Location	Past results in training studies	On array?	Reference(s)
Alpha actinin-3 (ACTN3)	rs1815739	C,T	Exon 16	Not conclusive	Yes	[18,19,34]
Angiotensin I converting enzyme (ACE)	rs4343	I/D	Intron 16, insertion of 287-bp repeat sequence	D allele favors hypertrophy	Yes	[13,39]
Ankyrin repeat domain 6 (ANKRD6)	rs61739327	C,T	Exon 16	T allele favors hypertrophy	No	[31]
Beta2-adrenergic receptor (ADRB2)	rs1042714	C,G	Present on sole exon	G allele favors hypertrophy	Yes	[25]
Bone morphogenic protein 2 (BMP2)	rs15705	A,C	Distal promoter	CC genotype favors hypertrophy	No	[21]
Bradykinin receptor B2 (BDKRB2)	rs5810761	+9(I)/-9(D)	Exon 1	-9/-9 genotype favors hypertrophy	No	[30]
Calcineurin B (PPP3R1)	rs3039851	I/D	Promoter, 5-bp insertion	I/I genotype favors hypertrophy	No	[14]
Fat mass and obesity-associated (FTO)	rs9939609	A,T	Intron 1	Not conclusive	Yes	[15]
	rs1421085	C,T			Yes	
	rs17817449	G,T			No	
	rs8050136	C,A			Yes	[27]
Follistatin (FST)	rs722910	A,C	3′ UTR	Not conclusive	Yes	[22]
Glucocorticoid receptor (NR3C1)	rs4634384	T,C	5′ UTR	T allele favors hypertrophy	No	[38]
IL-15 receptor-α (IL15RA)	rs2296135	C,A	Exon 7	AA genotype favors hypertrophy	Yes	[6]
Insulin-like growth factor 1 (IGF-1)[a]	rs10665874	192 allele (I)/--(D)	Promoter, 16-22 CA repeats	I allele favors hypertrophy	No	[16]
Leptin receptor (LEPR)	rs1137101	A,G	Exon 6	G allele favors hypertrophy	Yes	[32]
Myostatin (MSTN)[b]	rs1805085	C,T	Exon 1	T allele favors hypertrophy	No	[7]
	rs1805086	A,G	Exon 2	G allele favors hypertrophy	No	[7,8]
Osteopontin (OPN)	rs28357094	T,G	Promoter	G allele favors hypertrophy	No	[33]
Vitamin D Receptor (VDR)	rs1544410	T,G	Intron 8	Not conclusive	Yes	[23]

Note: This list was compiled from a systematic literature review that yielded 183 records, and 30 studies fitting our criteria. While some of these studies interrogated other polymorphisms (CCL2, CCR4, CNTF, MCR4, PTK2, SLC30A8, and UCP2), these variants were not included in our list because the associated polymorphisms did not differentiate hypertrophic responses.[24,26,28,29,35-37]

[a]Indicates rs5742692 for IGF-1 was interrogated.; [b]Indicates rs72909336 for MSTN was interrogated.

shipment to Thermo Fisher Scientific for quality assessment and DNA SNP array assays. Genome-wide interrogation of 315 505 SNPs for each sample was performed using the Axiom PMDA.r6 array (Thermo Fisher Scientific, Waltham, MA, USA). Following data procurement, raw data files were analyzed at Auburn University using the Axiom Analysis Suite v4.0.3.3 (Thermo Fisher Scientific) by creating a workflow that contained all participants and querying individual targets. Notably, raw data files were deposited to the National Institutes of Health (NIH) Gene Expression Omnibus (GEO) repository, and can be found online (https://www.ncbi.nlm.nih.gov/geo/).

