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IGF-II gene region polymorphisms related to exertional muscle damage

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Devaney JM, Hoffman EP, Gordish-Dressman H, Kearns A, Zambraski E, Clarkson PM. IGF-II gene region polymorphisms related to exertional muscle damage. *J Appl Physiol* 102: 1815–1823, 2007. First published February 8, 2007; doi:10.1152/japplphysiol.01165.2006.—We examined the association of a novel single-nucleotide polymorphism (SNP) in IGF-I (IGF-I −C1245T located in the promoter) and eight SNPs in the IGF-II gene region with indicators of muscle damage [strength loss, muscle soreness, and increases in circulating levels of creatine kinase (CK) and myoglobin] after eccentric exercise. We also examined two SNPs in the IGF binding protein-3 (IGFBP-3). The age, height, and body mass of the 151 subjects studied were 24.1 ± 5.2 yr, 170.8 ± 9.9 cm, and 73.3 ± 17.0 kg, respectively. There were no significant associations of phenotypes with IGF-I. IGF-II SNP (G12655A, rs3213216) and IGFBP-3 SNP (A8618T, rs6670) were not significantly associated with any variable. The most significant finding in this study was that for men, IGF-II (C13790G, rs3213221), IGF-II (ApaI, G17200A, rs680), IGF-II antisense (IGF2AS) (G11711T, rs7924316), and IGFBP-3 (−C1592A, rs2132570) were significantly associated with muscle damage indicators. We found that men who were *1*) homozygous for the rare IGF-II C13790G allele and rare allele for the ApaI (G17200A) SNP demonstrated the greatest strength loss immediately after exercise, greatest soreness, and highest postexercise serum CK activity; *2*) homozygous wild type for IGF2AS (G11711T, rs7924316) had the greatest strength loss and most muscle soreness; and *3*) homozygous wild type for the IGF2AS G11711T SNP showed the greatest strength loss, highest muscle soreness, and greater CK and myoglobin response to exercise. In women, fewer significant associations appeared.

insulin-like growth factor-II; single-nucleotide polymorphism

ECCENTRIC, muscle-lengthening contractions produce high levels of strain on muscle, resulting in damage that is manifested in prolonged losses in strength and range of motion, development of delayed-onset muscle soreness, and increases in muscle proteins [e.g., creatine kinase (CK) and myoglobin (Mb)] in the circulation (6, 23, 29–31, 35). We have found that individual responses to a standardized elbow flexion eccentric exercise are highly variable (7). To explain this phenomenon, we examined whether polymorphisms of two proteins coding for components of the sarcomere were associated with muscle damage after eccentric exercise in a sample size of 151 men and women who performed the standard elbow flexion eccentric exercise protocol (5). We found an association between a polymorphism in the sarcomeric protein myosin light chain kinase (MLCK; currently abbreviated MYLK) (2 SNPs tested; C49T and C37885A) and changes in blood CK and Mb and isometric strength. Following the eccentric exercise, those subjects homozygous for the MLCK 49T rare allele had a significantly greater increase in CK and Mb compared with the heterozygotes (CT), and those heterozygous for MLCK C37885A had a significantly greater increase in CK compared with the homozygous wild type (CC). MLCK C37885A was also associated with postexercise strength loss; the heterozygotes demonstrated greater strength loss compared with the homozygous wild type. These results show that variations in genes coding for a specific myofibrillar protein influence the phenotypic response to muscle-damaging exercise. Since variations in these genes explained less than 16% of the variation in the various phenotype measures, other genetic factors must contribute to this variability as well.

It is well known that muscles increase in size in response to a resistance exercise program, and the stimulus for hypertrophy is considered to be muscle damage, likely from eccentric contractions (11). We therefore considered whether polymorphisms in genes that are involved in muscle growth, specifically insulin-like growth factors (IGFs), could help to explain the variability in response to eccentric exercise. IGFs are members of a family of proteins involved in satellite cell differentiation and proliferation (12, 16). In animal models, IGFs play a key role in growth (32) and have been found to increase in response to degeneration and regeneration, thereby influencing satellite cell activation (1, 2, 12, 24, 26). Research on pigs has shown that there are quantitative trait loci affecting growth, muscle mass, or fat deposition that map to the IGF-I and IGF-II loci (3, 28, 43).

Polymorphisms have been identified in the IGF-I and IGF-II genes in humans that are associated with muscle phenotypes. Kostek et al. (20) found that the development of muscular strength in response to a resistance training program was associated with the IGF-I dinucleotide repeat polymorphism located in the promoter region such that carriers for the 192 allele gained significantly more strength than noncarriers of this allele. Two studies reported significant associations of IGF-II ApaI polymorphism and muscle phenotypes. First, Sayer et al. (39) found the ApaI polymorphism of the IGF-II gene was associated with grip strength for men but not women in a large sample of 693 men and women in the United Kingdom. Second, Schrager et al. (40) genotyped subjects for the IGF-II ApaI polymorphism from cohorts of prior studies and tested for associations with arm and leg strength and body composition. For men, arm strength was greater for the homozygous wild-type than the homozygous rare allele group, whereas for women, the homozygous rare allele group showed

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lower arm and leg strength and lower total body fat-free mass compared with homozygous wild type.

