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High Prevalence of Connective Tissue Gene Variants in Professional Ballet

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Background: There is a high prevalence of hypermobility spectrum disorder (HSD) in dancers. While there is no known genetic variant for HSD, hypermobile Ehlers-Danlos syndrome is a genetic disorder that exists within HSD. There are many connective tissue disorders (CTDs) with known (and unknown) genes associated with hypermobility. Hypermobility has distinct advantages for participation in flexibility sports, including ballet.

Purpose: To determine the prevalence of gene variants associated with hypermobility in a large professional ballet company.

Study Design: Cross-sectional study; Level of evidence, 3.

Methods: In this cross-sectional investigation, 51 professional male and female dancers from a large metropolitan ballet company were eligible and offered participation after an oral and written informed consent process. Whole blood was obtained from peripheral venipuncture, and DNA was isolated. Isolated DNA was subsequently enriched for the coding exons of 60 genes associated with CTD that included hypermobility as a phenotype, including Ehlers-Danlos syndromes, osteogenesis imperfecta, Marfan syndrome, and others. Genes were targeted with hybrid capture technology. Prepared DNA libraries were then sequenced with next-generation sequencing technology. Genetic database search tools (Human Gene Mutation Database and e!Ensembl, http://useast.ensembl.org/) were used to query specific variants. Descriptive statistics were calculated.

Results: Of 51 dancers, 32 (63%) agreed to participate in DNA analysis (mean ± SD age, 24.3 ± 4.4 years; 18 men, 14 women). Twenty-eight dancers had at least 1 variant in the 60 genes tested, for an 88% prevalence. A total of 80 variants were found. A variant in 26 of the 60 genes was found in at least 1 dancer. Among the 28 dancers with variants, 16 were found in the *TTN* gene; 10 in *ZNF469*; 5 in *RYR1*; 4 in *COL12A1*; 3 in *ABCC6* and *COL6A2*; 2 in *ADAMTS2, CBS, COL1A2, COL6A3, SLC2A10, TNC*, and *TNXB*; and 1 in *ATP6V0A2, B4GALT7, BMP1, COL11A1, COL5A2, COL6A1, DSE, FBN1, FBN2, NOTCH1, PRDM5, SMAD3*, and *TGFBR1*. Nine variants found in this population have never been reported. No identified variant was identical to any other variant. No identified variant was known to be disease causing. In the general population, the prevalence of each variant ranges from *never reported* to 0.33%. In the study population, the prevalence of each variant was 3.13%. There was no association between hypermobility scores and genetic variants.

Conclusion: Genetic variants in CTD-associated genes are highly prevalent (88%) in professional ballet dancers. This may significantly account for the high degree of motion in this population.

Keywords: ballet; dance; hypermobility; genetics; connective tissue disorders

Ballet dancers demand extreme ranges of motion to execute an array of complex movements and positions. The wide spectrum of motion attained by dancers is determined by osseous anatomy and soft tissue properties, including the morphology of the articulating bones, musculotendinous unit length and flexibility, capsuloligamentous laxity, and the presence or absence of pain (and the ability to tolerate it).[14,26,34] These properties may be genetically ingrained or acquired through training.

Multiple previous studies have demonstrated a higher prevalence of hypermobility spectrum disorder (HSD; formerly, generalized joint hypermobility or benign joint hypermobility syndrome) in ballet dancers as compared with controls, ranging from 24% to 92%.[6,7,25,35,41,43,44,47] These conditions are considered genetically determined.[30,66] Additionally, hypermobile Ehlers-Danlos syndrome (formerly, Ehlers-Danlos hypermobility type) is synonymous with HSD but lacks any known genetic variant.[66] While hypermobility has a heritability of 70%,[22] there are no known specific gene variants responsible for the hypermobility phenotype.[30] However, hypermobility, as a phenotype in general, is very common among connective tissue disorders (CTDs), for which there are several

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known genetic variants.[31] Some of the CTDs that include a phenotype of hypermobility are Ehlers-Danlos syndrome, osteogenesis imperfecta, Marfan syndrome, and many others.[7] To our knowledge, there have been no other reports of genetic variants in the dance population. The prevalence of HSD is known to be higher in dancers than the general population; thus, genes associated with the phenotype of hypermobility are likely to have a higher prevalence of variants in this population. The purpose of the current investigation was to determine the prevalence of gene variants associated with hypermobility in a professional ballet company. We hypothesized that variants within hypermobility-associated genes would be overrepresented in professional ballet dancers.

