

Genetic Work-Up for the Rare New Mutations Causing Musculoskeletal and Spine Pain

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Received: 10 Apr 2023

Accepted: 02 May 2023

Published: 10 May 2023

J Short Name: ACMCR

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Citation:

Lewandrowski KU, Genetic Work-Up for the Rare New Mutations Causing Musculoskeletal and Spine Pain. *Ann Clin Med Case Rep.* 2023; V10(19): 1-7

Keywords:

Genetic factors; Gene mutations; Screening, Counseling, Unexplained painful musculoskeletal and spinal pain, Targeted therapies and Pain management

1. Abstract

1.1. Background: Spine pain is widespread due to degenerative disc disease and facet arthropathy. Most patients improve with supportive conservative care measures, including non-steroidal anti-inflammatories, physical therapy, short episodes of rest, and activity modification. Medical and interventional pain management is reserved for those patients who do not improve within 4 to 6 weeks of standard spine care managed within the primary care setting, after which patients are typically referred to a specialist. Genetic conditions are rarely considered early in the differential diagnosis and may be easily missed in non-responsive patients.

1.2. Methods: We briefly describe two illustrative cases of patients with a history of chronic musculoskeletal and spinal pain, whose delayed diagnosis led to improper utilization of medical resources until they were diagnosed correctly and targeted personalized care for their painful syndromes could be instituted.

1.3. Results: After a long history of physiotherapy for several years to alleviate muscular and spine pain, a patient with lack

of proper control of the trunk and leg muscles causing difficulties with getting up from a sitting position, walking, and climbing stairs was diagnosed with a new mutation in the KLHL40 gene associated with the pain syndrome of Nemaline Myopathy. A second case a severe scoliosis with short femur leading to dwarfism through sonography investigations was diagnosed via mutation in exon 5 of the FGFR3 gene by WES and Sanger sequencing tests. An aborted fetus whose parents did not carry the mutant allele associated with Autosomal dominant thanatophoric dysplasia type I due to a de novo mutation of the FGFR3 gene associated with increased sensitivity of nociceptors such as TNF- α .

1.4. Conclusions: Genetic factors may play a more significant role in unrelenting musculoskeletal and spinal pain syndromes in individuals unresponsive to standard conservative care measures than previously thought. Genetic screening, counseling, and a combination of targeted interventions aimed at alleviating the harmful effect of the underlying gene defect and the disability associated with painful conditions of the musculoskeletal system affecting lo-

comotion and spinal deformity and balance should be considered early on mainly if the index of suspicion for an underlying genetic condition is high.

2. Introduction

A multidisciplinary, multinational research study investigating the possibility that spine disease and disc degeneration, in particular, could be more genetically influenced was conducted from 1991 to 2009. Investigators from Canada, Finland, and the United States came together to identify genes that have a higher association with spine pain related to degeneration of the intervertebral disc than other everyday environmental and activity or work-related factors play a role. [1-6] The study found that disc degeneration appears to be significantly determined. The Twin Spine Study did find evidence that back pain runs in families and that certain forms of the disease may, in fact, be inherited. While the investigators were unable to conclusively demonstrate any correlation between the severity of the disease or responsiveness to treating the symptoms – mainly pain – with any specific gene abnormalities, it became clear that patients with lumbar disc disease are more likely to have family members with the same symptomatic affliction with a significantly higher risk in close and distant relatives. A similar study of 2256 women including 371 and 698 monozygotic and dizygotic twin pairs and 29 sibling pairs and 60 singletons with a mean age of 50 years (18–84) corroborated the Twin Study findings by identifying higher risk for low back pain in monozygotic co-twin and dizygotic co-twin. [7] Published in 2011, risk factor adjusted regression analysis showed a 3.2 higher odds of low back pain (LBP) with advanced lumbar degenerative disc disease (LDD; 90% vs 10%). A statistically significant ($p < 0.001$) genetic correlation between LBP and LDD was also found. These findings suggested that approximately 11–13% of the genetic effects are shared by LDD and LBP. [7] Additional twin registries with similar findings do exist [1, 2, 5].

