RESEARCH ARTICLE

eQTL variants in COL22A1 are associated with muscle injury in athletes

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Submitted 24 September 2020; accepted in final form 1 November 2020

Miyamoto-Mikami E, Kumagai H, Kikuchi N, Kamiya N, Miyamoto N, Fuku N. eQTL variants in COL22A1 are associated with muscle injury in athletes. Physiol Genomics 52: 588–589, 2020. First published November 9, 2020; doi:10.1152/physiolgenomics.00115.2020.—The myotendinous junction (MTJ) is at high risk of muscle injury, and collagen XXII is strictly expressed at tissue junctions, specifically at the MTJ. We investigated the hypothesis that single-nucleotide polymorphisms (SNPs) related to collagen type XXII α-1 chain gene (COL22A1) mRNA expression are associated with susceptibility to muscle injury in athletes. History of muscle injury was assessed in 3,320 Japanese athletes using a questionnaire, and two expression quantitative trait loci (eQTL) SNPs for COL22A1 (rs11784270 A/C and rs6577958 T/C) were analyzed in 468 Japanese athletes using a questionnaire. A total of 1,601 athletes had a history of muscle injury. The odds ratio of muscle injury was significantly higher in athletes with the A allele of rs11784270 (OR = 1.45, 95% CI = 1.10–1.94, P = 0.0071) and in athletes with the C allele of rs6577958 (OR = 1.48, 95% CI = 1.17–1.90, P = 0.0017). These results suggest that the expression level of COL22A1 at the MTJ influences muscle injury risk in athletes.

COL22A1; expression quantitative trait loci; muscle injury; myotendinous junction; rs11784270; rs6577958

BACKGROUND/MOTIVATION FOR THE STUDY

Muscle injury is one of the most common injuries in sports and negatively influences the performance of athletes and teams due to missed training time and unavailability for competition. Although the preventive effect of strength training on muscle injury has been demonstrated, the incidence of muscle injury has not decreased (3). Understanding the susceptibility to muscle injury will contribute to the development of a more effective prevention modality.

The myotendinous junction (MTJ) is at high risk of muscle injury (1), and thus, the strength of this tissue could affect the risk of muscle injury. Collagen XXII is strictly localized at tissue junctions, specifically at the MTJ (4). The knockdown of collagen type XXII α-1 chain gene (COL22A1) in zebrafish resulted in a muscular dystrophy-like phenotype and decreased force production, due to the destabilization of the myosepta (2), suggesting that this collagen is important for the structural integrity and stabilization of the MTJ. Collectively, it is possible that quantitative diversity of collagen XXII at the MTJ influences the susceptibility to muscle injury in athletes by altering the properties of the MTJ. Genetic variants influencing the expression of COL22A1 are likely one of the determinants of the quantitative diversity of collagen XXII. We investigated the hypothesis that the expression quantitative trait loci (eQTLs) for COL22A1 are associated with the risk of muscle injury in athletes.

PHENOTYPE

The history of up to three sports-related injuries in descending order of severity was assessed using a questionnaire as described previously (5). Briefly, injured body part, type of injury, cause of injury, time of injury, time lost due to the injury, number of injuries of the same type at the same site, and whether or not a medical practitioner had diagnosed the injury were asked for each injury. The present study focused only on noncontact muscle injuries diagnosed by medical practitioners. Information regarding the main sport, competitive level, and playing years was also asked in the questionnaire.

Cohort details. A total of 3,020 Japanese athletes participated in this cohort. Written consent was obtained from all participants. The procedure was approved by the Ethics Committees of Juntendo University, Nippon Sport Science University, and Tenri University and was performed in accordance with the Declaration of Helsinki. Exclusion criteria were as follows: 1) less than 3 yr of competition in their main sports (n = 201), 2) a lack of questionnaire data (n = 22), and 3) contact muscle injury or muscle injury not diagnosed by medical practitioners (n = 160). After exclusion, data from 2,637 athletes were available. This study was part of the Japanese Human Athlome Project (J-HAP) in “Athlome Project Consortium” (http://www.athlomeconsortium.org/).

Type of study. This was a “Candidate SNP” study. A saliva sample (2 mL) was collected from each participant for genotypic analysis. Total DNA was isolated from the saliva using the Oragene DNA Collection Kit (DNA Genotek, Ontario, Canada). The DNA samples were quantified using a NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Fisher Scientific) and stored at 4°C until use. Two single-nucleotide polymorphisms (SNPs; A/C, rs11784270; T/C, rs6577958) located on COL22A1 were genotyped in each participant using the TaqMan SNP Genotyping Assay [Assay ID: C_176045196_10 (rs11784270), C__29400116_10 (rs6577958)] and QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). PCR was performed as described previously (5). Two to four negative controls were included in each plate. Genotypes were called based on the TaqMan assay results using the QuantStudio Design and Analysis Software (v 1.2, Thermo Fisher Scientific).