METHODS

A cross-sectional investigation was designed, and institutional review board approval was obtained. Genetic counseling was available for any individual found to have a disease-causing variant, and all results were made available to participants at their request. A total of 51 professional adult ballet dancers from a metropolitan ballet company in the United States (ages, 18-35 years; mean age, 23.9 years; men, n = 26; women, n = 25) were eligible and offered participation after an oral and written informed consent process. Dancers were divided into 3 ranks depending on their position in the company. Rank 1 included principal dancers and first soloists. Rank 2 included soloists and demi-soloists, and rank 3 included corps de ballet and apprentices. Turnout was measured in degrees via a protractor, with participants performing turnout to the ballet-first position on low-friction rotation discs (FitterFirst). Total turnout was measured by adding the measurement from the right and left sides.

Approximately 4 mL of whole blood was obtained in an EDTA vacutainer tube from each dancer who chose to have DNA analysis performed (referred to as a "participant"; samples were evaluated by Fulgent Diagnostics—a fully accredited laboratory with endorsements by the Centers for Medicare and Medicaid Services and the College of American Pathologists and licensed by multiple state agencies). DNA was isolated from whole blood samples through an automated liquid handling system (AnaPrep, Z1322002; BioChain). The DNA isolation method is bead based, and all components of the reagent kits used were also manufactured by BioChain (no third-party reagents were used). Isolated DNA was subsequently enriched for the coding exons of the 60 genes listed in Table 1. Exon enrichment was performed with a capture-based method. Reagents and protocols were based on Illumina's Nextera Rapid Capture Kit (FC-140-1003). They were targeted with hybrid capture technology. Prepared DNA libraries were then sequenced with next-generation sequencing technology. Sanger sequencing was used to confirm variants with low-quality scores and to meet coverage standards.[18]

All dancers were evaluated with a Beighton score[4] and Brighton criteria.[20] A positive Beighton score for HSD was defined as a score $\geq$5 (out of 9 total).[30] Brighton criteria were considered positive if the participant met 2 major, 1 major and 2 minor, or 4 minor criteria. The Hakim and Grahame score was also obtained and was considered positive if $\geq$2 answers were "yes."[23] Participants completed multiple patient-reported outcome scores: International Hip Outcome Tool–12 (iHOT-12),[21] Tegner activity score,[45] and American Orthopaedic Foot & Ankle Society (AOFAS) ankle-hindfoot score.[24]

Based on the syndrome or function with which they are associated, gene variants were separated into 7 groups for statistical analysis (Table 2). Following the rationale for combining rare mutations presented by Li and Leal,[29] the number of mutations present among genes associated with the considered syndromes were summed to develop a composite score. These composite scores were then correlated with the measured quantitative musculoskeletal traits for the participants. Data were analyzed with the *t* test, analysis of variance, and Spearman rank correlation for continuous, categorical, and correlation data, respectively. Spearman correlation and Somers *D* analysis was performed for the Hakim and Graham score, Beighton score, Brighton criteria, total turnout, iHOT-12, and AOFAS with each gene group.

RESULTS

All dancers agreed to have demographic data and noninvasive testing measures recorded. Of 51 dancers, 32 chose to undergo DNA analysis (participants). Those who chose not to undergo DNA analysis either simply did not want blood drawn owing to their aversion to needles (n = 10) or did not provide a reason (n = 9). Characteristics in the overall population and participant population are presented in Table 3. Turnout measurements and patient-reported outcome scores are summarized in Table 4.

No dancer was found to possess a disease-causing variant (ie, no dancer had a disease caused by the genetic variants found). Of 32 dancers, 28 had at least 1 variant in the 60 genes tested, for an 88% prevalence (Table 5). A variant in 26 of the 60 genes were found in at least 1 dancer (Table

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TABLE 1
Gene Data[a]