2.4 | Systematic literature review for previously identified muscle hypertrophy-associated polymorphisms candidates

A systematic literature review was performed to query hypertrophy-associated polymorphisms for the current analysis. Specifically, Medline was queried using the search terms "polymorphism" and "resistance exercise", and EndNote v.X8 (Thomson Reuters; Philadelphia, PA, USA) was used to screen and filter relevant studies. Polymorphisms were included in our candidate list if the following criteria were met: (a) the study involved interrogating the polymorphism associated with hypertrophic outcomes following a resistance training intervention in non-diseased, college-aged or older populations, and (b) the gene polymorphism was shown to be associated with hypertrophic outcomes. We also ensured that all relevant studies performed from the FAMuSS multicenter trial were considered when developing our candidate list.[12] Using these search criteria we identified 183 records dating from 1994-2019, and of these, 158 records were removed for not meeting the inclusion criteria. Additional search tools (ie, searching relevant search terms through Google Scholar and hand-searching reference lists of identified papers) were used to add 5 additional records,[8,13-16] and this resulted in a total of 30 scientific studies containing relevant criteria.[6-8,13-39] From these studies, 20 gene candidates were identified, and of these, the 10 polymorphisms present on the array were interrogated in the current study (Table 1). Given that the IGF-1 and MSTN polymorphisms were not on the array, and numerous studies have implicated these genes being involved in the hypertrophic response to resistance training, we examined one IGF-1 probe set as well as one MSTN probe set that detected intron polymorphisms of these genes that were present on the array. Thus, a total of 12 polymorphisms were interrogated with our targeted analysis.

2.5 | GWA for novel SNP identification

As previously mentioned, Axiom Analysis Suite v4.0.3.3 (Thermo Fisher Scientific) was used to filter raw data files and identify candidates that passed all quality control (QC) checkpoints established by the manufacturer. Following data normalization, QC of genotype data was implemented at both individual and SNP levels, using Plink (version 1.9).[40] We controlled for high levels of missingness, indicative of poor DNA quality or technical problems (--geno 0.02 and --mind 0.02 for SNPs and individuals, respectively); we also controlled for minor allele frequency (--maf 0.05). Lastly, we controlled for Hardy-Weinberg equilibrium (HWE) rule (P value $< 1.0 \times 10^{-5}$). All individuals and SNPs that did not pass the QC were removed from further analysis. We used quantile–quantile (Q–Q) plots of observed versus expected $-\log_{10}$ (P-value) to examine the genome-wide distribution of P-values and Manhattan plots to report genome-wide P-values, using R qqman package (https://doi.org/10.1101/005165) with annotatePval set to 0.00001. Given the exploratory nature of this analysis and with limited sample size (n = 106) for GWA studies (typical sample size being 2500-250 000), we set a P-value cutoff of $P < 1 \times 10^{-5}$ a priori, which is less stringent than that of normal GWA studies ($P < 5 \times 10^{-8}$).

2.6 | Statistics

Independent samples t-tests were performed on mean fCSA and DXA LSTM change scores between training site cohorts. Changes in certain dependent variables between genotypes were examined using two-way ANOVAs (genotype × time). LSD post hocs were used to decompose the model within and between genotype groups. Statistical significance for these analyses was established as $P < .05$. Cohen's d effect sizes within each genotype were calculated under certain circumstances by taking the mean difference between Pre and Post and dividing it by the pooled standard deviation at both time points. Cohen's d values between 0.500-0.799 were considered moderate effects, and d values above 0.800 were considered large effects as we have previously reported.[41] For GWA studies, statistical significance was established a priori at $P < .10^5$ as described above. All data are presented either means ± standard deviation values (in text), or as box and whisker plots including the median (line), interquartile range (boxes), and range (whiskers indicating minimum and maximum values).

3 | RESULTS

3.1 | General characteristics of participants

Participants' mean age was 22 ± 2 y, mean height was 1.80 ± 0.07 meters, mean weight was 80.3 ± 12.1 kg, and mean body mass index (BMI) was 24.7 ± 3.1 kg/m^2.