Since the publication of these twin studies, many articles have been published on the specific genes causing early and advanced disc degeneration or causing increased pain after spine surgery. For example, the IL6 and IL1B gene polymorphisms have been associated with less favorable long-term outcomes of degenerative lumbar spine surgeries. [8] Another genetic association exists between Vitamin D receptor (VDR) gene polymorphisms and the risk of intervertebral disc degeneration. A recent meta-analysis 23 VDR studies concluded that VDR FokI polymorphisms are associated with a higher risk of intervertebral disc degeneration among Caucasians but not Asians. VDR TaqI polymorphisms, on the other hand, are associated with disc degeneration risk among Asians but not Caucasians. The VDR ApaI polymorphism is associated with disc degeneration risk among Asians and Caucasians. [9] Many other examples of genetic mutations affecting the intervertebral disc exist. [10, 11] Even neuropathic pain syndromes have recently been linked to genetic factors [12].

The trend toward analysis of the genetic basis to pain is a paradigm shift from the traditional school of thought that age-related structural disintegration of the intervertebral disc, for example, from mechanical insults and injuries, is the primary reason for musculoskeletal and spinal pain syndromes. Understanding the involvement of specific gene mutations may offer personalized prevention and targeted treatment strategies for clinical conditions focused primarily on mechanical factors as primary causes of pain and painful degeneration. In this article, the authors present two new genetic mutation examples to illustrate their case for the search for newer treatments outside the traditional “injury model.” They are presenting a case of a patient with painful novel homozygote mutation of the FGFR3 gene causing autosomal dominant thanatophoric dysplasia (TD) type I, and another case of painful a new mutation in the KLHL40 gene that is homozygous recessive consistent with Nemaline Myopathy 8 (NM-8).

3. Materials and Methods

3.1. Study Design & Informed Consent

We briefly describe two illustrative cases of patients with a history of chronic musculoskeletal and spinal pain, whose delayed diagnosis led to improper utilization of medical resources until they were diagnosed correctly and targeted personalized care for their painful syndromes could be instituted. Patients volunteered for the study and consented in writing according to the declaration of Helsinki, last amended by the World Medical Association General Assembly in Fortaleza, Brazil, in October 2013. Study patients were adequately informed of the aims, methods, and the lack of funding for the study, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study, and the discomfort it may entail. The study was approved by the Institutional Review Board of Colsonitas University (protocol code CEIFUS 106-19 approved on February 12, 2019, for studies involving humans).

3.2. Sequencing Techniques Cohen’s Syndrome

WES was applied step by step as follows to identify any possible pathogenic mutations in the dead fetus and the parents: The cases’ samples used to extract and purify genomic deoxyribonucleic acid (gDNA), which was then quantified. Every specimen had an overall volume of 1.0 gDNA that was utilized to prepare the DNA. The Agilent SureSelect Human All ExonV7 Kit (Agilent Technologies, CA, USA) was used to construct sequencing databases, and x-index codes were appended to the sample attribute sequences. To summarize, 180-280 bp fragments were generated utilizing hydrodynamic shear system (Covaris, Massachusetts, USA). Exonuclease/polymerase activities blunted the residual overhangs, and enzymes were eliminated. Adapter oligonucleotides were ligated after the three ends of the DNA fragments were adenylated. In a PCR procedure, DNA fragments containing adapter molecules attached at both ends were specifically selected. To prepare for

hybridization, captured libraries were enriched in a PCR reaction with index tags. The products were purified using the AMPure XP system (Beckman Coulter, Beverly, USA) and quantified on the Agilent Bioanalyzer 2100 System using the Agilent High Sensitivity DNA Assay. Qualified libraries were fed into the Illumina NovaSeq 6000 sequencers. Data quality control, processing, and interpretation were then performed on the HP server's Generation G9 utilizing a Unix-based operating system. In silico analyses by VarSome (<https://varsome.com/>) and Franklin (<https://franklin.genoox.com/clinical-db/home>) categorized R158Q as a variant of uncertain significance (VUS). Data mining on the computational predictions from these two important databases showed an overall impact of damaging resulted from the amino acid substitution from Arginine (R) to Glutamine (Q)