Genes Tested	Variants Detected, n	Percentage in General Population
TTN	22	Never reported to 0.0007
ZNF469	12	Never reported to 0.0007
RYR1	6	Never reported to 0.0004
COL12A1	4	<0.01 to 0.0003
TNXB	3	Never reported to 0.0003
COL6A3	3	0.0004 to 0.001
ABCC6	3	0.0001 to 0.0003
COL6A2	3	<0.0001 to 0.0002
SLC2A10	2	Never reported to <0.0001
CBS	2	0.0033 to 0.0003
ADAMTS2	2	0.0001 to 0.0012
PRDM5	2	<0.0001 to 0.0009
COL1A2	2	<0.0001
TNC	2	<0.0001
FBN2	1	Never reported
SMAD3	1	Never reported
TGFBR1	1	Never reported
ATP6V0A2	1	<0.0001
DSE	1	<0.0001
FBN1	1	<0.0001
NOTCH1	1	<0.0001
BMP1	1	0.001
COL6A1	1	0.0006
COL11A1	1	0.0003
COL1A1	1	0.0003
COL5A2	1	0.0003
B4GALT7	1	0.0001
ACTA2	0	NA
ACVR1	0	NA
ACVR2B	0	NA
B3GALT6	0	NA
CHST14	0	NA
COL2A1	0	NA
COL3A1	0	NA
COL4A1	0	NA
COL5A1	0	NA
CYP21A2	0	NA
ELN	0	NA
FBLN5	0	NA
FKBP14	0	NA
FLNA	0	NA
ITGA9	0	NA
LOXL2	0	NA
LZTS1	0	NA
MED12	0	NA
MMP3	0	NA
MSTN	0	NA
MYH11	0	NA
MYLK	0	NA
PKD2	0	NA
PLOD1	0	NA
RPSA	0	NA
SEPN1	0	NA
SKI	0	NA
SLC39A13	0	NA
SLC39A14	0	NA
SMAD4	0	NA
TGFB2	0	NA
TGFB3	0	NA
TGFBR2	0	NA

[a]List of all genes tested, the number of variants found in each gene, and the percentage range of all variants found in the general population. NA, not applicable.

1). All variants were heterozygous, and no 2 variants were identical. The prevalence of the detected variants in the database of >60,000 patients ranged from *never reported* to 0.33%.[12] In the study population, each variant had a prevalence of 3.13% (1 of 32). Eighty variants were found in the participant population (Table 1). Nine variants have never been reported in the Human Gene Mutation Database or e!Ensembl.

Brighton criteria were positive in 31.3% (10 of 32) of participants; 65.6% (21 of 32) had a Beighton score $\geq$4; and 53.1% (17 of 32) had Beighton score $\geq$5. No significant difference ($P > .05$) was found between Beighton score or Brighton criteria and any gene group. There was no significant difference ($P > .05$) between total number of variants, Beighton score, or Brighton criteria and rank. Participants with a variant in the Marfan syndrome gene group had a decreased total turnout as compared with those without a variant in this group (119.6° $\pm$ 22.8° vs 139.6° $\pm$ 23.6°; $P = .03$). There was no difference ($P > .05$) in turnout among participants with or without variants in all other gene groups.

The Somers *D* statistic for total turnout versus Loeys-Dietz syndrome and Marfan syndrome was -0.14 ($P = .05$) and -0.19 ($P = .04$), respectively. The Somers *D* statistic for iHOT-12 versus morphology-of-muscle and morphology-of-skeleton gene groups was -0.34 ($P = .001$) and -0.24 ($P = .03$). Similarly, the Somers *D* statistic for AOFAS versus morphology-of-muscle and morphology-of-skeleton gene groups was -0.38 ($P = .003$) and -0.29 ($P = .01$). No other significant associations were found with Somers *D* analysis.

Significant inverse associations were found between iHOT-12 and the morphology-of-muscle and morphology-of-skeleton gene groups ($r = -0.51$; $P = .003$, and $r = -0.41$; $P = .02$, respectively). Similarly, significant inverse associations were found between AOFAS and the morphology-of-muscle and morphology-of-skeleton gene groups ($r = -0.51$; $P = .003$, and $r = -0.44$; $P = .01$).

No participant reported a family history of Marfan syndrome[36] or Ehlers-Danlos syndrome.[37]

DISCUSSION

The present investigation found that genetic variants in CTD-associated genes are highly prevalent (88%) in professional ballet dancers, thus confirming our hypothesis. Additionally, 9 gene variants were discovered that had not been previously reported in the Human Gene Mutation Database[12] or e!Ensembl[15] at the time of analysis. The current article is the only report to date on connective tissue gene variants in a population of professional ballet dancers. These results give insight into the possibility of a genetic bias to succeeding in a profession requiring extreme flexibility such as ballet.

