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RESEARCH ARTICLE

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Polygenic mechanisms underpinning the response to exercise-induced muscle damage in humans: In vivo and in vitro evidence

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Abstract

We investigated whether 20 candidate single nucleotide polymorphisms (SNPs) were associated with in vivo exercise-induced muscle damage (EIMD), and with an in vitro skeletal muscle stem cell wound healing assay. Sixty-five young, untrained Caucasian adults performed 120 maximal eccentric knee-extensions on an isokinetic dynamometer to induce EIMD. Maximal voluntary isometric/isokinetic knee-extensor torque, knee joint range of motion (ROM), muscle soreness, serum creatine kinase activity and interleukin-6 concentration were assessed before, directly after and 48 h after EIMD. Muscle stem cells were cultured from *vastus lateralis* biopsies from a separate cohort ($n = 12$), and markers of repair were measured in vitro. Participants were genotyped for all 20 SNPs using real-time PCR. Seven SNPs were associated with the response to EIMD, and these were used to calculate a total genotype score, which enabled participants to be segregated into three polygenic groups: 'preferential' (more 'protective' alleles), 'moderate', and 'non-preferential'. The non-preferential group was consistently weaker than the preferential group (1.93 ± 0.81 vs. 2.73 ± 0.59 N · m/kg; $P = 9.51 \times 10^{-4}$) and demonstrated more muscle soreness ($p = 0.011$) and a larger decrease in knee joint ROM ($p = 0.006$) following EIMD. Two *TTN-AS1* SNPs in linkage disequilibrium were associated with in vivo EIMD (rs3731749, $p \leq 0.005$) and accelerated muscle stem cell migration into the artificial wound in vitro (rs1001238, $p \leq 0.006$). Thus, we have identified a polygenic profile, linked with both muscle weakness and poorer recovery following EIMD. Moreover, we provide evidence for a novel *TTN* gene-cell-skeletal muscle mechanism that may help explain some of the interindividual variability in the response to EIMD.

KEYWORDS

eccentric exercise, extracellular matrix (ECM), fibroblast, myoblast, single-nucleotide polymorphism (SNP), total genotype score (TGS)

1 | INTRODUCTION

Resistance exercise is an effective way of improving muscle strength and it represents an attractive and low-cost strategy to prevent skeletal muscle wasting and metabolic diseases in various populations (Clegg et al., 2013). Unaccustomed resistance exercise, however, can lead to acute exercise-induced muscle damage (EIMD) (Friden & Lieber, 2001). It is thought that excessive sarcomere strain due to EIMD is the primary cause of microscopic muscle tears, and it is feasible that poorly managed training with insufficient recovery between exercise sessions can lead to (overuse) muscle injury at the macroscopic scale (Gabbett et al., 2016). Several investigations have revealed that EIMD is mainly induced by eccentric contractions, i.e. movements that forcibly lengthen the contracting muscle fibres (Clarkson & Hubal, 2002). Whilst we know that a wide range of interindividual variability exists in the response to unaccustomed intense exercise (Baumert et al., 2016), the mechanism(s) underlying the individual response to EIMD is poorly understood.

The response to EIMD is very complex, as several structural elements contribute to the transmission of force from the muscle to the bone, including skeletal muscle fibres, the extracellular matrix (ECM), and tendon (Hyldahl & Hubal, 2014). All these structural elements play a potential role in the damage/recovery response. Indeed, the response to EIMD can manifest in a variety of symptoms, including prolonged loss of maximal strength, decreased range of motion (ROM), ultrastructural damage, delayed onset muscle soreness, localised inflammation and release of muscle-specific proteins into the circulatory system (e.g., creatine kinase and interleukin-6), which can be detected for days or even for weeks after exercise (Clarkson & Hubal, 2002). All of these variables are considered to be biomarkers of EIMD.

Recent evidence from our laboratory suggests that the variable response to EIMD is based on different muscle characteristics. A larger muscle physiological cross-sectional area appears to protect against immediate loss of maximum strength, while a relatively high proportion of muscle connective tissue/ECM stem cells (fibroblasts) compared to activated satellite cells (myoblasts) appears to support the recovery of maximum strength in the days after EIMD (Baumert et al., 2021). Furthermore, some of the variable response to EIMD can be explained by age, ethnic origin, training status and sex, and there is also evidence to suggest that some of this interindividual variability might be explained by differences in genetic make-up (Ahmetov et al., 2014; Baumert et al., 2016; Clarkson et al., 2005). Indeed, several single nucleotide polymorphisms (SNPs), i.e., common variations in the sequence of nucleotides within genes that encode proteins in skeletal muscle (Baumert et al., 2017; Clarkson et al., 2005), tendon and muscle ECM (Barfield et al., 2014; Baumert et al., 2018), and inflammation (Ahmetov et al., 2014), have been associated with changes in various biomarkers of EIMD following eccentric exercise. Therefore, it is important to understand that any genetic association with damage to the muscle-tendon unit is likely to be polygenic.

To our knowledge, only two studies have investigated a link between the combination of candidate SNPs and the response to EIMD (Del Coso et al., 2017, 2020). Both studies investigated the combined association of seven candidate SNPs with plasma creatine kinase concentration in applied settings (marathon running and half-ironman), where the exercise was not standardised, thus making it difficult to accurately characterise the interindividual variability in the response to EIMD. Further, whilst a plethora of genetic association studies have been performed (for a review, please see Baumert et al., 2016), only a few studies have investigated the mechanisms underpinning the effect of genetic variation in the context of exercise and EIMD (Barfield et al., 2014; Seto et al., 2011). Identifying genetic associations with the recovery following EIMD (in vivo) and artificially induced damage in human muscle stem cells (in vitro) could identify novel mechanisms and improve our understanding of EIMD aetiology.

Therefore, the primary aim of this study was to investigate the polygenic association with EIMD, and the objective was to determine if a combination of candidate SNPs could distinguish between high and low responders to EIMD. A second aim was to investigate whether those SNPs associated with in vivo EIMD were also linked to the rate of repair in an in vitro human skeletal muscle stem cell wound healing assay, thus potentially elucidating novel mechanisms underpinning EIMD in vivo. We hypothesised that candidate SNPs (n = 20), chosen for their potential link with the skeletal muscle/ECM/tendon/inflammation response to EIMD, would be individually associated with EIMD, and that the combination of those SNPs could be used to predict the response to EIMD. We further hypothesised that at least one of these SNPs would be associated with the rate of repair in the in vitro wound healing assay, thus linking the in vivo and in vitro investigations.

2 | METHODS

2.1 | Participants

This investigation comprised two studies (one in vivo and one in vitro), for which two separate cohorts of young, healthy male and female participants were recruited. All participants provided written informed consent before participation in the study, which complied with the Declaration of Helsinki and was approved by the Research Ethics Committee of Liverpool John Moores University (protocol number: 13/SEC/056). Further, a prebiopsy screening was performed by a physician for each participant of the Muscle Stem Cell cohort (in vitro study). Participant inclusion criteria for both studies comprised: (i) aged 18–35 years; (ii) no history of strength training 6 months before the study; (iii) no history of lower extremity musculoskeletal injuries 12 months before the study; (iv) 'average' level of habitual physical activity, assessed via questionnaire (Baecke et al.); and (v) no use of purported anabolic supplements. The in vivo study cohort (Genetics of Recovery after Exercise, G-REX) comprised 65 (39 female and 26 male) participants, while the in vitro (Muscle Stem Cell) study cohort comprised 12 (four female and eight male) participants (Table S3).