Details of the SNPs studied. We searched for SNPs associated with COL22A1 mRNA expression in human skeletal
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muscle using the GTEx Portal (https://www.gtexportal.org/home/), and there were 142 eQTLs that passed the GTEx criteria (false discovery rate ≤ 0.05). Most of these eQTL SNPs were in high linkage disequilibrium (LD), and mainly two loci on COL22AI were strongly associated with COL22AI mRNA expression. Therefore, we selected a representative SNP from each locus as the candidate SNPs (rs11784270 and rs6577958). rs11784270 has a stronger association with each locus as the candidate SNPs (rs11784270: rs6577958). rs11784270 expression in skeletal muscle than rs6577958 (rs11784270: P = 2.5 × 10−16, rs6577958: P = 6.4 × 10−15) according to the GTEx Portal. The rs11784270 and rs6577958 SNPs are located in the intronic regions of COL22AI on chromosome 8 at positions 138,908,388 and 138,903,960 (GRCh38.p12), respectively. The minor allele frequencies of rs11784270 (C allele) and rs6577958 (C allele) were 0.11 and 0.16, respectively, in the Japanese population according to the Japanese Multi Omics Reference Panel (https://jmorp.megabank.tohoku.ac.jp/202001/variants).

Analysis model. LD calculations for the COL22AI SNPs were conducted by LDlink (https://ldlink.nci.nih.gov/?tab=home) using data from the Japanese population from the 1000 Genomes Project. The Hardy-Weinberg equilibrium (HWE) of SNPs were conducted by LDlink (https://ldlink.nci.nih.gov/?tab=home) using data from the Japanese population from the 1000 Genomes Project. The Hardy-Weinberg equilibrium (HWE) of each SNP was assessed using a χ² test. Comparisons between groups were conducted using unpaired Student’s t test or χ² test. Logistic regression analysis was applied to investigate the associations of SNPs with a history of muscle injury using an additive genetic model. In the analysis, sex, playing years, and main sport (track and field or others) were included as covariates. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Statistical significance was set at P < 0.025 for the SNP-phenotype association analysis and at P < 0.05 for the others. Statistical analyses were performed using JMP Pro version 12 (SAS Institute, Cary, NC).

RESULTS

Participant characteristics are shown in Supplemental Table S1 (all Supplemental Material is available at https://doi.org/10.6084/m9.figshare.12996875.v1). The COL22AI rs11784270 and rs6577958 genotype frequencies in the control group did not deviate from HWE (P = 0.1952 and P = 0.9094), and there was a low LD between the SNPs (D’ = 0.873 and R² = 0.2413; P < 0.0001). The rs11784270 and rs6577958 SNPs were significantly associated with muscle injury in athletes. The A allele of rs11784270 and the T allele of rs6577958 were associated with muscle injury under the additive genetic model (rs11784270: OR = 1.45, 95% CI = 1.10–1.94, P = 0.0083; Supplemental Tables S2 and S3). When analyzed separately by sex, the associations of these SNPs with muscle injury were prominent in female athletes (rs11784270: OR = 5.12, 95% CI = 2.08–17.05, P < 0.0001; rs6577958: OR = 2.46, 95% CI = 1.35–4.96, P = 0.0024; Supplemental Tables S2 and S3).

INTERPRETATION

We found that the A allele of rs11784270 and the T allele of rs6577958 were significantly associated with muscle injury in athletes. These muscle injury-related alleles are associated with high expression of COL22AI mRNA according to the GTEx Portal. Our results suggest that high mRNA expression of COL22AI at the MTJ influences muscle injury risk in athletes. However, muscle injury assessment using a questionnaire could not evaluate the detailed location of injury. Further studies are required to clarify what type of muscle injury is affected by the high expression of COL22AI mRNA and to examine the mechanisms of their association.

ACKNOWLEDGMENTS

The authors thank Editage (www.editage.jp) for English language editing.

GRANTS

This work was supported by JSPS KAKENHI Grant Numbers JP20H04081, JP17H04752, JP18H03155, JP18K17863, and MEET-Supported Program for the Private University Research Branding Projects (Juntendo University).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.M-M., N.M., and N.F. conceived and designed research; E.M-M., H.K., N.K., N.K., N.M., and N.F. performed experiments; E.M., H.K., and N.K. analyzed data; E.M-M., H.K., N.M., and N.F. interpreted results of experiments; E.M. drafted manuscript; E.M-M., H.K., N.M., and N.F. revised manuscript; E.M-M., H.K., N.K., N.M., and N.F. approved final version of manuscript.

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