The present study found a significant inverse association between total turnout and genes associated with Loeys-Dietz syndrome and Marfan syndrome. These 2 gene groups have many of the same genes, and the clinical presentations of the 2 syndromes are very similar as well. While they both may involve hypermobile joints, they are also associated with acetabular protrusio.[3,16] This suggests

TABLE 2
Gene Groups for Statistical Analysis[a]

Syndrome or Function	Genes
Ehlers-Danlos syndrome	*ADAMTS2, B3GALT6, B4GALT7, CHST14, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, DSE, FBN2, FKBP14, FLNA, NOTCH1, PLOD1, PRDM5, SLC39A13, TNXB, ZNF469*
Loeys-Dietz syndrome	*ACTA2, COL3A1, COL5A2, FBN1, MYH11, MYLK, SMAD3, TGFB2, TGFB3, TGFBR1, TGFBR2*
Marfan syndrome	*ACTA2, COL1A2, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MYH11, MYLK, NOTCH1, SMAD3, TGFBR1, TGFBR2*
Bethlem myopathy	*COL12A1, COL6A1, COL6A2, COL6A3*
Morphology of connective tissue	*ACTA2, ACVR2B, CBS/CBSL, COL12A1, COL1A1, COL2A1, COL5A2, DSE, ELN, FBLN5, FBN1, MMP3, MSTN, NOTCH1, SMAD3, TGFB2, TGFB3, TGFBR2*
Morphology of muscle	*ACVR2B, COL12A1, COL6A1, ELN, FBN1, MSTN, MYH11, NOTCH1, PLOD1, RYR1, SELENON, SKI, SMAD4, TGFB2, TGFBR2, TTN*
Morphology of skeleton	*ACVR2B, COL12A1, COL1A2, COL2A1, FBN1, MSTN, NOTCH1, RYR1, SKI, SLC39A14, SMAD3, TGFB2*

[a]Genes found to have variants in the population were distributed into groups based on the syndrome or function with which they are associated. This was performed for statistical analysis.

TABLE 3
Characteristics[a]

	Eligible Population (n = 51)	Participants (n = 32)	*P* Value
Male	26	18	
Female	25	14	
Rank 1	10	8	
Rank 2	10	5	
Rank 3	31	19	
Age, y	23.9 ± 4.5	24.4 ± 4.4	.6
Body mass index, kg/m^2	20.4 ± 2.3	20.3 ± 2.4	.9
Beighton score	4.3 ± 2.5	4.3 ± 2.5	.9
Hakim and Grahame score	2.32 ± 0.80	2.10 ± 0.64	.3
Dancers meeting positive Brighton criteria	12	10	

[a]Values are presented as n or mean ± SD.

TABLE 4
Turnout Measurements and Patient-Reported Outcome Scores[a]

	Mean	SD
Total turnout (left + right), deg	134.1	24.7
iHOT-12	84.0	25.0
Tegner	9.5	1.4
AOFAS ankle-hindfoot	88.3	10.3

[a]Turnout measurements and patient-reported outcome scores for all study participants. AOFAS, American Orthopaedic Foot & Ankle Society; iHOT-12, International Hip Outcome Tool Short Version.

TABLE 5
Number of Variants per Participant

Variants, n	Dancers, n	Percentage
0	4	12.5
1	6	18.8
2	5	15.6
3	10	31.3
4	3	9.4
5	3	9.4
6	0	0.0
7	0	0.0
8	1	3.1

that there may be an association with variants of the genes in these groups that result in a deeper acetabulum, limiting hip external rotation and resulting in decreased total turnout.

This study found an inverse relationship between variants in the morphology-of-muscle and morphology-of-skeleton gene groups and patient-reported outcome scores regarding the hip, foot, and ankle. This suggests that participants with gene variants in these groups experience more pain in their hips, feet, and ankles than do those without such gene variants. These findings are consistent with studies that have shown a correlation between hypermobility and chronic or severe pain.[8,9,38] Di Stefano et al[13] found that patients with joint hypermobility syndrome may have widespread pain owing to sensitization from joint abnormalities that produce persistent painful stimuli. Furthermore, many of the genes in these 2 gene groups are involved in the

structure and function of the extracellular matrix and are important for tissue strength and repair.[50,54,57-60] Thus, variations in these genes may result in a poor healing response that allows microtrauma to progress to chronic pain in commonly overused joints within this population.

In the present study, no participant was known or suspected to have a genetic disorder or disease of any kind before genetic testing. However, an investigation into participants' genetic profiles based on a nonpathologic phenotype is likely to reveal unexpected findings. This can be seen in the identification of the variant in *ABCC6* with a deletion of exon 23 to 29 (Appendix Table A1, available in the online version of this article). This variant demonstrates compound heterozygosity; thus, it has been found to be pathologic only in the setting of additional *ABCC6* mutations,[46] which was not the case in our 1 participant demonstrating this variant. Furthermore, dedicated genetic studies that identify pathogenic genetic variants in affected populations are subject to inherent "post hoc, ergo propter hoc" bias. In a patient with disease, a genetic variant finding does not necessarily indicate the cause of disease (ie, correlation does not indicate causation). Thus, it is important to emphasize that participants in the present study do not have disease and that the findings are correlations among a nonpathologic phenotype, hypermobility, and variants in hypermobility-associated genes.