Exertional muscle damage through eccentric exercise has been found to activate satellite cells (8, 10). Crameri et al. (8) reported that muscle biopsies taken after subjects performed eccentric exercise showed a significant increase in mononuclear cells staining positive for neural cell adhesion molecule, an abundant protein that had been found on the surface of early embryonic myotubes, and fetal antigen 1, indicating activation of satellite cells (38). Quiescent satellite cells are likely activated by growth factors, such as IGF-I, released from damaged muscle fibers (18, 25). Keller et al. (18) found that after contraction-induced damage in murine soleus muscle, there was a significant increase in IGF-I and IGF-II in the days following the injury. Developmental myosin heavy chain staining also increased and was coexpressed with both IGFs.

Because of the involvement of IGF-I and IGF-II in the muscle damage and repair process, we examined the association of a novel SNP in IGF-I (reference sequence: NM_000618.2; −C1245T) and examined eight total SNPs in the entire IGF-II gene region {5 SNPs in the IGF-II gene [reference sequence (Ref Seq): NM_001007139.3; G12655A, T13705C, C13790G, C16646T, and the ApaI SNP (G17200A)]; 2 SNPs in the IGF-II antisense gene (IGF2AS) (NM_016412; A1364C, G11711T), and 1 SNP in the INS gene (Ref Seq: NM_000207.2; C1045G)}, and two SNPs in IGF binding protein-3 (IGFBP-3) (Ref Seq: NM_001013398.1; A8618T, −C1592A) with indicators of muscle regeneration: strength recovery, muscle soreness, and increases in circulating levels of CK and Mb (see Table 1 for SNP positions and SNP identification numbers).

The IGF-II gene region includes three genes [IGF-II, IGF2AS, and the insulin gene (INS)] (22, 36). Human IFG2AS is translated from an antisense transcript of the IGF-II gene in the reverse orientation and share common genetic regions with IGF-II (34). The IGF2AS gene is ubiquitously expressed in normal tissue; however, its abundance is only 1–10% of IGF-II (44). Both IGF-II and IGF2AS are paternally imprinted and appear to be part of the same chromatin domain (28). The INS gene is the best-known regulator of intermediary metabolism (29). In addition, INS is a promoter of growth in utero and insulin secretion (28).

Table 1. *Information for SNPs: gene, position, SNP identification number, and location*

Gene	SNP	SNP ID No.	Location of SNP
IGF-II	C16646T	rs2230949	3′-UTR
IGF-II	C13790G	rs3213221	1st Intron
IGF-II	T13705C	rs3213220	1st Intron
IGF-II	G12655A	rs3213216	1st Intron
IGF-II	G17200A (ApaI)	rs680	3′-UTR
IGFBP-3	A8618T	rs6670	3′-UTR
IGFBP-3	−C1592A	rs2132570	Promoter
IGF-1	−C1245T	rs35767	Promoter
IGF2AS	A1364C	rs4244808	1st Intron
IGF2AS	G11711T	rs7924316	3-Downstream
INS	C1045G	rs3842748	2nd Intron

SNP, single-nucleotide polymorphism; ID, identification; IGF, insulin-like growth factor; IGF2A, IGF-II antisense; IGFBP-3, IGF binding protein-3; INS, insulin; UTR, untranslated region.

We hypothesized that strength loss, muscle soreness, and increases in circulating levels of CK activity and Mb concentration in response to muscle-damaging eccentric exercise would differ among the genotype groups for the IGF-I and IGF-II gene region SNPs. Any SNP that interferes with the muscle regeneration activity of IGF-I or IGF-II would be detrimental to muscle function; therefore, we hypothesized that the rare alleles of the SNPs in IGF-I and IGF-II would cause increases in muscle soreness, circulating levels of CK, and Mb concentration. Because IGFBPs are thought to localize IGFs to the receptor, regulate IGF activity, and thereby modulate IGF action on muscle size and phenotype (41), we also examined two SNPs in IGFBP-3 (−C1592A, A8618T). We hypothesized these SNPs may affect the function of IGFBP-3, including the binding to IGF-I, thus increasing muscle soreness, circulating levels of CK, and Mb concentration.

METHODS

The blood samples used in this study were derived from a larger clinical trial, and further details are presented in our earlier work (5, 7). Briefly, in that study (5), we applied one dosage of a topical nonsteroidal anti-inflammatory drug analgesic to the elbow flexors approximately 12–14 h after the exercise. In the statistical analyses for associations in the present study, treatment was included as a covariate. We took an additional sample of blood for DNA genotyping. Subjects gave informed consent for this additional sample, and the protocol and informed consent document for both the clinical trial and the additional blood draw were approved by the University of Massachusetts Human Subjects Review Committee. Of the 208 subjects who participated in the clinical trial, 181 subjects agreed to have the additional blood sample taken for DNA testing. Viable DNA samples were isolated from 156 subjects, 78 men and 78 women, but the number of subjects differs for each SNP. Within this study sample, there were approximately 73% Caucasians, 3% African-Americans, 4% Hispanics, 13% Asians, and 7% who self-classified their race as "other." We have previously reported data on associations of α-actinin 3 and MLCK with markers of muscle damage in this same subject group (5). Subject inclusion criteria is described in our previous reports (5, 7).