3.2 | Comparison of fCSA changes between study sites

Participants from both sites demonstrated significant Pre to Post increases in mean fCSA (both sites $P < .001$), and changes from an absolute or percentage basis were not significantly different between sites ($P = .125$ and $P = .359$, respectively; Table 2). Participants from both sites also demonstrated significant Pre to Post increases in DXA LSTM (both sites; $P < .001$). However, changes in LSTM from an

TABLE 2 Pre- to post-training changes in mean fCSA and DXA LSTM between study sites

Variable	Auburn study (n = 66)	McMaster study (n = 43)	*P* value between sites
Δmean fCSA (μm^2)	+614 ± 1026	+938 ± 1134	$P = .125$
Δmean fCSA (%)	+9.8 ± 18.5	+12.9 ± 16.0	$P = .359$
ΔDXA LSTM (kg)	+2.6 ± 2.0	+1.2 ± 1.4	$P < .001$
ΔDXA LSTM (%)	+4.2 ± 3.2	+1.9 ± 2.1	$P < .001$

Note: Δ, pre- to post-training change.

Abbreviation: fCSA, fiber cross-sectional area.

absolute or percentage basis were significantly greater in Auburn University versus McMaster University participants ($P < .001$) (Table 2).

3.3 | Mean fCSA and DXA LSTM changes with training relative to SNP candidates identified through the systematic literature review

Mean fCSA and DXA LSTM changes with training relative to SNP candidates is depicted in Table 3. None of the SNP candidates from our targeted analysis demonstrated genotype × time interactions for mean fCSA or DXA LSTM.

3.4 | Genome-wide association with changes in mean fCSA and DXA LSTM

Our GWA studies in 109 participants indicated that two SNP exceeded the threshold of significance for change in mean fCSA ($P = 6.9 \times 10^{-7}$ for rs4675569, and $P = 1.7 \times 10^{-6}$ for rs10263647) (Figure 1A). We used Q–Q plots to illustrate observed versus expected $-\log_{10}$ (*P* value) to evaluate the genome-wide distribution of *P* values, which presented as normal, demonstrating a lack of skewness in the data. The former target (chr2:205936846 (GRCh38.p12)) is not annotated. The latter target (chr7:41971865 (GRCh38.p12)) is an intron variant of the GLI Family Zinc Finger 3 (GLI3) gene. Given that the former target was not annotated, we focused our statistical analysis on the GLI3 variant. There was a genotype × time interaction for mean fCSA (Figure 1B). Follow-up analyses indicated that mean fCSA increases occurred in the T/C and C/C GLI3 genotypes ($P < .001$ and $P < .001$, respectively), whereas no change occurred in the T/T GLI3 genotype ($P = .936$). Allele frequency for the studied subjects was calculated to be 58% for cytosine and 42% for thymine, and it has been estimated that the cytosine allele frequency is ~40% whereas the thymine allele frequency is ~60%.[42,43]

No targets exceeded the threshold of significance for changes in LSTM; thus, a Manhattan plot was not generated.

3.5 | Changes in myonuclear and satellite cell numbers between GLI3 genotypes

Given that the GLI3 gene has been linked to muscle progenitor cell physiology as discussed below, we sought to determine if the myonuclear and satellite cell responses to training were different between GLI3 genotypes. Notably, we had these data on hand from our previous publication in the Auburn participants[44]; thus, only the Auburn participants were included in this analysis. There were no significant genotype x time interactions for number myonuclei per type I fiber ($P = .237$, Figure 2A), number of myonuclei per type IIa fiber ($P = .206$, Figure 2b), and satellite cell number ($P = .402$, Figure 2c). However, given that each variable demonstrated significant time effects ($P < .001$), we implemented forced post hoc tests within each genotype from Pre to Post. Interestingly, these post hoc tests indicated that all three of these variables increased in the GLI3 T/C and C/C genotypes ($P < .05$), whereas none of these variables increased in the T/T genotype. Moreover, Cohen's d effect sizes indicated increases in these variables yielded large effects in the GLI3 T/C and C/C genotypes, whereas only small effects were observed in the T/T genotype.