Variants in *TTN, ZNF469, RYR1, COL12A1, COL6A2, COL6A3, TNXB*, and *ABCC6* made up 69% (56 of 81) of all the variants found in this population. When these genes and their functions were examined, 2 common themes developed for an explanation of their association with a population possessing an increased joint range of motion. The first commonality is the association between skeletal muscle and the *TTN* and *RYR1* genes. The *TTN* gene encodes for a large protein of striated muscle and plays a substantial role in the elasticity of muscle.[48,62] The *RYR1* gene encodes for the ryanodine receptor isoform 1, which is a transmembrane calcium channel in skeletal muscle.[64] Some of the clinical manifestations of *RYR1* variants include joint laxity and acetabular dysplasia. At a histologic level, there is a type 1 muscle fiber predominance in individuals with *RYR1* vriants.[65] Interestingly, the type of titin found in type 1 muscle fiber is longer and more extensible,[39,52] and the extensibility of titin is dependent on calcium concentrations.[11,27] Thus, it is possible that the *TTN* and *RYR1* gene variants in this population allow the dancers greater elasticity in their muscles (and thus greater range of motion) through variations in titin subtype or calcium channel mechanisms, allowing more elasticity in the titin molecules present within the muscle.

The second commonality is the association of extracellular matrix and the *ZNF469, COL12A1, COL6A2, COL6A3, TNXB*, and *ABCC6* genes. The *ZNF469* gene encodes for a zinc finger protein that is thought to function as a transcription factor or extranuclear regulator for the synthesis or organization of collagen fibers.[63] Alterations in the ZNF469 protein are thought to disrupt the formation of extracellular matrix proteins.[40] The *COL12A1* gene encodes for collagen XII alpha 1 subunit, which functions to maintain extracellular matrix integrity in load-bearing connective tissues of the musculoskeletal system.[33] *COL6A2* and *COL6A3* encode the alpha 2 and alpha 3 chains of type VI collagen, which bind extracellular matrix proteins, making it important in organizing matrix components.[55,56] Collagen VI has been found to have a role in the physiology of the synovial joint,[2] the tensile strength of skin,[28] muscle regeneration,[49] and tendon fibroblast renewal.[42] The *TNXB* gene codes for the extracellular matrix glycoprotein tenascin.[61] Tenascin has antiadhesive properties and is thought to function in matrix maturation during wound healing.[61] The *ABCC6* gene encodes for a protein in the superfamily of ATP-binding cassette (ABC) transporters, which act to transport molecules across cellular membranes.[53] It is thought to be essential for extracellular matrix deposition or turnover of connective tissue and to specifically affect elastic fiber assembly.[5,17] HSDs are thought to be caused by disorganization of several extracellular matrix components and alterations in connective tissue architecture, homeostasis, and inflammatory response.[10,65] Thus, each of these gene variants likely plays a role in the extensile properties of the extracellular matrix, allowing dancers with these variants a greater range of motion through variable but equally beneficial molecular pathways.

The present study demonstrates results similar to those of prior studies, with generalized joint hypermobility in 65.6% of dancers and joint hypermobility syndrome in 31.3%.[25,35,41,44] However, no association was found between CTD gene variants and hypermobility scores. This does not mean that these gene variants do not influence dancers' mobility. CTDs have been shown to express genetic heterogeneity,[40] and unique variants among CTD genes exhibit incomplete penetrance or variable expressivity in clinically unaffected individuals.[1] As such, the variants found in this population may produce traits on the spectrum of connective tissue laxity that benefit dancers in their careers without being pathologic. The inability to find an association between hypermobility scores and genetic variants is likely due to the small sample size, lack of control group, or lack of comparison with a population without CTD gene variants. Hypermobility is a complex trait, and there are likely multiple rare unknown variants that develop independently and contribute to ligamentous laxity.[19,51] The variants found in this study may be some of those rare variants.