Subjects performed 50 maximal isotonic eccentric (muscle lengthening) contractions of the elbow flexor muscles of their nondominant arm on a modified preacher curl bench (31). Details of the exercise can be found in our earlier work (5, 7). Subjects performed three maximal isometric contractions at an elbow angle of 90° (3 s each with 1 min between trials) before and immediately after exercise, and the average was used as the criterion measure. Subjects returned to the laboratory 4, 7, and 10 days postexercise for assessment of strength and to have a blood sample drawn. Muscle soreness was assessed preexercise and 3, 4, 7, and 10 days postexercise using a visual analog scale, a 100-mm line on which subjects place a mark between 0 (no soreness) to 100 mm (unbearable pain). Blood samples were analyzed for CK activity and Mb concentration (5). Another blood sample was drawn in EDTA-containing vacutainer tubes and shipped deidentified to Children's National Medical Center, Washington, DC, for DNA extraction and subsequent genotyping.

Selection of SNPs and genotyping. The SNPs for this study were selected on the basis of two criteria: position in the gene and conservation (42). For example, Fig. 1 shows that the SNPs selected for IGF-II, IGF2AS, and INS are all located within the 1st or 2nd introns and untranslated regions (UTRs) except for rs7924316. The SNPs rs7924316 and rs2067051 were selected for genotyping on the basis of conservation information (33). This is an attempt to discover a genetic variant that might have some function in controlling gene expression (9). In addition, the SNPs picked for the IGFBP-3 and IGF-I are within UTRs or the promoter region of the two genes.

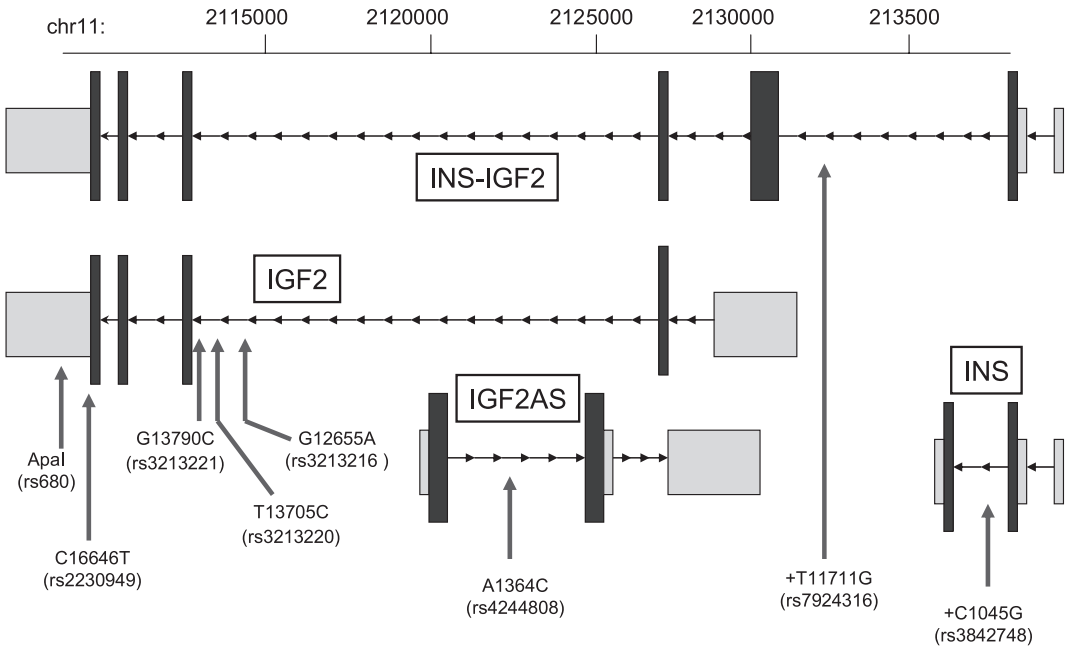

Fig. 1. Schematic of genes in the chromosome 11 region and single-nucleotide polymorphisms (SNPs) for this study. The dark boxes represent coding sequence, and the light boxes represent untranslated regions (UTRs). IGF2, insulin-like growth factor-II gene; IGF2AS, IGF-II antisense gene; INS, insulin gene.

The methods for genomic DNA extraction and genotyping have been previously described (8). For each SNP, the primers and probes are listed in Table 2.

Statistics. The phenotypes that were tested for association with genotype were baseline isometric strength [maximum voluntary contraction (MVC)]; relative (compared with baseline) MVC immediately after the eccentric exercise; relative MVC at 4 days postexercise; relative MVC at 7 days postexercise; relative MVC at 10 days postexercise; baseline blood CK activity; CK activity at 4, 7, and 10 days postexercise; and blood Mb concentration at 4 days postexercise.