4 | DISCUSSION

This is the first GWA study examining how polymorphisms are related to change in hypertrophy measures following a resistance training program. Interestingly, our targeted analysis revealed that none of the common SNP identified from the scientific literature review were associated with hypertrophic outcomes. While this is difficult to reconcile, differences between our data and others may be attributed to differences in study design, particularly the type and length of training. It should also be noted that conflicting results exist between various gene candidate studies. For example, some have reported that the ACTN3 gene is associated with strength outcomes,[18,45] while others have not.[46,47] There are also equivocal findings when examining the effects of the ACE I/D genotype on endurance performance.[48] However, while no targets exceeded the level of significance for change

TABLE 3 Mean fCSA and DXA LSTM changes with training relative to SNP candidates identified through the systematic literature review

Gene	SNP ID	n-size for genotype	Genotype × time *P* value (fCSA changes)	Genotype × time *P* value (DXA LSTM changes)
Alpha actinin-3 (ACTN3)	rs1815739	TT: 18 TC: 51 CC: 40	.922	.111
Angiotensin I converting enzyme (ACE)	rs4343	Ins/Ins: 27 Ins/Del: 55 Del/Del: 27	.130	.918
Beta2-adrenergic receptor (ADRB2)	rs1042714	CC: 37 CG: 49 GG: 23	.375	.667
Fat mass and obesity-associated (FTO)	rs9939609	AA: 24 AT: 46 TT: 39	.896	.596
	rs1421085	TT: 39 TC: 45 CC: 25	.897	.423
	rs8050136	AA: 23 AC: 47 CC: 39	.972	.325
Follistatin (FST)	rs7229102	AA: 109 AC: 0 CC: 0	ND	ND
IL-15 receptor-α (IL15RA)	rs2296135	AA: 41 AC: 45 CC: 23	.336	.239
Insulin-like growth factor 1 (IGF-1)	rs5742692	AA: 104 AG: 5 GG: 0	.275	.976
Leptin receptor (LEPR)	rs1137101	TT: 0 TC: 1 CC: 108	.453	.506
Myostatin (MSTN)	rs72909336	AA: 0 AG: 22 GG: 87	.170	.262
Vitamin D Receptor (VDR)	rs1544410	TT: 15 TC: 53 CC: 41	.657	.462

Note: This table provides genotype × time interaction *P* values for each single nucleotide polymorphism for pre- to post-training changes in mean fiber cross sectional area (fCSA) values as well as DXA lean soft tissue mass (LSTM) values.

Abbreviation: ND, not defined due to all participants having one genotype.

in whole-body LSTM, we discovered an intron variant of the GLI3 gene as a potential marker that may differentiate the training-induced change in mean fCSA.

The GLI3 gene encodes a DNA-binding transcription factor that mediates Sonic hedgehog (Shh) signaling.[49] It has also been demonstrated in Hela cells that GLI3 nuclear localization is regulated in an mTORC1-dependent manner, and GLI3 DNA binding increases Cyclin D1 mRNA expression.[50] These findings are interesting given the role that mTORC1 has in promoting muscle protein synthesis.[51] Moreover, the ability of GLI3 to drive Cyclin D1 mRNA expression is intriguing given that cyclin D1 has been shown