Limitations of the current study include the small sample size, the lack of a dedicated control group, and the limited existing genetic data on the identified variations and their clinical implications. Furthermore, the average genome differs from the reference genome by approximately 4 million sites, and each individual's DNA has between 100 and 120 loss-of-function variants.[32] Thus, the variants found in the study population may be inconsequential variants that are on a spectrum of normal. Finally, while there may be an advantage for hypermobile dancers in attaining professional status, hypermobility was not found to have an influence on their career progression beyond this with regard to rank within the company.

CONCLUSION

Genetic variants in CTD-associated genes are highly prevalent (88%) in professional ballet dancers. This may

significantly account for the high degree of motion in this population.

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REFERENCES

1. Alazami AM, Al-Qattan SM, Faqeih E, et al. Expanding the clinical and genetic heterogeneity of hereditary disorders of connective tissue. *Hum Genet*. 2016;135(5):525-540.
2. Alexopoulos LG, Youn I, Bonaldo P, Guilak F. Developmental and osteoarthritic changes in *Col6a1* knockout mice: the biomechanics of collagen VI in the cartilage pericellular matrix. *Arthritis Rheum*. 2009;60(3):771-779.
3. Al Kaissi A, Zwettler E, Ganger R, et al. Musculo-skeletal abnormalities in patients with Marfan syndrome. *Clin Med Insights Arthritis Musculoskelet Disord*. 2013;6:1-9.
4. Beighton P, Solomon L, Soskolne CL. Articular mobility in an African population. *Ann Rheum Dis*. 1973;32(5):413-418.
5. Bergen AAB, Plomp AS, Schuurman EJ, et al. Mutations in *ABCC6* cause pseudoxanthoma elasticum. *Nat Genet*. 2000;25(2):228-231.
6. Castori M, Colombi M. Generalized joint hypermobility, joint hypermobility syndrome and Ehlers-Danlos syndrome, hypermobility type. *Am J Med Genet Part C Semin Med Genet*. 2015;169(1):1-5.
7. Castori M, Tinkle B, Levy H, Grahame R, Malfait F, Hakim A. A framework for the classification of joint hypermobility and related conditions. *Am J Med Genet Part C Semin Med Genet*. 2017;175(1):148-157.
8. Cattalini M, Khubchandani R, Cimaz R. When flexibility is not necessarily a virtue: a review of hypermobility syndromes and chronic or recurrent musculoskeletal pain in children. *Pediatr Rheumatol*. 2015;13(1):40.
9. Celletti C, Castori M, La Torre G, Camerota F. Evaluation of kinesiophobia and its correlations with pain and fatigue in joint hypermobility syndrome/Ehlers-Danlos syndrome hypermobility type. *Biomed Res Int*. 2013;2013:580460.
10. Chiarelli N, Carini G, Zoppi N, et al. Transcriptome-wide expression profiling in skin fibroblasts of patients with joint hypermobility syndrome/Ehlers-Danlos syndrome hypermobility type. *PLoS One*. 2016;11(8):e0161347.
11. Colombini B, Nocella M, Bagni MA. Non-crossbridge stiffness in active muscle fibres. *J Exp Biol*. 2016;219(2):153-160.
12. Cooper DN, Ball EV, Stenson PD, et al. HGMD home page. http://www.hgmd.cf.ac.uk/ac/index.php. Accessed July 23, 2017.
13. Di Stefano G, Celletti C, Baron R, et al. Central sensitization as the mechanism underlying pain in joint hypermobility syndrome/Ehlers-Danlos syndrome, hypermobility type. *Eur J Pain (United Kingdom)*. 2016;20(8):1319-1325.
14. Duthon VB, Charbonnier C, Kolo FC, et al. Correlation of clinical and magnetic resonance imaging findings in hips of elite female ballet dancers. *Arthroscopy*. 2013;29(3):411-419.
15. Ensembl Genome Browser. http://useast.ensembl.org/index.html Accessed July 23, 2017.
16. Erkula G, Sponseller PD, Paulsen LC, Oswald GL, Loeys BL, Dietz HC. Musculoskeletal findings of Loeys-Dietz syndrome. *J Bone Joint Surg Am*. 2010;92(9):1876-1883.
17. Finger RP, Issa PC, Ladewig MS, et al. Pseudoxanthoma elasticum: genetics, clinical manifestations and therapeutic approaches. *Surv Ophthalmol*. 2009;54(2):272-285.
18. Fulgent Diagnostics Connective Tissue NGS Panel. https://www.fulgentgenetics.com/connective-tissue. Published 2019.