Hardy-Weinberg equilibrium was determined for each SNP using a χ^2-test to compare the observed genotype frequencies to those expected under Hardy-Weinberg equilibrium. Normality of each quantitative trait was tested using the Shipiro-Wilk normality test. Mean quantitative muscle measurements were compared in relation to SNP genotypes using analysis of covariance (ANCOVA) methods. The ANCOVAs used Sidak post hoc tests to control for multiple tests. To account for any possible effects of sex, body mass, and treatment, analyses were conducted in sex-specific cohorts while covarying for body mass, age, and treatment. The percent variation attributable to each SNP was determined with a likelihood-ratio test comparing the

Table 2. *TaqMan primer sets for genotyped SNPs*

Gene	SNP	Forward Primer	Reverse Primer	WT Allele Probe (5′-VIC)	MT Allele Probe (5′-FAM)
IGF-I	−C1245T	GGATTTCAAGCAGAAC-TGTGTTTTCA	GGTGGAAATAACCTGGAC-CTTGAAT	CCTGAGAGTCATGTGG-AAA	CTGAGAGTCATGC-GGAAA
IGF-II	G12655A	CTTCGGCCCCCTCCTT	TGCGGAGCAGGTGAGG	TCAGGGCTTTTCC	TCAGGACTTTTCC
IGF-II	T13705C	CCTGAGAAGTGGCGAT-GTGA	CGACCTGGCCTCCGT	CCCCATGGTGTCATAG	CCATGGCGTCATAG
IGF-II	G13790C	CCCCTGCAGCTGTGGAT	CCTTCCATTTGCAAGAAG-CACTAG	AATAATGACCGTGCAG-ATG	AATGACCGTGGAGATG
IGF-II	ApaI	TGGACTTGAGTCCCTG-AACCA	GTGCCCACCTGTGATTTCTG	AGAGAAAAGAAGGGCC	AAAGAGAAAAGAA-GGACC
IGF-II	C16646T	GGCCAGCAATCGGAAG-TGAG	CTGATGGAAACGTCCGTG-GTC	GCCCGGCGCCAC	AGCCCGGTGCCAC
IGFBP-3	−C1592A	GGTGCTGGGCCTTCGT	GGACCTCTACAAATAAAA-AGAGACCAAAGA	CAATGGAGGGCCCCG	ACAATGGAGTGCCCCG
IGFBP-3	A8618T	GCCCCAAATATAGTAA-GATCTATACTAGATA-ATCCT	GGCGTGAGCTCCTTTCCT	CACAGTTGTATCATAT-AGCA	CAGTTGTATCAAA-TAGCA
IGF2AS	A1364C	GACGACATCCAGGAAA-ATATATAGTCTTTT-TCTTA	CCTGGAGTGTGATCAGTA-CATTTGG	TTTTCTATGCTAATTT-TTAAATGA	CTATGCTAATTTG-TAAATGA
IGF2AS	G11711T	GCACCGCCAGTAAATC-CATATTG	CCTCTTCTCTCAATTCCC-AAGGTTT	CCATGACCGTAGCTAC	CATGACCGGAGCTAC
INS	C1045G	GGAAGGAGGTGGGACA-TGTG	GGCTGGACCCAGGTTAGAG	CCCACAGTGGGTGTG	CCCACACTGGGTGTG

WT, wild type; MT, mutant; FAM, fluorescein; VIC, 2′chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein.

Table 3. *LD (r^2) between IGF-II SNPs and other SNPs in the same gene region*

	INS (C1045G)	IGF2AS (A1364C)	IGF2AS (G11711T)	IGF-II (G12655A)	IGF-II (T13705C)	IGF-II (C13790G)	IGF-II (C16646T)
IGF2AS (A1364C)	0.18						
IGF2AS (G11711T)	0.23	0.56					
IGF-II (G12655A)	0.12	0.76	0.37				
IGF-II (T13705C)	0.01	0.00	0.00	0.00			
IGF-II (C13790G)	0.03	0.08	0.10	0.02	0.01		
IGF-II (C16646T)	0.01	0.01	0.01	0.03	0.00	0.04	
IGF-II (G17200A)	0.04	0.29	0.24	0.22	0.00	0.56	0.03

Linkage disequilibrium.

full model containing genotype and all covariates with the constrained model containing only covariates.

Data are reported as means ± SE or adjusted means ± SE where appropriate. All analyses were performed using Stata Version 8.2 (StataCorp, College Station, TX). We set the significance level at $P < 0.05$, but given that we performed multiple statistical tests, those tests under $P < 0.01$ should be viewed with caution and require validation in future studies.

Linkage disequilibrium and haplotypes. Pairwise tests for linkage disequilibrium (LD) were done for all eight polymorphisms within the IGF-II gene region using Lewontin's D', odds ratio, and R^2 calculation on each pairwise combination in the 151 subjects from this study cohort (Table 3 and Fig. 2).