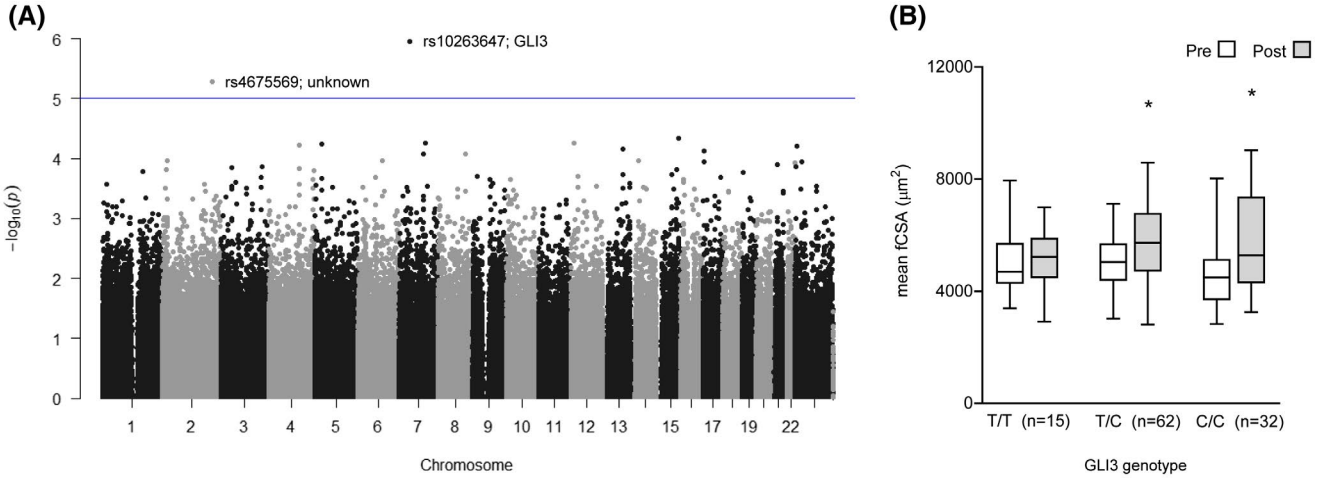

FIGURE 1 Manhattan plot for mean fCSA change in all participants. *Note:* These data show a Manhattan plot for mean fCSA change in all participants (panel A) as well as mean fCSA changes in the GLI3 intron variant (panel B). *, increase from pre- to post-training within the T/C or C/C genotype ($P < .05$)

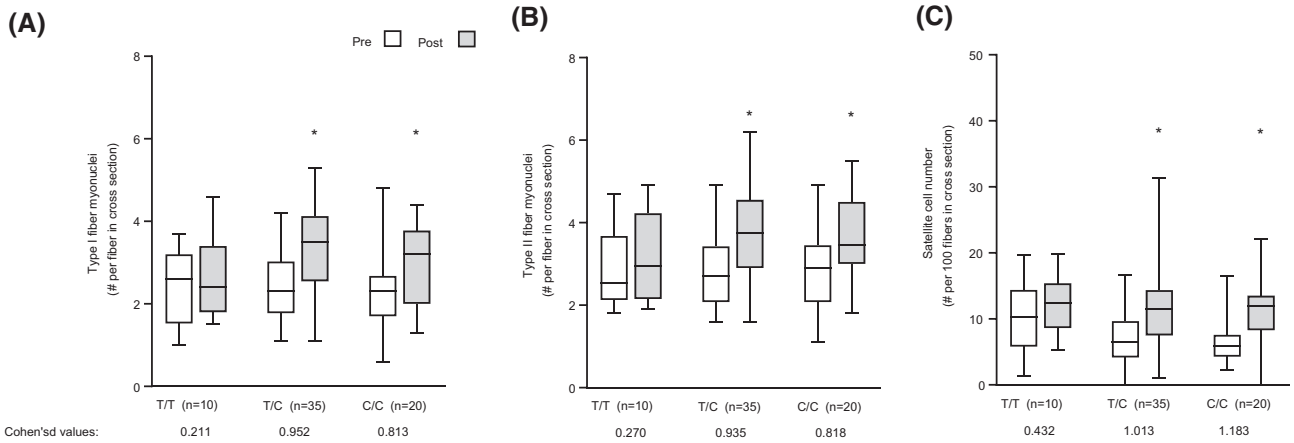

FIGURE 2 Type I/IIa myonuclear number and satellite cell changes in the Auburn cohort based on GLI3 genotype. *Note:* These data illustrate the Auburn cohort only for pre- to post-training changes in type I fiber myonuclear number (panel A), type IIa fiber myonuclear number (panel B), and total (type I/II fiber) satellite cell number (panel C) according to GLI3 genotype (rs10263647). While there were no significant interactions, forced post hoc tests were performed within each genotype given that significant time effects were observed for these three variables. Moreover, Cohen's d values indicated that the T/C and C/C genotypes exhibited large effects for increases in type I/II myonuclear number as well as satellite cell number with training. *, increase from pre- to post-training within the T/C or C/C genotype ($P < .05$)