3.3. Sequencing Techniques Nemaline Myopathy 8 (NM-8)

For the patient diagnosed with Nemaline Myopathy 8 (NM-8), a WES test for detecting plausible mutations or potential mutations was performed in the following order to identify potentially pathogenic variants in the case. A filter-based technique was used to extract and purify genomic deoxyribonucleic acid (gDNA) from the case's blood sample, which was then measured. For DNA preparation, a total of 1.0g of gDNA was employed. The Agilent Sure-Select Human All ExonV7 Kit (Agilent Technologies, CA, USA) was used to construct sequencing databases, and x-index codes were appended to the sample at-tribute sequences. The hydrodynamic shear system (Covaris, Massachusetts, USA) was used to create 180-280 bp pieces. Exonuclease/polymerase activities attenuated the residual overhangs, and enzymes were removed. After adenylation of the 3' ends of the DNA fragments, adapter oligonucleotides were ligated; DNA fragments with adaptor molecules attached at both ends were preferentially selected in a PCR reaction. Captured libraries were enriched in a PCR reaction using index tags to prepare for hybridization. The products were purified by the AMPure XP system (Beckman Coulter, Beverly, USA) and quantified through the Agilent High Sensitivity DNA Assay on the Agilent Bioanalyzer 2100 System. Qualified libraries were fed into the NovaSeq 6000 Illumina sequencer. Quality control of data, analysis, and interpretation was then carried out on Generation G9 from the HP server by a Unix-based operating system. For the in silico analysis, homology modeling for KLHL40 protein structures in wild-type (Leu19) and mutant (Arg19) models was performed for 621 amino acid Kelch-like protein 40 (UniProt ID: Q2TBA0) through SWISS-MODEL and also, assessed through Molprobit and ProSA online software (available at <https://prosa.services.came.sbg.ac.at/prosa.php> and (<https://prosa.services.came.sbg.ac.at/prosa.php>)).

4. Results

The authors report on two cases in whom the patients were diagnosed with a new mutation of the KLHL40 gene associated with

Nemaline Myopathy (case 1) and in the FGFR3 gene causing a new lethal form of Autosomal dominant thanatophoric dysplasia type I (case 2).

4.1. Case I - Nemaline Myopathy

A 14-year-old female complaining of myopathy was sent for genetic testing because a diagnostic workup for common reason musculoskeletal pain in newborns did not reveal any viable avenues for treatment. Genetic counseling suggested that the patient could have a hidden recessive mutation. As a result, the WES test was performed to detect plausible or potential mutations. The patient's parents reported that within the first few hours of her birth and after breastfeeding for the second time, she had to be admitted to the hospital for painful aspiration-related symptoms. She remained in neonatal intensive care for dilation of esophageal stricture and surgical treatment of a lung abscess that resulted in pneumothorax that required surgical repair. Cardiac ultrasound revealed that the foramen ovale had closed spontaneously at birth. The patient showed delayed development for walking and standing compared to her peers. She had apparent pain with ambulation and fell frequently. As a child, she could not climb stairs without assistance and had a dropped jaw with deformity contributing to speech delay. Several years of occupational and physical- and speech therapy provided some benefits (Figure 1). Genetic testing revealed Nemaline Myopathy without mental retardation, aberrant balance, or neurological issues.

The WES test findings indicated a mutation in exon 1st of the KLHL40 gene (c. 56T>G:p.L19R) related to NM-8 with an autosomal recessive inheritance model. WES screening of KLHL40 revealed genotype GG for the patient (Homozygote), while Sanger sequencing revealed genotype TG for both her father (Heterozygote) and mother (Heterozygote) (Figure 2). Overall, preliminary medical genetic counseling is strongly advised for the interpretation of test findings or the decision to conduct genetic testing in the case of a prenatal diagnosis for her mother's or her future pregnancies.