2.2 | Experimental design

Participants of the in vivo study reported to the laboratory on three separate occasions: (i) familiarisation to the isometric and isokinetic knee extension maximum voluntary contraction (MVC) assessments on an isokinetic dynamometer (IKD); (ii) maximal knee extension eccentric contractions on an IKD to induce EIMD ('EIMD-intervention') in the right leg plus assessments before (PRE) and directly after (POST); and (iii) 48 h after (POST48) the EIMD-intervention. The assessments of muscle damage indices comprised isometric and isokinetic MVC torque, muscle soreness (assessed via visual analogue scale), knee joint ROM (assessed on the IKD) and serum samples (for analysing interleukin-6 concentration and creatine kinase activity). All tests were performed on the right leg and at the same time of day for each participant. Participants were instructed to maintain their normal dietary behaviour (consumption of 'recovery' supplementation, e.g., protein, antioxidants, etc., caffeine and antiinflammatory medicine was not permitted), to refrain from drinking alcohol and to avoid any strenuous physical activity for at least 48 h before and throughout the study.

2.3 | In vivo EIMD-intervention

The in vivo EIMD-intervention comprised 12 sets of 10 maximal and continuous knee extension eccentric contractions on an IKD (either a Humac Norm, CSMI or Biodex Multi-Joint System 3 Pro). The two IKDs show similar isometric, concentric and eccentric peak torques with high to very high reproducibility (Alvares et al., 2015) and each participant performed all their strength assessments and damage protocol on the same dynamometer. We decided to use 120 knee extensor eccentric MVCs following extensive pilot work, which demonstrated that this number of contractions caused considerable loss of torque, with no further significant loss following additional contractions. Moreover, the DOMS reported by our pilot participants after 120 MVCs was substantial 48 h post EIMD. Participants rested for 30 s between sets, except after every fourth set, when participants rested for 3 min. The angular velocity during the eccentric contraction was set to 30°·s^{-1} (ROM: 30°–100° knee flexion; 0° = full knee extension) and participants were instructed to maximally resist throughout the entire range of movement. Participants received verbal encouragement and biofeedback throughout the EIMD-intervention.

2.4 | Knee joint ROM and MVC torque

The participant was seated on the IKD with the hips flexed to 85° (180° = supine position) and securely fastened with inextensible straps at the chest and waist, whilst the arms were held crossed over the chest. The mid-tibiofemoral contact point was aligned with the axis of rotation of the lever arm on the dynamometer, and the bottom of the shin pad on the lever arm was strapped to the leg. Participants fully extended and flexed their leg without resistance or assistance, and the maximal positions in each direction were recorded to calculate ROM in degrees. Before performing MVCs, participants underwent a standardised warm up, comprising 10 submaximal isokinetic leg extensions/flexions (60°·s^{-1}). Participants then performed three isometric MVCs (lasting 2–3 s and interspersed by 60 s rest), with the knee joint angle set at 80° knee flexion (0° = full extension). Afterwards, three isokinetic MVCs (70° ROM, from 100° to 30° knee flexion angle; velocity 60°·s^{-1}) were performed consecutively. Participants received verbal encouragement and biofeedback throughout the assessment and the highest isometric and isokinetic MVC were used for subsequent analysis.

2.5 | Delayed onset muscle soreness

Participants were asked to score their perceived muscle soreness and pain with a visual analogue scale following three repetitions of bilateral bodyweight squat exercises, after which participants were asked to rate their perceived quadriceps muscle soreness using a visual analogue scale (scale 0–10 cm; 0 cm = no soreness; 10 cm = maximal soreness).

2.6 | Muscle biopsy procedure, extraction, and expansion of human muscle-derived cells

This methodological approach has been described in detail elsewhere (Baumert et al., 2021). Briefly, Muscle Stem Cell study participants were instructed to avoid any strenuous exercise 48 h before the biopsy procedure. Biopsies of the *vastus lateralis* muscle were obtained approximately halfway between the anterior superior iliac spine and the patella under local anaesthesia by using the Weil-Blakesley conchotome technique (Baczynska et al., 2016). The biopsies were isolated and cultured (Baumert et al., 2021) with growth media (Hams F-10 nutrient mix [Lonza] with added L-glutamine [2.5 mM], 10% heat-inactivated fetal bovine serum [Gibco, Thermo Fisher Scientific], 1% penicillin-streptomycin [Life Technologies], and 1% L-Glutamine [Gibco]). After the extraction procedure, cells were washed every 48 h following two brief washes with phosphate-buffered saline (Sigma-Aldrich) and were passaged via trypsinisation until 80% confluency for cell expansion. Cells were incubated in a HERAcell 150i CO_2 Incubator (Thermo Scientific) at 37°C/5% CO_2. All experiments were performed on cells between passages 3 and 6 of their growth kinetics to ensure consistency and to avoid potential issues of senescence.

2.7 | Characterization of human muscle-derived cells

The mixed population of human skeletal muscle-derived myoblasts and fibroblasts were characterised by immunofluorescent staining for

desmin (polyclonal rabbit anti-human antibody [1:200; Cat# ab15200, RRID: AB_301744; Abcam] and secondary TRITC polyclonal goat anti-rabbit [1:200; Cat# A16101, RRID: AB_2534775; Fisher Scientific]), to determine the percentage of myoblast positivity, as reported previously (Baumert et al., 2021). A total of four randomly selected areas per immunostained well were analysed per participant. Grohmann et al. (2005) revealed that passaging cells from young populations does not change the ratio of myoblasts to fibroblasts.

2.8 | Wound-Healing assay, migration, and differentiation analysis

This method has been described in detail elsewhere (Baumert et al., 2021). We chose to investigate SNPs in a mixed population of myoblast and fibroblasts, as we showed previously that the inherent muscle stem cell composition (i.e., myoblast:fibroblast ratio) was associated with the rate of muscle recovery following an artificial wound healing assay in vitro and EIMD in vivo (Baumert et al., 2021). Briefly, cells were seeded onto gelatinised six-well plates (Nunc) and expanded until cell monolayers reached a confluent state. Growth media was removed, monolayers were washed with phosphate-buffered saline and cells were damaged by scraping with a 1 ml pipette tip (width of the wound area, mean ± SEM: 896.4 ± 21.24 µm). Phosphate-buffered saline was aspirated, damaged cell monolayers were washed twice with phosphate-buffered saline to remove cell debris and 2 ml differentiation media (same composition as GM but consisted only 1% heat-inactivated fetal bovine serum) was added. Monolayers were imaged using live imaging microscopy (Leica) for the analysis of cell migration immediately, 24 and 48 h after the wound healing assay. Previous studies from our laboratory demonstrated that the relative proportion of migrating cells (i.e., between myoblasts and fibroblasts) does not differ over 48 h (Owens et al., 2015). TIF images were exported from Leica Application Suite and loaded as TIF image stacks in ImageJ with a cell counter plug-in. The wound itself was divided into an outer and inner segment, to track migrated cell numbers, and cells in the outer and inner segments were counted (Figure 1). A total of two images per well were analysed.

2.9 | Blood samples

Venous whole blood samples were obtained from all participants (for genotyping purposes), while serum creatine kinase activity and interleukin-6 concentration were determined from a subgroup of the in vivo cohort ($n = 38$). All blood samples were drawn from an antecubital vein and collected into a 10 ml ethylenediaminetetraacetic acid (EDTA) (genotyping sample) vacutainer (BD Vacutainer Systems) and serum (creatine kinase and interleukin-6) vacutainer (BD Vacutainer Systems). The genotyping blood sample was taken during the familiarisation session. Serum samples were obtained at each time point (pre, post and 48 h post-EIMD), and left in a temperature controlled laboratory (between 22°C and 24°C) for 30 min to allow clotting, and then centrifuged at 1300*g* for 15 min at 4°C. All samples were then aliquoted into 1.5 ml microcentrifuge tubes (Axygen [Corning]) and stored at −80°C until subsequent analysis (see below).