to promote ribosome biogenesis in skeletal muscle cells.[52] To our knowledge, no studies have primarily sought to determine how GLI3 mRNA expression, protein expression, and/or DNA binding are affected in response to one or multiple bouts of resistance training. Using available microarray data, we examined how GLI3 mRNA levels responded both acutely and chronically with resistance training in younger men.[53] Transcript levels were not altered 24 hours following the first bout of training (expression value units at Acute Pre = 4.75 ± 0.12, values at 24-hours Post = 4.91 ± 0.31; $P = .167$), or after 10 weeks of chronic training (expression value units at Chronic Pre = 4.75 ± 0.12, values at Chronic Post = 4.74 ± 0.203; $P = .910$). Nonetheless, a relationship between GLI3 gene expression and skeletal muscle processes related to hypertrophy has been reported. For instance, Chaillou et al[54] examined the time-course response in the plantaris muscle transcriptome following synergist ablation, and *Gli3* mRNA levels were elevated at 3, 5, 7, 10 and 14 days following surgery compared to muscle from mice that underwent sham operations. Renault et al[55] have also demonstrated that the *Gli3* gene is essential for muscle repair following a hypoxic insult in *Gli3*-knockout mice. These authors also reported that the *Gli3* gene regulates satellite cell differentiation and fusion by affecting the expression of myogenic regulatory factors (ie, *Myf5, Myog, and Myod1*). Others have shown the *Gli3* gene is involved with skeletal muscle development in utero, and

this gene was found to operate by increasing the expression of certain myogenic regulatory factors in muscle progenitor cells.[56] Thus, the aforementioned studies have shown that the GLI3 gene may be critical for skeletal muscle hypertrophy given that it is a downstream effector of mTORC1, and the GLI3 transcription factor has been shown to drive the expression of cyclin D1 as well as myogenic regulatory factors which, in turn, increase ribosome biogenesis and satellite cell proliferation.

While speculative, individuals harboring the GLI3 T/T genotype may have an altered expression of this gene in satellite cells in response to resistance training. Such an impairment may cause a diminished satellite cell response and myonuclear accretion to resistance training, and we propose this might affect exercise-induced changes in mean fCSA. It is notable that data in Figure 2C provide supporting evidence for this hypothesis, given that those with T/T alleles showed a diminished satellite cell response to training compared to the T/C and C/C genotypes who showed increases. In addition, the T/C and C/C genotypes achieved myonuclear addition in response to training, whereas the T/T cohort did not (Figure 2A,B). It is notable that the GLI3 rs10263647 polymorphism exists in an intron region. Thus, the transcript and resulting protein may not be functionally affected. However, it is notable that other intron polymorphisms have been linked to phenotype outcomes. For instance, while the ACE I/D allele is located in intron 16 of the gene, there is evidence to suggest that the presence of the D allele increases ACE enzyme activity and leads to the higher production of blood angiotensin II levels.[57] There is also recent evidence from sequencing endeavors to suggest that intronic nucleotide variation in the ERCC8 gene can alter splicing events and lead to the differential insertion of exons in the mature mRNA transcript.[58] Therefore, it remains possible that the GLI3 rs10263647 polymorphism could alter the protein structure and activity of the encoded transcription factor. While provocative, these data are preliminary and warrant future research into the potential role that the GLI3 intron variant has on the myofiber response to resistance training. Additionally, future studies that isolate and grow primary human muscle cells from participants harboring the C/C and T/T GLI3 genotypes will yield informative data regarding whether differences in satellite cell proliferation and/or myotube formation are impaired in response to anabolic stimuli in vitro.