19. Grahame R. Joint hypermobility and genetic collagen disorders: are they related? *Arch Dis Child*. 1999;80(2):188-191.
20. Grahame R, Bird HA, Child A. The revised (Brighton 1998) criteria for the diagnosis of benign joint hypermobility syndrome (BJHS). *J Rheumatol*. 2000;27(7):1777-1779.
21. Griffin DR, Parsons N, Mohtadi NG, et al. A short version of the International Hip Outcome Tool (iHOT-12) for use in routine clinical practice. *Arthroscopy*. 2012;28(5):611-616.
22. Hakim AJ, Cherkas LF, Grahame R, Spector TD, MacGregor AJ. The genetic epidemiology of joint hypermobility: a population study of female twins. *Arthritis Rheum*. 2004;50(8):2640-2644.
23. Hakim AJ, Elizabeth S. A simple questionnaire to detect hypermobility: an adjunct to the assessment of patients with diffuse musculoskeletal pain. *Int J Clin Pract*. 2003;57(3):163-166.
24. Kitaoka HB, Alexander IJ, Adelaar RS, Nunley JA, Myerson MS, Sanders M. Clinical rating systems for the ankle-hindfoot, midfoot, hallux, and lesser toes. *Foot Ankle Int*. 1994;15(7):349-353.
25. Klemp P, Learmonth ID, Learmonth D. Hypermobility and injuries in a professional ballet company. *J Sports Med*. 1984;18(3):143-148.
26. Kolo FC, Charbonnier C, Pfirrmann CWA, et al. Extreme hip motion in professional ballet dancers: dynamic and morphological evaluation based on magnetic resonance imaging. *Skeletal Radiol*. 2013;42(5):689-698.
27. Labeit D, Watanabe K, Witt C, et al. Calcium-dependent molecular spring elements in the giant protein titin. *Proc Natl Acad Sci U S A*. 2003;100(23):13716-13721.
28. Lettmann S, Bloch W, Maaß T, et al. Col6a1 null mice as a model to study skin phenotypes in patients with collagen VI related myopathies: expression of classical and novel collagen VI variants during wound healing. *PLoS One*. 2014;9(8):e105686.
29. Li B, Leal SM. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am J Hum Genet*. 2008;83(3):311-321.
30. Malfait F, Francomano C, Byers P, et al. The 2017 international classification of the Ehlers-Danlos syndromes. *Am J Med Genet Part C Semin Med Genet*. 2017;175(1):8-26.
31. Malfait F, Hakim AJ, De Paepe A, Grahame R. The genetic basis of the joint hypermobility syndromes. *Rheumatology*. 2006;45(5):502-507.
32. Marian AJ. Sequencing your genome: what does it mean? *Methodist Debakey Cardiovasc J*. 2014;10(1):3-6.
33. Matthias C, Birk DE, Bonnemann CG, Manuel K. Molecules in focus: collagen XII. Protecting bone and muscle integrity by organizing collagen fibrils. *Int J Biochem Cell Biol*. 2014;53:51-54.
34. Mayes S, Ferris AR, Smith P, Garnham A, Cook J. Bony morphology of the hip in professional ballet dancers compared to athletes. *Eur Radiol*. 2017;27(7):3042-3049.
35. McCormack M, Briggs J, Hakim A, Grahame R. Joint laxity and the benign joint hypermobility syndrome in student and professional ballet dancers. *J Rheumatol*. 2004;31(1):173-178.
36. McKusick VA, Kelly J. Marfan syndrome; MFS. Online Mendelian Inheritance in Man. https://www.omim.org/entry/154700. Published 2015. Accessed July 1, 2016.
37. McKusick VA, Sobreira N. Ehlers-Danlos syndrome, classic type. Online Mendelian Inheritance in Man. https://www.omim.org/entry/130000. Published 2016. Accessed July 1, 2016.
38. Mulvey MR, Macfarlane GJ, Beasley M, et al. Modest association of joint hypermobility with disabling and limiting musculoskeletal pain: results from a large-scale general population-based survey. *Arthritis Care Res (Hoboken)*. 2013;65(8):1325-1333.
39. Prado LG, Makarenko I, Andresen C, Krüger M, Opitz CA, Linke WA. Isoform diversity of giant proteins in relation to passive and active contractile properties of rabbit skeletal muscles. *J Gen Physiol*. 2005;126(5):461-480.
40. Rohrbach M, Spencer HL, Porter LF, et al. ZNF469 frequently mutated in the brittle cornea syndrome (BCS) is a single exon gene possibly regulating the expression of several extracellular matrix components. *Mol Genet Metab*. 2013;109(3):289-295.
41. Sanches SB, Oliveira GM, Osorio FL, Crippa JAS, Martin-Santos R. Hypermobility and joint hypermobility syndrome in Brazilian students and teachers of ballet dance. *Rheumatol Int*. 2015;35(4):741-747.