2.10 | Serum creatine kinase activity

Creatine kinase activity was measured using a commercially available kit (Catachem Inc.), as described in detail elsewhere (Baumert et al., 2021). Briefly, 10 µl serum from the in vivo cohort and 10 µl cell lysate from the in vitro Muscle Stem Cell cohort were loaded onto a 96-well UV plate. The creatine kinase reaction reagent and diluent (Catachem) were prepared as per the manufacturer's instructions and heated for 2 min at 37°C. The reconstituted reagent contained the following active ingredients: 30 mmol/L PCr, 2 mmol/L ADP, 5 mmol/L AMP, 2 mmol/L NAD, 20 mmol/L N-acetyl-L-cystine, 3000 U/L hexokinase, 2000 U/L G-6-PDH, 10 mmol/L 1 Mg2, 20 mmol/L D-glucose, 10 mol/L di (adenosine 5 =) pentaphosphate, and 2 mmol/L EDTA, buffered to pH 6.7. The reagent mixture was added to the samples and the change in absorbance monitored continuously over 20 min in a Thermo Multiskan Spectrum plate reader at a wavelength of 340 nm.

2.11 | Serum Interleukin-6 concentration

Serum samples were analysed for interleukin-6 concentration using commercially available human interleukin-6 enzyme linked immunosorbent assay (ELISA) kits (Quantikine®, R&D systems), according to the manufacturer's instructions. Briefly, the serum samples were thawed and 200 µl aliquots of each sample, positive control or standard, were plated on a coated (monoclonal antibody specific for human interleukin-6) 96-well microtitre plate for 2 h. After washing, human interleukin-6 conjugate (200 µl) was added to each well and incubated for 2 h at room temperature (between 22°C and 24°C), with mixing on an orbital shaker. After the wells were washed, substrate solution (200 µl) was added to each well at room temperature (between 22°C and 24°C) and protected from light. After adding 50 µl stop solution to each well, plates were measured with a Thermo Multiskan Spectrum microplate reader (Thermo Fisher Scientific) at 450 nm and values were calculated with Excel 365 (Microsoft, v. 365) by generating a four-parameter logistic (4-PL) curve fit. The minimum detectable dose of human interleukin-6 was 0.70 pg/ml.

2.12 | Genotyping

DNA extraction from whole blood was performed with a QIAamp DNA Blood Mini Kit (Qiagen), following the manufacturer's QIAamp spin column protocol for DNA purification from whole blood.

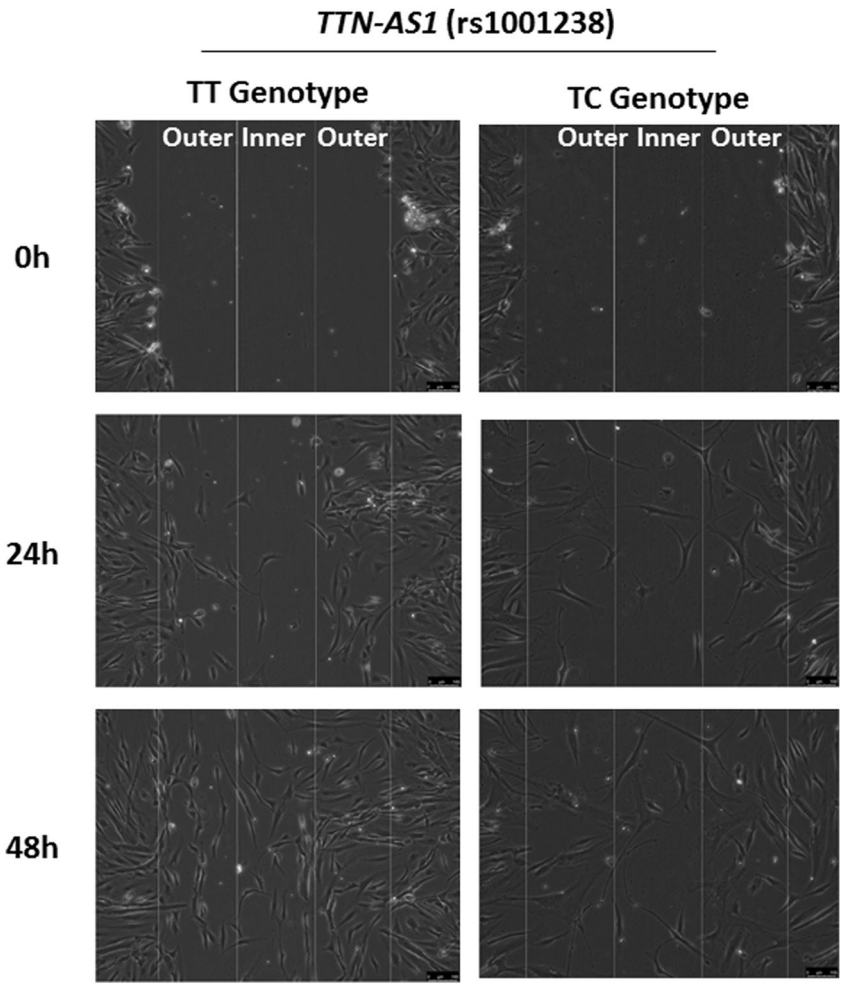

FIGURE 1 Representative images regarding cell migration of skeletal muscle cells between the TTN-AS1 (rs1001238; in linkage disequilibrium with rs3731749) genotypes into the artificial wound zone (wound area is about 900 µm in width and split into 3 ×300 µm segments) within 48 h after implementing the artificial wound. Magnification is ×10.5, and scale bar is 100 µm

Real-time polymerase chain reaction (PCR) was performed using a Rotor-Gene Q PCR machine (Qiagen) to define the genotype for each SNP for each participant. Reactions were completed on a 72-well rotor-disc. Each 10 µl reaction volume contained: 5 µl Genotyping Master Mix (Applied Biosystems), 3.5 µl nuclease-free H_2O (Qiagen), 0.5 µl genotyping assay (Applied Biosystems), which included the SNP-specific TaqMan primers and probes, and 1 µl DNA. A list of the 20 SNPs analysed can be found in Table 1. For negative control wells, 1 µl nuclease-free H_2O replaced the DNA template. Positive controls were also used to provide further confidence in our results. The following PCR protocol was used: 50 cycles of incubation at 92°C for 15 s (denaturation) then annealing and extension at 60°C for 1 min. Last, genotype was determined using Rotor-Gene Q Software 2.3.1. All samples and controls were analysed in duplicate and there was 100% agreement between all duplicate samples.

2.13 | Total genotype score (TGS) calculation

Using a two-level approach to compute a TGS (Williams & Folland, 2008), 10 previously EIMD-associated SNPs (*ACTN3*, rs1815739; *COL1A1*, rs1800012; *COL1A1*, rs2249492; *COL2A1*, rs2070739; *COL5A1*, rs12722; *IGF2-AS*, rs4244808; *IL6*, 1800795; *MYLK*, rs2700352; *MYLK*, rs28497577; *TRIM63*, rs2275950) (Baumert et al., 2017, 2018; Clarkson et al., 2005; Devaney et al., 2007; Yamin et al., 2008), plus 10 SNPs not yet associated with EIMD, which we selected for their potential effect on EIMD due to their genetic location (*AGT*, rs699; *CCL2*; *DES*, rs12621188; *MMP3*, rs679620; *NOS3*, rs2070744; *PAX7*, rs485874; *TNF*, rs1799964; *TTN-AS1*, rs1001238; *TTN-AS1*, rs3731749; *VDR*, rs2228570), were each individually investigated for an association with our standardised EIMD-intervention, while controlling for multiple comparisons using a 20% false discovery rate (FDR). Heterozygote genotypes were pooled with one of the two homozygotes of the same SNP, which showed a similar pattern, except for SNPs possessing rare homozygotes $n \leq 2$ (*COL1A1*, rs1800012; *COL2A1*, rs2070739; *MYLK*, rs28497577; *MYLK*, rs2700352), for which a recessive model was used. SNP associated with EIMD following FDR were then used to calculate our TGS model. Each genotype for each SNP was given a score between 0 and 2, based on the response to our EIMD-intervention. For each SNP, the homozygote genotype that showed a beneficial ('protective') effect was given a score of 2 and the homozygote genotype associated with a detrimental effect was given a score of 0. The heterozygote genotype received a score of 1, except for *TTN-AS1* (rs3731749), as this SNP showed an over-dominance for the heterozygote genotype, which received a score of 0. Total values