RESULTS

The age, height, and body mass of the 151 subjects (78 women and 73 men) from which viable DNA could be isolated were 22.8 ± 4.6 yr (mean ± SD), 159.2 ± 6.8 cm, and 64.5 ± 11.4 kg (for women) and 25.3 ± 5.4, 170.5 ± 7.9 cm, 81.9±17.2 kg (for men), respectively. The responses of the total subject population for strength, CK, and Mb to the eccentric exercise regimen have been previously reported (5). Here we report responses for the sex-specific cohorts. Briefly, the baseline and 4, 7, and 10-day postexercise average CK values were 86.2 ± 38.1, 5,484.5 ± 6,914.3, 2,052.0 ± 2,620.0, 327.6 ± 351.1 U/l, respectively, in women and 146.1 ± 74.6, 10,174.0 ± 15,042.5, 4,169.7 ± 8,983, 660.8 ± 990.9 U/l, respectively in men. Because myoglobin at baseline and at 10 days postexercise was often below the detection limit of the assay (27 ng/ml), only the 4 and 7 days postexercise measures (343.1 ± 375.9 and 70.9 ± 201.8 ng/ml, respectively, in women and 420.9 ± 487.8 and 123.6 ± 114.5 ng/ml, respectively, in men) were analyzed for their association with genotype. The average percent strength losses immediately after exercise and 4, 7, and 10 days postexercise were 56.3 ± 17.9, 56.8 ± 18.1, 41.5 ± 28.4, and 26.9 ± 28.2%, respectively, in females and 112.9 ± 32.6, 47.6 ± 18.3, 33.0 ± 29.3, and 21.6 ± 30.1%, respectively, in men. Soreness was elevated to 51 ± 15 and 55 ± 22 mm, respectively, in women and 49 ± 19 and 50 ± 23 mm, respectively, in men, on 3 days and 4 days postexercise, respectively, and returned to baseline by 7 days postexercise. Table 4 shows the genotype and allele frequencies for IGF-I (C1245T), IGF-II SNPs, and IGFBP SNPs.

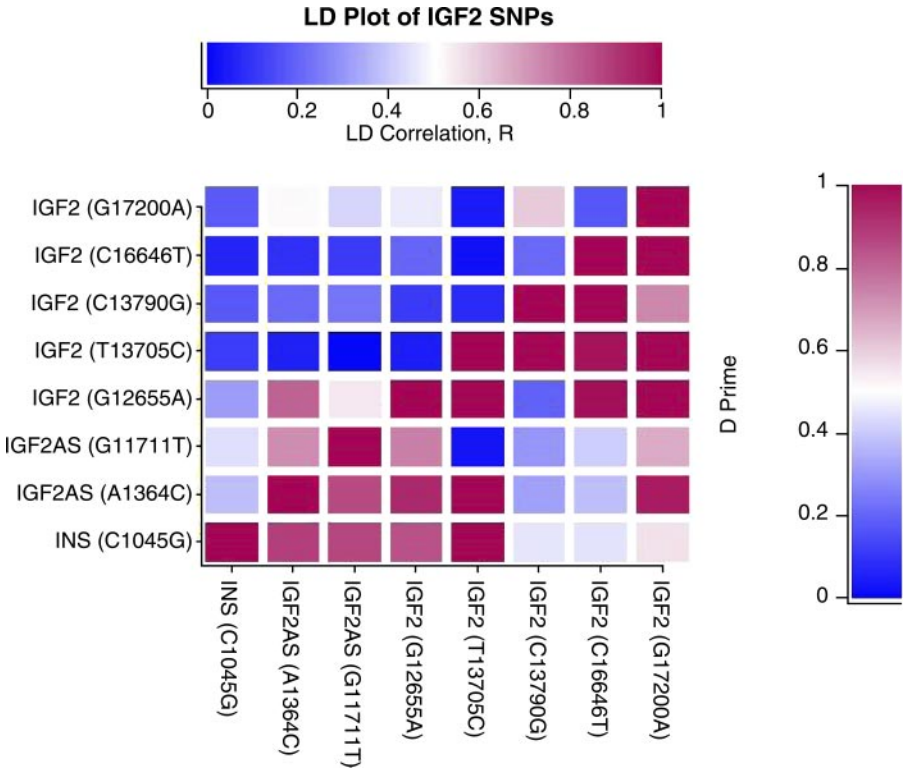

Fig. 2. Linkage disequilibrium (LD) plot of SNPs in the IGF-II gene region. This plot shows LD correlation (R) and D' for 7 SNPs within the IGF-II gene region.

Table 4. *Genotype distributions of the sample population*

Gene	SNP	Homozygous Wild Type	Heterozygous	Homozygous Rare Allele	Allele Frequency	
					Wild Type	Rare
IGF-II	C16646T	135 (87.1%, CC)	19 (12.3% CT)	1 (0.6%, TT)	0.932	0.068
IGF-II	G13790C	69 (44.2%, GG)	58 (37.2%, GC)	29 (18.6%, CC)	0.628	0.372
IGF-II	T13705C	153 (99.3%, TT)	1 (0.7%, TC)	0 (CC)	0.997	0.003
IGF-II	G12655A	70 (45.4%, GG)	60 (39.0%, GA)	24 (15.6%, AA)	0.649	0.351
IGF-II	G17200A	89 (54.6%, GG)	56 (34.4%, GA)	18 (11.0%, AA)	0.718	0.282
IGFBP-3	A8618T	85 (56.7%, AA)	50 (33.3%, AT)	15 (10.0%, TT)	0.733	0.267
IGFBP-3	−C1592A	111 (70.3%, CC)	42 (26.6%, CA)	5 (3.2%, AA)	0.835	0.165
IGF-I	−C1245T	103 (65.6%, CC)	44 (28.0%, CT)	10 (6.4%, TT)	0.796	0.204
IGF2AS	A1364C	55 (36.2%, AA)	68 (44.7%, AC)	29 (19.1%, CC)	0.586	0.414
IGF2AS	G11711T	45 (27.8%, GG)	75 (46.3%, GT)	42 (25.9%, TT)	0.509	0.491
INS	C1045G	106 (65.5%, CC)	46 (28.4%, CG)	10 (6.17%, GG)	0.796	0.204