42. Sardone F, Traina F, Tagliavini F, et al. Effect of mechanical strain on the collagen VI pericellular matrix in anterior cruciate ligament fibroblasts. *J Cell Physiol*. 2014;229(7):878-886.
43. Scheper MC, Devries JE, De vos R, Verbunt J, Nollet F, Engelbert RHH. Generalized joint hypermobility in professional dancers: a sign of talent or vulnerability? *Rheumatol (United Kingdom)*. 2013;52(4):651-658.
44. Steinberg N, Hershkovitz I, Zeev A, Rothschild B, Siev-Ner I. Joint hypermobility and joint range of motion in young dancers. *J Clin Rheumatol*. 2016;22(4):171-178.
45. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. *Clin Orthop Relat Res*. 1985;198:43-49.
46. Terry SF, Bercovitch L. *Pseudoxanthoma Elasticum*. Seattle, WA: University of Washington; 1993. http://www.ncbi.nlm.nih.gov/pubmed/20301292. Accessed August 2, 2019.
47. Tinkle B, Castori M, Berglund B, et al. Hypermobile Ehlers-Danlos syndrome (aka Ehlers-Danlos syndrome type III and Ehlers-Danlos syndrome hypermobility type): clinical description and natural history. *Am J Med Genet Part C Semin Med Genet*. 2017;175(1):48-69.
48. TTN titin (Homo sapiens [human]). https://www.ncbi.nlm.nih.gov/gene/7273. Published 2017. Accessed July 29, 2017.
49. Urciuolo A, Quarta M, Morbidoni V, et al. Collagen VI regulates satellite cell self-renewal and muscle regeration. *Nat Commun*. 2013;4:1964.
50. US National Library of Medicine. Loeys-Dietz syndrome. Genetics Home Reference. https://ghr.nlm.nih.gov/condition/loeys-dietz-syndrome#sourcesforpage. Published 2018.
51. Vitart V, Benčić G, Hayward C, et al. New loci associated with central cornea thickness include *COL5A1, AKAP13* and *AVGR8. Hum Mol Genet*. 2010;19(21):4304-4311.
52. Wang K, Mccarter R, Wright J, Beverly J, Ramirez-Mitchell R. Regulation of skeletal muscle stiffness and elasticity by titin isoforms: a test of the segmental extension model of resting tension. *Proc Natl Acad Sci U S A*. 1991;88(16):7101-7105.
53. Weizmann Institute of Science. *ABCC6* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=ABCC6&keywords=abcc6. Published 2019.
54. Weizmann Institute of Science. *COL12A1* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL12A1. Published 2018.
55. Weizmann Institute of Science. *COL6A2* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL6A2&keywords=col6a2. Published 2019.
56. Weizmann Institute of Science. *COL6A3* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL6A3&keywords=col6a3. Published 2019.
57. Weizmann Institute of Science. *FBN1* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=FBN1. Published 2018.
58. Weizmann Institute of Science. *MSTN* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=MSTN. Published 2018.
59. Weizmann Institute of Science. *RYR1* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=RYR1. Published 2018.
60. Weizmann Institute of Science. *SKI* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=SKI. Published 2018.
61. Weizmann Institute of Science. *TNXB* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=TNXB&keywords=tnxb. Published 2019.
62. Weizmann Institute of Science. *TTN* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=TTN&keywords=ttn. Published 2019.
63. Weizmann Institute of Science. *ZNF469* gene. GeneCards Human Gene Database. https://www.genecards.org/cgi-bin/carddisp.pl?gene=ZNF469&keywords=znf469. Published 2019.
64. Witherspoon JW, Meilleur KG. Review of RyR1 pathway and associated pathomechanisms. *Acta Neuropathol Commun*. 2016;4(1):121.
65. Zoppi N, Chiarelli N, Binetti S, Ritelli M, Colombi M. Dermal fibroblast-to-myofibroblast transition sustained by αvß3 integrin-ILK-Snail1/Slug signaling is a common feature for hypermobile Ehlers-Danlos syndrome and hypermobility spectrum disorders. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(4):1010-1023.
66. Zweers MC, Hakim AJ, Grahame R, Schalkwijk J. Joint hypermobility syndromes: the pathophysiologic role of tenascin-X gene defects. *Arthritis Rheum*. 2004;50(9):2742-2749.