TABLE 1 Investigated single nucleotide polymorphisms

Symbol	Gene	rs-number	REF > ALT	Chromosome (position)	REF Freq	Protein function	SNP function
ACTN3	Actinin Alpha 3	rs1815739	C > T	11 (66,560,624)	0.62	Links the Z-line to actin in type II skeletal muscle fibres, and it inhibits slow myogenic programme	Nucleotide substitution with thymine leads to a stop codon at amino acid 577, preventing protein production (Seto et al., 2011)
AGT	Angiotensinogen	rs699	A > G	1 (230,710,048)	0.54	Precursor of angiotensin II, which modulates inflammatory responses and muscle homeostasis	A-allele is associated with an increase of inflammatory markers after a marathon (Sierra et al., 2019)
CCL2	C-C Motif Chemokine Ligand 2	rs2857656	G > C	17 (34,254,988)	0.75	Following muscle damage, CCL2 attracts macrophages to the injury site to provide an adequate inflammatory response	GG genotype is associated with severe muscle injuries (Pruna et al., 2013)
COL1A1	Collagen Type I Alpha 1 Chain	rs1800012	C > A	17 (50,200,388)	0.83	Major component of type I collagen, a structural protein found in most connective tissues, including the perimysium	Not yet known but intronic SNPs have the potential to influence gene expression and mRNA stability (Tabor et al., 2002)
COL1A1	Collagen Type I Alpha 1 Chain	rs2249492	C > T	17 (50,185,660)	0.46		
COL2A1	Collagen Type II Alpha 1 Chain	rs2070739	C > T	4 (47,974,193)	0.92	Major component of type II collagen, commonly expressed in cartilage. Chronic stress can induce an upregulation of *COL2A1* in tendon	CC genotype is associated with improved speed (Murtagh et al., 2020) but the SNP is not linked with EIMD so far
COL5A1	Collagen Type V Alpha 1 Chain	rs12722	C > T	9 (134,842,570)	0.48	Major component of type V collagen, which regulates the diameter of collagen fibrils	T-allele is associated with the severity of skeletal muscle injuries (Massidda et al., 2015)
DES	Desmin	rs12621188	C > G	2 (219,423,170)	0.30	Surrounds the Z-discs to each other and links them to the sarcolemma, and desmin remodelling is associated following eccentric exercise	Not yet known but intronic SNPs have the potential to influence gene expression and mRNA stability (Tabor et al., 2002)
IGF2-AS	IGF2 Antisense RNA	rs4244808	T > G	11 (2,141,880)	0.51	Linked with satellite cell differentiation and proliferation and possibly with ECM integrity	Associated with the response to EIMD (Devaney et al., 2007)
IL6	Interleukin 6	rs1800795	C > G	7 (22,727,026)	0.42	Important function in systemic inflammation and mediates other cytokines	Located within the promoter region. SNP is associated with EIMD (Yamin et al., 2008) and muscle injury (Hall et al., 2021)
MMP3	Matrix Metallopeptidase 3	rs679620	T > C	11 (102,842,889)	0.44	Following muscle damage, MMP3 degrades components of the ECM (e.g., collagen), and regulates the remodelling of skeletal muscle cells	Located within the gene's promoter region. C-allele is linked with decreased gene transcription, and elevated hamstring injury risk (Larruskain et al., 2018)
MYLK	Myosin Light Chain Kinase	rs2700352	G > A	3 (123,831,616)	0.78	The enzyme is activated by Ca2+ binding to calmodulin and it phosphorylates the myosin regulatory light chain that improves the interaction with actin filaments during contractions	GG genotype is associated with lower CK activity and myoglobin concentration after EIMD (Clarkson et al., 2005)

(Continues)

TABLE 1 (Continued)

Symbol	Gene	rs-number	REF > ALT	Chromosome (position)	REF Freq	Protein function	SNP function
MYLK	Myosin Light Chain Kinase	rs28497577	G > T	3 (123,793,780)	0.90	AT-allele is linked with with elevated EIMD markers (Clarkson et al., 2005).	
NOS3	Nitric Oxide Synthase 3	rs2070744	C > T	7 (150,992,991)	0.37	Endothelial Nitric oxide synthase (eNOS) contributes to skeletal muscle repair after injury	T-allele increases gene promoter activity, thus increasing eNOS and NO synthesis (Nakayama et al., 2000)
PAX7	Paired Box 7	rs485874	A > G	1 (18,746,432)	0.44	Transcription factor Pax7 plays a role in satellite cell survival and proliferation	Not yet known but SNPs in the 3′untranslated region may interfere with mRNA stability and translation (Ryan et al., 2010)
TNF	Tumour Necrosis Factor	rs1799964	T > C	6 (31,574,531)	0.79	The proinflammatory cytokine TNF is released from neutrophils after muscle damage	C allele is associated with muscle wasting in cancer patients (Johns et al., 2017)
TRIM63	Tripartite Motif Containing 63	rs2275950	T > C	1 (26,058,512)	0.77	The E3 ubiquitin-protein ligase has an important function in muscle atrophy via the ubiquitin-proteasome pathway	Previous analysis from the same cohort showed an association between the T-allele and greater muscle strength and less muscle pain after EIMD (Baumert et al., 2017)
TTN-AS1	Titin Antisense RNA 1	rs1001238	T > C	2 (178,599,800)	0.72	Stress-sensing proteins along the titin molecule activate muscle remodelling processes after intense exercise in vivo, and differently expressed titin isoforms change the stiffness of cardiac muscle cells in vitro	Both TTN SNPs are associated to distal muscle weakness (Seo et al., 2014) but not to EIMD, yet. Intronic SNPs have the potential to influence gene expression and mRNA stability (Tabor et al., 2002)
TTN-AS1	Titin Antisense RNA 1	rs3731749	C > T	2 (178,541,464)	0.82		
VDR	Vitamin D Receptor	rs2228570	A > G	12 (47,879,112)	0.37	Vitamin D receptor is involved in immune function, calcium homeostasis, bone health and muscle function	G-allele leads to a three amino acid-shorter VDR compared to the A-allele and it is associathed with enhanced transactivation capacity as a transcription factor (Kerr Whitfield et al., 2001)

Note: Chromosome positions are based on NCBI 154. All alleles refer to the forward DNA strand.

Abbreviations: ECM, extracellular matrix; EIMD, exercise-induced muscle damage; mRNA, messenger RNA; REF/ALT, reference allele and alternative allele; REF Freq, reference allele frequency of this study; SNP, single-nucleotide polymorphism.

were converted to a percentage based on the number of EIMD-associated alleles. Combining the scores of each significant SNP gave a TGS within the range of 0%–100%, with the best possible polygenic TGS defined as 100% (containing all beneficial genotypes), and the worst possible TGS as 0% (containing all of the detrimental genotypes). Based on the TGS distribution, we divided the cohort into three categories, namely a 'preferential' (high TGS), 'moderate', and 'non-preferential' (low TGS) genetic profile. The thresholds of these three TGS groups were determined according to the $\bar{x}$ of the TGS ± 1 *SD* (i.e., non-preferential genotype group ≤ $\bar{x}$ −1*SD*; $\bar{x}$ −1*SD* > moderate genotype group < $\bar{x}$ + 1 *SD*; preferential genotype group ≥ $\bar{x}$ + 1 *SD*).

2.14 | 'Metamex' meta-analysis to investigate human skeletal muscle gene expression changes following a single bout of resistance exercise

We performed a meta-analysis using the metamex application (Pillon et al., 2019) to investigate if the expression of genes linked to those SNPs associated with EIMD in our study changed following an acute bout of resistance exercise. This meta-analysis included 10 studies that assessed changes in gene expression from skeletal muscle biopsies collected before and after a single bout of resistance exercise in men and women, aged 18–35 years, with a sedentary to physically active lifestyle, and no restriction of body mass index (FDR <0.05; GSE1832, GSE4249, GSE7286, GSE19062, GSE23697, GSE24235, GSE28422, GSE59088, GSE106865, GSE107934; data analysed 30/10/2020).