All SNPs were in Hardy-Weinberg equilibrium. Table 5 presents the significant associations of IGF-II and IGFBP SNPs with changes in CK and strength loss. There were no significant associations of muscle phenotypes with genetic variations in IGF-I. Also, other IGF-II SNPs (G12655A) and IGFBP-3 SNP (A8618T) were not significantly associated with any outcome variable. For men, the IGF-II SNPs G13790C, C16646T, and ApaI were significantly associated with strength, serum CK activity, and soreness. However, for women, fewer significant associations were found.

Men homozygous for the rare allele for IGF-II G13790C showed greater strength loss immediately after exercise, greater soreness at 3 days postexercise, and higher serum CK activity at 7 days postexercise. Men homozygous for the IGF-II ApaI polymorphism had greater strength loss immediately postexercise, greater soreness at 4 days postexercise, and higher serum CK activity at 7 days postexercise. Men homozygous for the wild-type IGF2AS SNP and G11711T SNP showed greater strength loss and more soreness compared with the heterozygotes. For the G11711T SNP, men homozygous for the wild-type alleles also showed higher CK and Mb response to exercise compared with the heterozygotes.

DISCUSSION

We found the population frequency of the ApaI SNP to be 54.6, 34.4, and 11.0% for GG, GA, and AA, respectively. These values are similar to those previously reported by Schrager et al. (40) of 49, 39, and 12% and Gaunt et al. (13) of 53, 40 and 7%. Frequencies from dbSNP, using data for HapMap (CEPH samples) or a Caucasian group, show similarities to our data (see Supplemental Table, available with the online version of this article).

The most significant finding in this study was that for men, IGF-II (G13790C), IGF-II (ApaI), IGF2AS (A1364C), and IGF2AS (G11711T) were significantly associated with muscle damage indicators.

There were fewer significant associations for women. Although there is no apparent explanation for the sex-specific associations, several gene association studies also find this to be true (4, 37, 47). One reason we did not find a significant association with the IGF-I SNP (−C1245T) or some of the IGF-II SNPs could be that these polymorphisms do not substantially alter the function of the IGF genes; further research is needed to determine whether this is a possibility.

We found a significant correlation of strength loss with serum CK activity at 4 days postexercise ($r = -0.43$, $P < 0.01$) and at 7 days postexercise ($r = -0.50$, $P < 0.01$). Both the strength loss and increased serum CK activity indicate muscle damage, although strength is considered a better indicator of damage (6, 45). Men homozygous for the rare allele of IGF-II (G13790C) demonstrated dramatically greater strength loss (58%) immediately postexercise and serum CK activity at 7 days postexercise (8818 U/l) compared with the other genotype groups (see Table 5 for the response data for all genotype groups). Likewise, men homozygous for the rare IGF-II ApaI allele demonstrated 60% strength loss immediately postexercise and 12,261 U/l CK activity at 7 days postexercise. For the IGF2AS A1364C SNP, men homozygous for the wild-type allele showed the greatest strength loss (58%) and greatest soreness (50 mm). For the C11711T SNP, men homozygous for the wild-type alleles showed greater strength loss (56%), more soreness (53 mm), and higher increases in blood CK (17,160 U/l) and Mb (659 ng/ml) than one or both other genotype groups. Thus individuals possessing specific alleles of IGF-II gene appear to be more susceptible to exertional muscle damage.

Interestingly, we discovered LD between some of the SNPs that show significant associations. The IGF2AS A1364C and the IGF2AS G11711T SNPs had an r^2 value of 0.56 and were both associated with strength loss and soreness in men. Additionally, the IGF-II ApaI (G17200A) SNP and the IGF-II G13790C SNP had an r^2 value of 0.56 and both genotypes were associated CK activity, soreness, and strength loss. It is very possible that there exist certain haplotypes in the IGF-II gene region that predispose individuals for strength, muscle soreness, and CK levels. Interestingly, we did not see any associations with the IGF-II G12655A and any of our variables, even though this SNP is in LD with two IGF2AS SNPs (A1364C and G11711T). However, the LD values for these SNPs are only in 151 individuals. SNP discovery remains to be completed on this genetic region and HapMap (17) data are available to be used to explore haplotype diversity among populations.