It should finally be noted that our laboratory and others have taken an interest in molecular attributes that delineate the hypertrophic response to resistance training,[3] and several biomarkers from the Auburn cohort were examined in a previous publication.[44] In our prior publication, participants who experienced greatest localized hypertrophy (ie, increases in vastus lateralis thickness) were classified as high responders, whereas those who experienced minimal changes in vastus lateralis thickness with training were classified as low responders. We back-tested the current GLI3 findings to that dataset, and found that 40% (4/10) of subjects in the T/T genotype were in the low responder group, whereas 33% (7/21) of C/C genotype were in the high responder group. It is logical to assume that higher percentages of participants would be in each respective group given the T/T genotype in the current study did not experience mean fCSA increases, whereas the C/C genotype did. However, it should be noted that these disparate findings are likely due to methodological differences between studies in determining hypertrophy (ie, histology versus ultrasound). In this regard, we authored a recent review that provides ample data to suggest histology and ultrasound measures show poor agreement in terms of tracking skeletal muscle hypertrophy.[59] Thus, the current data should be interpreted with these collective findings in mind. Specifically, the identified GLI3 allele may delineate the hypertrophic response in relation to histological attributes (ie, mean fCSA, myonuclear number, satellite cells), but this polymorphism may be relatively poor at predicting the hypertrophic response if other methods are being used to track training adaptations (eg, ultrasound or DXA).

4.1 | Limitations

A limitation to the current study, along with previous GWA studies using chip-based assays, is the limited availability of probe sets to interrogate inter-individual base differences across genomes. Indeed, half of the previously interrogated polymorphisms related to skeletal muscle hypertrophy identified in our literature search were not present on our utilized array. Further, while our array was relatively extensive in the number of targets contained on the array, this limitation speaks to the need to perform whole-genome sequencing to identify novel polymorphisms linked to various traits as has been done in other areas.[60] Thus, next-generation sequencing efforts with larger and more diverse sample sizes may have improved our ability to detect SNP associations (particularly those of less-common frequencies) with exercise-induced phenotype variation. Readers should also be aware that the sample size in the current study is relatively small compared to other SNP interrogations or GWA studies, but this is a function largely of the logistics of running supervised resistance training studies with multiple muscle biopsies. It is notable that other training interventions have performed GWA studies using less than 150 participants,[61,62] and these studies have yielded insightful information. Finally, we lacked enough sample from the McMaster University cohort to perform histological assays seen in Figure 2 from the Auburn cohort. Indeed, the relationship between GLI3 genotype and satellite cell as well as myonuclear adaptations with training would have been strengthened with the inclusion of both

cohorts. Given these limitations, we posit that the current results are preliminary and require further validation.

5 | CONCLUSIONS

This is the first study to adopt a genome-wide analytical approach to validate previously identified SNP candidates while identifying a new potential gene candidate that may affect the hypertrophic response to resistance training. More research is needed to validate if different variants of the GLI3 gene differentially affect the hypertrophic responses to resistance training.

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CONFLICT OF INTEREST

No authors have financial or other conflicts of interest to report with regard to these data.

AUTHOR CONTRIBUTIONS

C. G. Vann, S. M. Phillips, and M. D. Roberts designed the research; R. W. Morton and C. B. Mobley were study coordinators of the Auburn University and McMaster studies, respectively, and C. T. Haun, P. A. Roberson, S. Y. Oikawa, and C. McGlory were critically involved in executing the training aspects of said studies; I. J. Vechetti and C. B. Mobley performed GWA analyses; B. K. Ferguson and M. D. Roberts performed SNP analyses; C. G. Vann, S. C. Osburn, C. L. Sexton, C. D. Fox, M. A. Romero performed pilot and scaled experiments to ensure appropriate DNA isolation; J. J. McCarthy, and K. C. Young provided critical insight regarding the writing of the manuscript. All co-authors reviewed the preliminary draft of the manuscript and approved the submission.

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