2.15 | Data processing and statistical analysis

Each SNP was tested for compliance with the Hardy-Weinberg equilibrium by using a χ^2 test. All parameters were normally distributed according to the Shapiro-Wilk test and by inspection of the Q-Q plots. Linkage disequilibrium was analysed via LDlink suite and data from the 1000 Genomes Project European ancestry populations (Machiela & Chanock, 2015). All analyses were performed using two-way-mixed analysis of covariance (ANCOVA), with genotype (between subjects factor: genotype groups) and time (within subjects factor: PRE, POST, and POST48), while sex (male/female) was included as a covariate. Repeated-testing error by using multiple ANCOVAs was controlled by using a 20% FDR. For post hoc analyses, either, independent *t* tests, or one-way ANOVAs were used, where appropriate. Regarding the primary muscle stem cells aspect of the study, two-way-mixed ANOVAs were used to analyse interactions and main effects for genotype (genotype groups: between subjects factor) and time (0, 24, 48 h: within subjects factor) regarding migration dynamics (total cell migration, proportion inner/outer segment), with wound zone width included as a covariate. A one-way ANOVA was performed to determine if there was a main effect for genotype group (between subject factor) regarding myoblast:fibroblast ratio. Repeated one-way ANOVAs were controlled for multiple comparisons by using a 20% FDR correction. All MVC torque values were analysed with AcqKnowledge software 4.4 (Biopac-Systems Inc.) and normalised to body mass unless otherwise stated. SPSS 23 Software (IBM Inc.) was used for statistical analysis. Results are expressed as mean ± *SD* except otherwise stated, with statistical significance set at $p < 0.05$.

3 | RESULTS

3.1 | Effect of EIMD-Intervention on biomarkers according to sex

3.1.1 | Isometric and isokinetic MVC

There was an interaction between sex and time for isometric ($F_{2,126} = 4.13$, $p = 0.018$) and isokinetic MVC torque ($F_{2,126} = 8.06$, $P = 5.08 \times 10^{-4}$; Table S4) normalised to body mass, i.e. isometric and isokinetic MVC torque was higher in men than women at PRE but not different at POST or POST48. Therefore, subsequent genetic analyses included sex as a covariate.

3.1.2 | Muscle soreness

There was no main effect of sex ($F_{1,63} = 0.15$, $p = 0.701$) and no interaction between sex and time ($F_{2,126} = 1.19$, $p = 0.309$) following EIMD.

3.1.3 | Knee joint ROM

There was no main effect of sex ($F_{1,38} = 0.15$, $p = 0.704$) and no interaction between sex and time ($F_{2,76} = 1.08$, $p = 0.343$) following EIMD.

3.1.4 | Blood biomarkers

There was no main effect of sex ($F_{1,36} = 2.77$, $p = 0.105$; $F_{1,36} = 3.51$, $p = 0.069$) and no interaction between time and sex ($F_{2,72} = 0.66$, $p = 0.521$; $F_{2,72} = 1.67$, $p = 0.202$) regarding serum interleukin-6 concentration or serum creatine kinase activity, respectively.

3.1.5 | Effect of EIMD-Intervention on EIMD biomarkers

Isometric and isokinetic MVC, muscle soreness (all $p < 0.001$) and serum creatine kinase activity ($p = 0.009$) showed a main effect of time, indicating EIMD had occurred (Table S4). Serum creatine kinase activity was not significantly elevated from pre- to post-EIMD ($p > 0.05$), but increased 48 h post-EIMD compared to baseline ($p = 0.003$). However, serum interleukin-6 concentrations did not show any changes at POST and POST48 compared to PRE EIMD-intervention ($p > 0.05$).

3.1.6 | Hardy-Weinberg equilibrium and linkage disequilibrium

The genotypes of all 20 SNPs were in Hardy-Weinberg equilibrium, except for *COL2A1* rs2070739 ($\chi^2 = 6.04$, $p = 0.014$) and *PAX7* rs485874 ($\chi^2 = 5.15$, $p = 0.023$). Linkage disequilibrium calculations revealed that the following SNPs were in linkage disequilibrium: both *COL1A1* (rs1800012 and rs2249492) SNPs ($D' = 0.736$ and $R^2 = 0.077$, $p < 0.001$); *MMP3* (rs679620) and *ACTN3* (rs1815739) SNPs ($D' = 0.068$ and $R^2 = 0.004$, $p = 0.046$); both *MYLK* (rs2700352 and rs28497577) SNPs ($D' = 0.482$ and $R^2 = 0.08$, $p < 0.001$); *PAX7* (rs485874) and *TRIM63* (rs2275950) SNPs ($D' = 0.134$ and $R^2 = 0.004$, $p = 0.004$); and both *TTN-AS1* (rs1001238 and rs3731749) SNPs ($D' = 1.0$ and $R^2 = 0.472$, $p < 0.001$).

3.1.7 | SNP associations with biomarkers of EIMD

From the 20 SNPs analysed, seven displayed significant interactions with time (Table 2) regarding muscle soreness (*COL2A1*, rs2070739; *COL5A1*, rs12722; and *TTN*, rs3731749), ROM (*COL5A1*, rs12722; *IGF2-AS*, rs4244808; *VDR;* rs2228570; and *TRIM63*, rs2275950) and isometric torque (*MMP3*, rs679620).

The responses of the in vivo cohort for both *COL1A1* (rs1800012 and rs2249492), *COL2A1* (rs2070739), *COL5A1* (rs12722) and *TRIM63* (rs2275950) SNPs to the eccentric exercise protocol have been reported previously (Baumert et al., 2017, 2018). However, for each SNP in the present study, we pooled the heterozygote group with the homozygote group that showed a similar response to that group (whereas genotypes were analysed on an individual basis previously). The *TRIM63* rs2275950 SNP demonstrated time × genotype interaction (CC homozygotes compared to T-allele carriers) regarding ROM, but no significant interaction in regard to soreness [as reported in Baumert et al. (2017), where the three genotypes were analysed separately]. The *VDR* (rs2228570) SNP demonstrated an interaction effect between ROM and time following EIMD using a two-way mixed ANCOVA (Table 2). However, post hoc analyses were not able to determine which genotype(s) were preferential or non-preferential. Interpretation of the data in Table 2 and Figure S3 suggest that G-allele carriers had a greater initial damage response immediately after the damage but recovered to near PRE values at 48 h postdamage. AA genotype, on the other hand, showed a different (delayed) damage response, i.e., no change immediately after damage but a detrimental response 48 h after damage. Therefore, we defined AA genotype as the non-preferential genotype for the purpose of our TGS.