Studies have reported significant associations of body mass, fat-free mass, and strength with IGF-II ApaI genotypes. Although Schrager et al. (40) found that IGF-II ApaI genotype was significantly associated with isokinetic arm strength in men, the AA genotype men had lower strength than the GG

Table 5. *Significant associations of IGF-II and IGFBP SNP genotypes with strength, muscle soreness, and serum CK and myoglobin*

SNP/Measurement	Sex	*F*-Test	*P* Value	*n;* Adjusted Mean±SE	%Variability Attributable to Genotype: LRT; *P* Value
IGF-II C16646T (rs2230949)					
Soreness 4 days postexercise	M	4.12	0.046	CC: $n = 64$; 39.83±2.85* CT: $n = 10$; 23.98±7.25*	5.5%; 0.038
IGF-II G13790C (rs3213221)					
Strength loss immediately postexercise	M	3.93	0.024	CC: $n = 33$; −44.36±3.06* CG: $n = 21$; −45.82±3.92 GG: $n = 19$; −58.12±4.07*	10.1%; 0.017
Soreness 3 days postexercise	M	3.38	0.040	CC: $n = 33$; 44.77±4.06* CG: $n = 21$; 50.22±5.18 GG: $n = 18$; 65.50±5.52*	9.2%; 0.030
CK activity at 7 days postexercise	M	3.56	0.034	CC: $n = 33$; 2,768.35±1,508.61 CG: $n = 20$; 2,263.79±1,970.92 GG: $n = 19$; 8,817.56±2,002.77	9.3%; 0.025
IGF-II T13705C (rs3213220)					
Baseline strength	F	6.43	0.013	TT ($n = 77$; 55.87±1.75)* TC ($n = 1$; 95.71±0.71)*	6.2%; 0.010
Myoglobin 4 days postexercise	F	4.15	0.045	TT ($n = 76$; 333.20±42.38)* TC ($n = 1$; 1099.72±373.72)*	5.3%; 0.038
CK activity 4 days postexercise	F	5.45	0.022	TT ($n = 76$; 5,278.3±766.3)* TC ($n = 1$; 21,152.7±6,756.6)*	6.7%; 0.018
IGF-II ApaI G17200A (rs680)					
Strength loss immediately postexercise	M	3.84	0.027	GG: $n = 41$; −44.67±3.85* GA: $n = 20$; −45.73±4.24 AA: $n = 12$; −60.91±5.24*	9.9%; 0.019
Soreness 4 days postexercise	M	3.22	0.046	GG: $n = 41$; 33.89±3.66* GA: $n = 20$; 35.60±5.44 AA: $n = 12$; 53.09±6.72*	8.6%; 0.035
CK activity 7 days postexercise	M	6.37	0.003	GG: $n = 40$; 2,439.6±1,364.9† GA: $n = 20$; 2,775.4±2,010.8* AA: $n = 12$; 12,260.8±2,474.4*†	15.5%; 0.002
IGFBP-3 C1592A (rs2132570)					
Baseline CK	M	5.80	0.005	CC: $n = 55$; 147.13±9.56† CA: $n = 17$; 120.69±17.21* AA: $n = 3$; 273.29±41.74*†	14.3%; 0.003
IGF2AS A1364C (rs4244808)					
Strength loss immediately postexercise	F	4.15	0.019	AA ($n = 29$; −58.57±3.24) AC ($n = 31$; −61.16±3.16)* CC ($n = 18$; −46.39±4.17)*	10.0%; 0.014
Strength loss immediately postexercise	M	3.55	0.034	AA ($n = 26$; −55.58±3.56)* AC ($n = 37$; −43.35±2.94)* CC ($n = 11$; −45.52±5.57)	9.1%; 0.025
Soreness 4 days postexercise	M	6.87	0.002	AA ($n = 26$; 50.36±4.30)* AC ($n = 37$; 29.72±3.55)* CC ($n = 11$; 34.50±6.72)	16.4%; 0.001
Baseline CK	M	5.92	0.004	AA ($n = 26$; 123.45±14.12)* AC ($n = 37$; 173.42±11.64)*† CC ($n = 11$; 104.52±22.07)†	14.7%; 0.003
IGF2AS G11711T (rs7924316)					
Strength loss immediately postexercise	M	3.70	0.030	GG ($n = 16$; −48.32±4.53) TG ($n = 38$; −43.15±2.90)* TT ($n = 20$; −56.86±4.09)*	9.5%; 0.022

Continued

Table 5.—*Continued*

SNP/Measurement	Sex	*F*-Test	*P* Value	*n;* Adjusted Mean ± SE	% Variability Attributable to Genotype: LRT; *P* Value
Strength loss 4 days postexercise	M	5.79	0.005	GG (n = 16; −35.37±7.10) TG (n = 38; −23.67±4.55)* TT (n = 20; −50.42±6.41)*	14.3%; 0.003
Strength loss 10 days postexericse	M	7.25	0.001	GG (n = 16; −26.60±6.99) TG (n = 37; −10.56±4.54)* TT (n = 20; −39.64 ±6.32)*	16.8%; <0.001
Soreness 3 days postexercise	M	4.88	0.010	GG (n = 16; 45.78±5.76) TG (n = 37; 44.99±3.75)* TT (n = 20; 64.58±5.23)*	12.6%; 0.007
Soreness 4 days postexercise	M	7.01	0.002	GG (n = 16; 31.36±5.47)* TG (n = 38; 31.95±3.50)† TT (n = 20; 53.64±4.94)*†	16.6%; 0.001
Myoglobin 4 days postexercise	M	3.89	0.025	GG (n = 13; 492.34±133.98) TG (n = 38; 289.77±77.74)* TT (n = 20; 659.27±109.46)*	10.5%; 0.018
CK activity at 4 days postexercise	M	3.60	0.033	GG (n = 13; 12,069.30±4,031.01) TG (n = 38; 6,247.44±2,340.97)* TT (n = 19; 17,160.60±3,390.07)*	9.6%; 0.024
CK activity at 7 days postexercise	M	4.14	0.020	GG (n = 15; 3,772.16±2,237.64) TG (n = 38; 1,867.10±1,392.59)* TT (n = 20; 8,841.29±1,960.74)*	10.5%; 0.014
INS C1045G (rs3842748)					
CK activity at 10 days postexercise	F	3.97	0.023	CC (n = 47; 267.97±50.07)* CG (n = 26; 359.61±67.56) GG (n = 5; 722.26±155.18)*	9.6%; 0.017