In addition to the interactions (Table 2), there were also main effects (Table S5) regarding the following SNPs for isometric torque (*COL1A1*, rs1800012; *COL1A1*, rs2249492; *TRIM63*, rs2275950), for isokinetic torque (*ACTN3*, rs1815739; *COL1A1*, rs1800012; *COL1A1*,

TABLE 2 SNP interactions with the EIMD-intervention after correction for multiple comparisons

Gene symbol	SNP rs-number	Interaction variable (units)	Genotype (*n*)	Time points			*F* test value	*p* value
				PRE	POST	POST48		
COL2A1	rs2070739	Muscle Soreness (cm)	CC (56)	0	3.17 ± 2.22[a]	3.58 ± 2.43	$F_{2,124} = 7.45$	< 0.001
			CT + TT (9)	0	6.00 ± 2.45[a]	3.56 ± 2.19		
COL5A1	rs12722	Muscle Soreness (cm)	CC + CT (49)	0	3.54 ± 2.35	3.13 ± 2.05[a]	$F_{2,124} = 4.22$	0.017
			TT (16)	0	3.63 ± 2.78	4.94 ± 2.84[a]		
COL5A1	rs12722	Range of Motion (°)	CC + CT (32)	118 ± 9[a]	109 ± 12	115 ± 10	$F_{2,74} = 3.42$	0.038
			TT (8)	131 ± 8[a]	116 ± 14	117 ± 15		
IGF2-AS	rs4244808	Range of Motion (°)	TT + TG (35)	119 ± 9[a]	110 ± 12	116 ± 10	$F_{2,74} = 3.99$	0.023
			GG (5)	133 ± 12[a]	111 ± 12	116 ± 19		
MMP3	rs679620	Isometric Torque (N · m)	TT (12)	3.15 ± 1.11	2.04 ± 0.54	2.91 ± 1.22[a]	$F_{2,124} = 5.10$	0.007
			TC + CC (53)	2.93 ± 1.00	2.04 ± 0.72	2.38 ± 0.83[a]		
TRIM63	rs2275950	Range of Motion (°)	TT + TC (38)	121 ± 10	110 ± 13	116 ± 10[a]	$F_{2,74} = 3.38$	0.039
			CC (2)	119 ± 1	113 ± 4	100 ± 14[a]		
TTN-AS1	rs3731749	Muscle Soreness (cm)	CC (45)	0	2.93 ± 2.03[a]	3.28 ± 2.24	$F_{2,124} = 5.46$	0.005
			CT + TT (20)	0	4.98 ± 2.73[a]	4.25 ± 2.59		
VDR	rs2228570	Range of Motion (°)	AG + GG (36)	121 ± 10	110 ± 13	116 ± 10	$F_{2,74} = 3.76$	0.028
			AA (4)	122 ± 12	117 ± 7	108 ± 16		

Abbreviations: EIMD, exercise-induced muscle damage; n, number of participants; SNP, single-nucleotide polymorphism.

[a]significant differences between genotype groups; top row of each SNP represents the beneficial genotype.

rs2249492; *MMP3*, rs679620; *TRIM63*, rs2275950), for muscle soreness (*NOS3*, rs2070744; *TTN-AS1*, rs3731749) and for ROM (*PAX7*, rs485874). To highlight, the COL5A1 (rs12722) SNP showed an interaction effect for both muscle soreness and ROM, whilst the TRIM63 (rs2275950) SNP revealed an interaction effect for ROM and a main effect for isometric torque.

3.1.8 | Exercise modulates the SNP-related gene expression in human skeletal muscle

To explore the gene expression patterns of those genes related to the SNPs associated with EIMD in this study, we performed a bioinformatics meta-analysis in skeletal muscle with respect to acute resistance exercise with transcriptomic data from 10 studies via the application MetaMex (Pillon et al., 2019). Of the genes that were related to our seven SNPs, which were associated with our in vivo EIMD-intervention, three genes (*MMP3*, *TRIM63*, and *VDR*) demonstrated an increased expression following acute resistance exercise in the meta-analysis (Table S6). Two additional genes (*ACTN3* and *NOS3*), of which related SNPs demonstrated a main effect following our EIMD-intervention (*ACTN3* rs1815739 with isokinetic strength and *NOS3* rs2070744 with muscle soreness), also showed changes in gene expression following acute resistance exercise. The messenger RNA (mRNA) expression of the *ACTN3* decreased by 22%, whilst *ACTN2*, a closely related gene to *ACTN3*, showed a higher mRNA abundance following a bout of resistance exercise.

3.1.9 | Artificial wound healing model to assess the genetic association with repair and regeneration

Damaged muscles exhibit immediate muscle strength loss after an EIMD-intervention but the recovery process of muscle strength is already remarkable within one day (Byrne et al., 2001). In contrast, the rise of inflammatory biomarkers in the blood, such as creatine kinase activity, start at a later time point and usually peak 48–72 h after the intervention, indicating that the time frame of muscle strength recovery is somewhat disconnected from the inflammatory response (Baumert et al., 2016; Lindsay & Peake, 2021). Further, the proteins associated with the seven SNPs that showed an EIMD-intervention interaction in vivo (Table 2), are predominantly located within the muscle tissue structure. To explore whether these SNPs are potentially capable of acting directly on the muscle cells per se and of potentially having an effect on the muscle regeneration process through mechanisms that are independent to the inflammatory response, we further investigated the seven SNPs in an in vitro artificial wound healing model. The mean ± *SD* of the myoblast:fibroblast ratio of the twelve participants revealed 1.26 ± 1.00 (range: 0.276–2.93). The *IGF2-AS* (rs4244808) SNP was not analysed, as one genotype group had a sample size of $n = 1$. *TTN-AS1* (rs3731749) did not show any association with artificial wound healing assay parameters (all $p > 0.05$) but there was an interaction between time and total cell migration into the wound zone ($F_{2,18} = 6.79$, $p = 0.006$) regarding *TTN-AS1* (rs1001238) genotype (Figure S4a–c). This latter SNP is in high linkage disequilibrium with *TTN-AS1* (rs3731749, please see section, Linkage Disequilibrium). In TT homozygotes ($n = 8$), more cells (24 h: 66.1 ± 9.56 cells; 48 h: 96.9 ± 10.0 cells) migrated into the wound compared to the cells in individuals with at least one C-allele ($n = 4$; 24 h: 57.0 ± 6.00 cells; 48 h: 78.8 ± 10.1 cells) (Figure 1). Further, there was a main effect for the *ACTN3* (rs1815739) and *COL1A1* (rs2249492) SNPs regarding myoblast:fibroblast ratio in the in vitro artificial wound healing assay. The CC genotype of the *ACTN3* (rs1815739) [CC ($n = 7$): 36.1 ± 18.8% vs. CT + TT ($n = 5$): 61.8 ± 20.9%; $F_{1,10} = 9.26$, $p = 0.012$] and *COL1A1* (rs2249492) [CC ($n = 2$): 17.5 ± 5.9% vs. CT + TT ($n = 10$): 52.7 ± 20.3%; $F_{1,10} = 5.50$, $p = 0.041$] SNPs showed a lower myoblast:fibroblast ratio compared to their respective T-allele carrier counterparts.

3.1.10 | Effect of TGS on biomarkers of EIMD

The seven SNPs (*COL2A1*, rs2070739; *COL5A1*, rs12722; *IGF2-AS*, rs4244808; *MMP3*, rs679620; *VDR*, rs2228570; *TRIM63*, rs2275950; and *TTN-AS1*, rs3731749) that presented significant interactions with either muscle soreness, isometric torque or ROM (Table 2) were used for the TGS analyses. Individuals were divided into three groups, depending on their TGS [see "Total Genotype Score calculation" non-preferential genotype group ≤49%; moderate genotype group; preferential genotype group ≥79%], above, i.e., non-preferential genotype group: $n = 10$ (females = 6; males = 4); moderate genotype group: $n = 37$ (females = 22; males = 15); and preferential genotype group: $n = 18$ (females = 11; males = 7)]. There were no differences between the groups regarding height, body mass and age (Figure S4d–f). Subsequently, there was a main effect for isometric ($F_{2,61} = 8.78$, $P = 4.45 \times 10^{-4}$, Figure 2a) and isokinetic ($F_{2,61} = 7.82$, $P = 9.51 \times 10^{-4}$) MVC torque. Individuals of the non-preferential (1.93 ± 0.81 Nm/kg) and moderate (2.28 ± 0.69 Nm/kg) genetic profile groups revealed weaker baseline isokinetic MVC torque values compared to the preferential genetic profile group (2.73 ± 0.59 Nm/kg; $p = 0.005$).

There was a TGS group × time interaction in regard to muscle soreness ($F_{4,122} = 3.44$, $p = 0.011$, Figure 2b), with greater soreness reported for the non-preferential genotype group compared to the moderate and preferential genetic genotype group at POST ($F_{2,64} = 4.87$, $p = 0.011$) and POST48 ($F_{2,64} = 6.42$, $p = 0.003$). Concerning ROM, there was a TGS group × time interaction ($F_{4,72} = 3.40$, $p = 0.006$), whereby the non-preferential genotype group (135.0 ± 8.66°) showed greater ROM PRE-EIMD compared to the moderate (MG: 118.3 ± 9.69°) and preferential genotype group (122.6 ± 8.70°; $F_{2,36} = 5.13$, $p = 0.011$, Figure 2c). However, the EIMD-intervention resulted in a reduced ROM for the non-preferential genotype group and, therefore, the non-preferential genotype group showed the same level of flexibility POST- and POST48-EIMD as the moderate and preferential genotype group ($p > 0.05$). It should be noted that the non-preferential genotype group comprised $n = 3$ female participants only but, as described above, there was no main effect of sex or interaction between sex

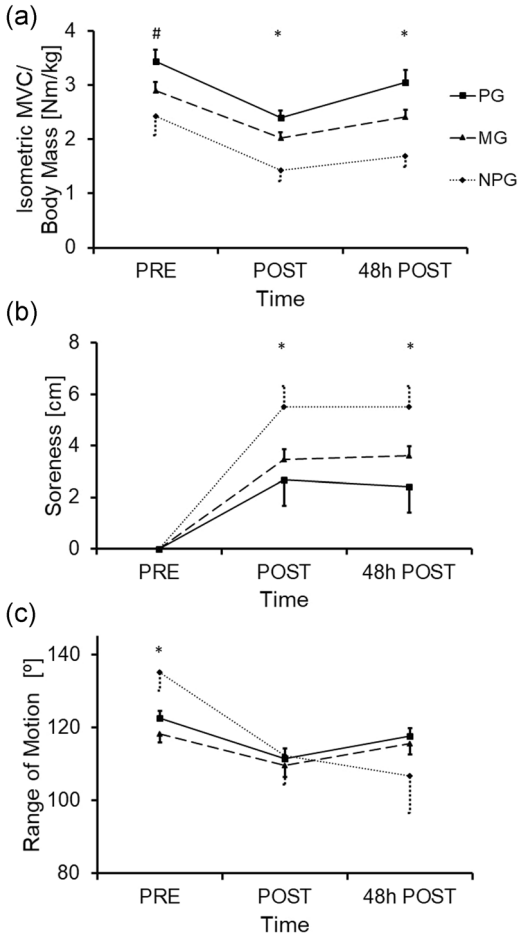

FIGURE 2 (a) Main effect for normalised isometric maximal voluntary contraction torque in regard to the polygenic profile. PG—Preferential Genotype Group ($n = 18$); MG—Moderate Genotype Group ($n = 37$); NPG—Non-Preferential Genotype Group ($n = 10$). #One-way ANOVA, NPG and MG are significant different compared to PG, $p < 0.05$; *One-Way ANOVA, significant differences between each group, $p < 0.05$, mean ± *SEM*. (b) Gene-Interaction effect (polygenic profile × time) for muscle soreness. PG—Preferential Genotype Group ($n = 18$); MG—Moderate Genotype Group ($n = 37$); NPG—Non-Preferential Genotype Group ($n = 10$); *one-way ANOVA, NPG are significant different compared to MG and PG, $p < 0.05$, mean ± *SEM*. (c) Gene-Interaction effect (polygenic profile × time) for range of motion (ROM). PG—Preferential Genotype Group ($n = 13$); MG—Moderate Genotype Group ($n = 24$); NPG—Non-Preferential Genotype Group ($n = 3$); *one-way ANOVA, NPG are significant different compared to MG and PG, $p < 0.05$, mean ± *SEM*. ANOVA, analysis of variance

and time regarding joint ROM. Further, all ANCOVAs were performed with sex as a covariate.

4 | DISCUSSION

The primary aim of this study was to investigate a polygenic association with standardised EIMD, and the main objective was to determine if a combination of candidate SNPs could distinguish between high and low responders to EIMD. To achieve this aim, a total genotype score (TGS; Williams & Folland, 2008) was applied to each participant of our in vivo cohort, based on their genetic profile for seven candidate SNPs that each demonstrated a genotype × EIMD-interaction with at least one EIMD-biomarker. The in vivo cohort was then divided into three sub-groups according to their TGS and the main finding was that the non-preferential polygenic group (i) demonstrated greater muscle soreness and the greatest reduction in knee joint ROM following EIMD; and (ii) were weaker compared to moderate and preferential polygenic groups. A second aim was to investigate whether the same seven SNPs associated with EIMD in vivo were associated with human skeletal muscle stem cell repair in an artificial wound healing assay in vitro. Two *TTN-AS1* SNPs were associated with muscle soreness following EIMD in the in vivo study (rs3731749 SNP), and with total cell migration into the wound zone in the Muscle Stem Cell study (rs1001238), with both SNPs being in high linkage disequilibrium. This information suggests that (i) the response to EIMD in young, healthy adults is polygenic and (ii) *TTN* SNPs may provide a novel mechanism to explain the EIMD response, i.e., *TTN* genotype may influence the elastic components of the muscle stem cell body, subsequently affecting the recovery phase following EIMD in vivo.

4.1 | Functional group of SNPs related to muscle soreness

The first functional group of SNPs comprised *COL2A1* (rs2070739), *COL5A1* (rs12722), and *TTN*-AS1 (rs3731749). These SNPs are located within genes, which contribute to the elastic properties of the muscle-tendon unit and may have a crucial role in storing and releasing energy during fast movements and/or stretch shortening cycles (Turrina et al., 2013). The risk alleles/genotypes of these three SNPs [T-allele of *COL2A1* (rs2070739); TT genotype of *COL5A1* (rs12722); T-allele of *TTN-AS1* (rs3731749)] demonstrated an interaction between perceived muscle soreness and time following EIMD. The three SNPs appear to negatively influence the elastic components of the muscle-tendon unit with a subsequent decreased capacity to store and release energy, making the non-preferential genotype group more prone to EIMD during eccentric contractions compared to the preferential polygenic group. Overstretched cytoskeleton/muscle ECM may result in an increase of extracellular inflammatory mediators, which might trigger the nociceptor response within the muscle, leading to elevated muscle soreness (Hyldahl & Hubal, 2014). In addition, the TT genotype of the *TTN-AS1* (rs1001238) SNP, which is in high linkage disequilibrium with the beneficial C-allele of *TTN-AS1* (rs3731749) SNP (suggesting they might be inherited together as a haplotype), showed an improved artificial wound closure in vitro. Successful muscle stem cell migration is dependent on the mechanical properties of the cell body (Friedl et al., 2011) and previous research showed that differently expressed titin isoforms change the stiffness of cardiac muscle cells (Cazorla et al., 2000). The TT genotype of the *TTN-AS1* (rs1001238) SNP could

potentially lead to more elastic and plastic properties of the muscle stem cell's body. This may positively affect the migration efficiency of the muscle stem cells on the cellular level, which in turn has an impact on the global muscle regeneration following an EIMD-intervention in vivo.

4.2 | Functional group of SNPs related to delayed muscle regeneration

The second functional group of SNPs, which are related to enzymes of the skeletal muscle catabolic (*MMP3*, rs679620; *TRIM63*, rs2275950) and anabolic (*VDR*, rs2228570; also referred to as *FOK1*) signaling pathways, were associated with biomarkers of EIMD in vivo 48 h after the EIMD-intervention. The transcriptional data of the MetaMex application (Pillon et al., 2019) revealed that the related genes (*MMP3*, *TRIM63*, and *VDR*) of these three SNPs increased expression following acute resistance exercise. It is possible that the preferential alleles of three of these SNPs [T-allele of *MMP3* (rs679620); T-allele of *TRIM63* (rs2275950); G-allele of *VDR* (rs22285700)] exert their beneficial effect by increasing their respective gene's transcription rate following the resistance-type exercise during our EIMD-intervention compared to their corresponding non-preferential alleles. The increased response potentially promotes repair and supports successful skeletal muscle remodeling mirrored by a faster recovery of ROM (*TRIM63* and *VDR* SNPs) and muscle strength (*MMP3* SNP) 48 h following EIMD. Further, individuals with the preferential allele of two of these three SNPs (*MMP3* and *TRIM63*) were generally stronger. Increased gene expression of the preferential alleles and a subsequent increased degradation activity of the corresponding enzymes after intense exercise might lead to a better turnover of the target proteins and, therefore, to a higher stability of the ECM, thus potentially explaining the greater strength of the 'preferential' polygenic group.