All phenotypes are adjusted by sex, body weight, treatment, and age. LRT = likelihood ratio test; CK, creatine kinase; M, male; F, female; Significantly different means: *$P < 0.05$, †$P < 0.01$.

genotypes. Women possessing the IGF-II ApaI AA genotype had lower fat-free mass and strength compared with the GG genotype. In the present study, strength loss immediately after exercise was not significantly correlated with initial strength ($r = -0.01$) or body weight ($r = 0.02$), and we found no significant association of baseline strength with genotype. Thus the association of IGF-II ApaI genotype with markers of muscle damage cannot be attributed to the influence of initial strength, although we have no measure of the amount of force exerted during the eccentric contraction.

The significant associations of IGF-II SNPs with indicators of muscle damage could be due to the role of IGFs in muscle repair. Adams (1) proposed that mechanical load would modulate IGF-I expression and that IGF-I would act in both an autocrine and paracrine manner to activate satellite cells to proliferate and differentiate. These activated satellite cells would then fuse to muscle fibers, and the process of hypertrophy would ensue. Bamman et al. (2) showed that a bout of eccentric exercise increased IGF-I mRNA in human skeletal muscle. The Bamman et al. study, however, focused on the subsequent responses of muscle (e.g., tissue regeneration) by increased IGFs. IGF-II may also be involved in regeneration; Wilson and Rotwein (46) found that IGF-II was involved in signaling via IGF-1 receptors and was essential for regulation of myogenic transcription factor function. However, the fact that in the present study the genotype groups differed in their response immediately postexercise suggests another possible mechanism of action of IGF-II.

Kotlyar et al. (21) delivered IGF-I, IGF-II, or albumin (control) into pig hearts during induced myocardial infarction. Serum cardiac troponin I concentrations were dramatically increased for the control at 3 h postinfarction but only minimally changed for the animals that received IGF-I or IGF-II. The investigators suggested that IGFs may prevent the initiation of events induced by myocardial infarction that lead to cell damage (21). Kinugawa et al. (19), examining whether IGF-I would have an effect on cardiac myocyte contractility, found that IGF-I exerted a positive inotropic effect by increasing the availability of Ca^{2+} to the cardiac cells. McLoon and Christiansen (27) found that IGF-II injected intramuscularly into rabbit extraocular muscles for 1 wk resulted in significantly increased single-twitch force and mean tetanic force generation compared with a saline control. In mdx mice, 8 wk of IGF-I administration by subcutaneous pump was found to improve fatigue resistance (15) and muscle contractile function (force per cross-sectional area) (14) in the diaphragm muscle. After 2 wk of IGF-I treatment, there was a fourfold increase in serum total IGF-I and no difference in muscle concentration of IGF-I in the treated animals compared with untreated controls (14). These data suggest that altered circulating levels of IGFs may influence muscle contractility.

Sayer et al. (39) and O'Dell et al. (33) reported that the mean serum IGF-II concentration in those homozygous for the IGF-II ApaI rare allele was higher than concentrations found for the homozygous wild-type group. However, Schrager et al. (40) found that subjects homozygous for the wild-type ApaI allele had 23.7% higher serum IGF-II levels compared with subjects homozygous for the rare allele, but this was not significant. Although not assessed in the present study, it is possible that IGF levels differed by genotype groups and that such a difference could affect muscle contraction during exercise, possibly by altering calcium handling. Gregorevic et al.

(14) suggested that IGF-I administration alters membrane-bound receptor/channel populations that regulate voltage- and ion-dependent calcium release in muscle cells. Thus skeletal muscle of IGF-II genotype groups may differ in their exposure to circulating IGF-II, which could alter their contractile properties and explain the significant associations between genotype and muscle damage. Some subjects may be able to generate more force during a contraction and place greater strain on the muscle.

In conclusion, our results show for the first time that specific SNPs in the IGF-II gene are associated with several markers of exertional muscle damage. These results help to explain why some individuals are more susceptible to damage when performing strenuous, unaccustomed exercise and shed light on understanding the rare cases of exertional rhabdomyolysis that have been reported in otherwise healthy people. Skeletal muscle of IGF-II genotype groups may differ in their exposure to circulating IGF-II, which could alter calcium handling and force per cross-sectional area during exercise and explain the significant associations between genotype and muscle damage.

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