4.3 | No polygenic association with strength loss following EIMD

It is thought that muscle force is transmitted predominantly laterally via the perimysium (surrounding muscle fascicles) to the tendon and ultimately to the bone (Hyldahl & Hubal, 2014). The preferential alleles/genotypes of the muscle structure-related SNPs [T-allele of *ACTN3* (rs1815739); CC homozygotes of *COL1A1* (rs1800012); and CC homozygotes of *COL1A1* (rs2249492)] showed a main effect for a greater MVC torque generating capacity in vivo. CC homozygotes of *COL1A1* (rs2249492) demonstrated a lower myoblast:fibroblast ratio in vitro. Collagen type I is predominantly present in the perimysial tissue and the beneficial alleles of both *COL1A1* SNPs might increase fibroblast activity, potentially causing stiffer perimysial components and subsequently leading to improved longitudinal and lateral force transmission. The structural role of α-actinin-3 (encoded by the *ACTN3* gene) is to anchor actin to the Z-line but presence of the protein also inhibits the conversion of type II to type I skeletal muscle fibres (Seto et al., 2013). The *ACTN3* rs1815739 SNP results in the substitution of a cytosine with a thymine, leading to a stop codon (X) instead of an arginine (R) at amino acid 577 of exon 16 on chromosome 11. Possessing two copies of the nonfunctional gene (i.e., XX genotype, referred to in this study as TT genotype) prevents the production of alpha-actinin-3, which can influence skeletal muscle phenotype and elite athlete status (Seto et al., 2011). Interestingly, we found that carriers of the *ACTN3* rs1815739 T-allele had a higher myoblast to fibroblast ratio, which may be linked to the SNP's influence on muscle fibre-type composition (Seto et al., 2013; Vincent et al., 2007) and the fact that muscles comprising predominantly type I fibres contain a greater number of satellite cells compared to muscles comprising mainly fast twitch fibres (Gayraud-Morel et al., 2017). Thus, having more slow twitch fibres might positively affect the amount of satellite cells within the skeletal muscle of T-allele carriers. However, our T-allele carriers demonstrated a nonsignificant tendency to lose more strength following EIMD in vivo (unadjusted $p = 0.022$) compared to CC (RR) homozygotes, which supports the findings of previous studies (Seto et al., 2011). The stiff α-actinin-3 in fast-twitch fibres (Broos et al., 2012) might increase the stability and rigidity of (type II) skeletal muscle fibres, making CC (RR) homozygotes more resistant to EIMD.

Regardless, the only SNP that demonstrated an interaction with time regarding MVC was *MMP3* rs679620. Matrix metalloproteinases (MMPs) are enzymes that are synthesised and secreted by fibroblasts, and whose main function is to breakdown ECM components (e.g., collagen) (Hyldahl & Hubal, 2014). The preferential *MMP3* T-allele might increase the enzyme activity to degrade proteins (such as collagen type III within the perimysium), potentially leading to faster turnover of ECM proteins. Together with other protein degradation-related SNPs, such as *TRIM63* rs2275950, this may help explain the faster recovery of MVC strength in *MMP3* TT homozygotes compared to their C-allele counterparts.

As well as *MMP3* rs679620, we have discussed the potential influence of three other SNPs (*COL2A1* rs2070739, *COL5A1* rs12722; *TTN-AS1* rs3731749) on the cytoskeleton and ECM, following our finding that all four SNPs were associated with the response to EIMD in vivo (and in vitro regarding *TTN-AS1* rs1001238). Damage to the ECM (specifically the perimysium) may be a determining factor in distinguishing the aetiology of EIMD from that of muscle strains (Balius et al., 2018). We hypothesise that EIMD-interventions induced by an IKD with low-speed eccentric MVCs (such as those used in this study) are not able to cause sufficient damage to the stiff components of the perimysium (such as the collagen type I fibrils), and that the MVC strength loss seen in this study might be more associated with damage to structural components of the myofibrillar apparatus, particularly those involved in the longitudinal force transmission (e.g., titin and nebulin) (Trappe et al., 2002). In contrast, repeated high external forces involved with high-speed contractions, such as sprinting, might have a greater risk of damage to components of the perimysium (Baumert et al., 2021).

Therefore, although this is the first study to investigate a polygenic association with a standardised form of EIMD, it should be noted that different types of eccentric exercise may damage different tissues/proteins (Coratella et al., 2019). Athletes conducting chronic training might adapt differently to specific stimuli, which could also affect the polygenic association. Thus, the polygenic profile associated with EIMD could be specific to the mode of exercise used to cause damage and to the training status of the athlete. By addressing these points, future studies will comprehensively further our understanding of EIMD aetiology.

4.4 | Limitations

We acknowledge that we have recruited both male and female participants and there is limited evidence that men and women respond differently to eccentric exercise (Sewright et al., 2008). However, the majority of studies attempting to address this question have shown no sex differences in the susceptibility to ultrastructural muscle damage (Sayers & Clarkson, 2001; Stupka et al., 2001). Furthermore, although our male participants were stronger than our female subjects in both absolute terms and relative to body mass, when the change in MVC at time points POST and POST48 was reported as a percentage change from baseline MVC, we observed no difference in strength loss between men and women. We, therefore, chose to analyse absolute strength normalised to body mass, using sex as a covariate. Moreover, each of our three polygenic groups contained a similar ratio of men to women, so it is unlikely that any sex differences influenced the outcome of our results. Finally, whilst these results offer greater external validity than previous studies due to the both controlled in vivo eccentric exercise intervention and in vitro skeletal muscle stem cell wound healing assay, replication by independent groups is required to support our findings.

5 | CONCLUSION/PERSPECTIVE

Our results demonstrate for the first time that seven SNPs in seven different genes, both on an individual and combined (polygenic) basis, were associated with the response to EIMD following strenuous exercise in healthy, young men and women. Individuals with a non-preferential genetic profile concerning changes in muscle soreness and ROM were weaker than individuals with a preferential genetic profile. Moreover, two *TTN* SNPs were associated with EIMD in vivo (rs3731749) and with changes in muscle stem cell migration following damage in vitro (rs1001238), and both SNPs were in high linkage disequilibrium, thus providing evidence for a novel genetic mechanism underpinning the EIMD response in humans. Further work is necessary to determine whether this non-preferential genetic profile (i) increases the risk of sustaining a muscle-tendon injury; and (ii) whether these different EIMD responses between polygenic groups are further amplified in older populations, in whom muscle-tendon properties are diminished and the negative response to EIMD appears to be augmented.,

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Robert M. Erskine, Philipp Baumert, and Claire E. Stewart: conceived and designed the research; **Philipp Baumert, Matthew Cocks, Juliette A. Strauss, and Sam O. Shepherd**: performed the experiments; **Philipp Baumert and Robert M. Erskine**: analyzed the data; **Robert M. Erskine, Philipp Baumert, and Claire E. Stewart**: interpreted the results of the experiments; **Philipp Baumert**: prepared the figures; **Philipp Baumert**: drafted the manuscript; **Robert M. Erskine, Philipp Baumert, Mark J. Lake, Barry Drust, Claire E. Stewart, Matthew Cocks, Juliette A. Strauss, and Sam O. Shepherd**: edited and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article (and its Supporting Information files).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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