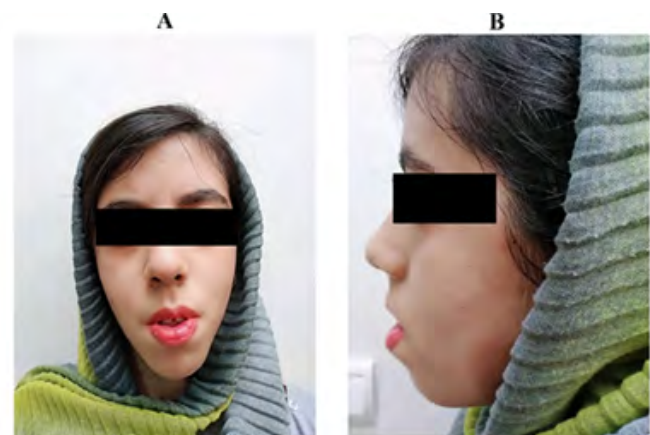


Figure 1: Image of 14-year-old patient with new mutation in KLHL40 gene (exon:1:c.56T>G: p.L19R) representing a definite deformity of jaw consistent with Nemaline Myopathy.

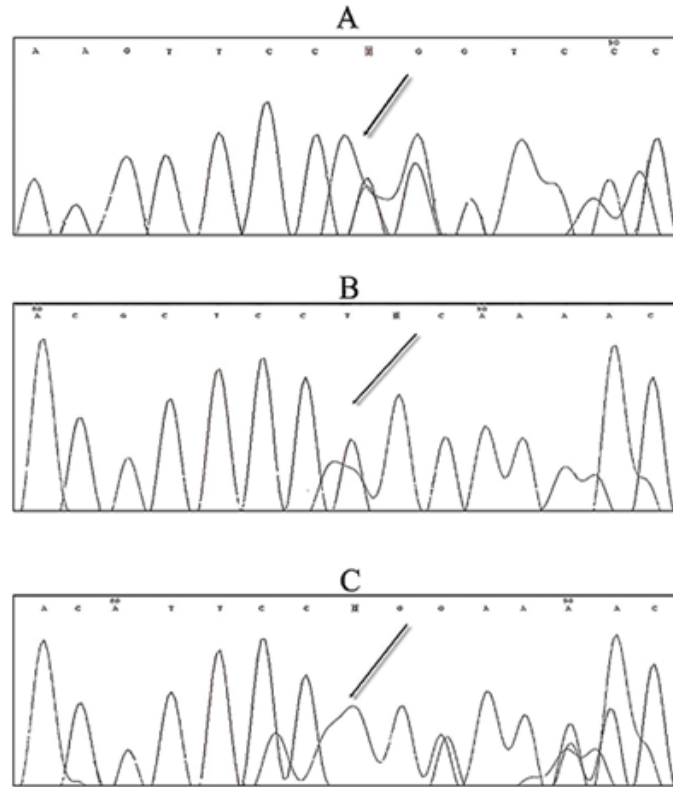


Figure 2: Mutation confirmation by Sanger sequencing in patient's parents (A and B) and herself (C). A and B show heterozygosity in the parents and C indicates homozygosity for mutated al-lele.

4.2. Case II - Autosomal Dominant Thanatophoric Dysplasia Type I

A 25-year-old woman referred to the genetic laboratory for the WES test after her abortion. The parents were both healthy with no manifestation of any distinct disease. Somehow, the dead fetus diagnosed with a severe scoliosis with short femur leading to dwarfism through sonography investigations. The final result of genetic counseling suggested the dead fetus to be investigated by WES for finding the putative mutation (s). WES results indicated that this fetus had a de novo mutation in the FGFR3 gene. To confirm the mutation, Sanger sequencing conformation was preformed. The results of WES test for the aborted fetus uncovered a new de novo mutation in exon 5th of FGFR3 gene (c.473G>A:p.R158Q) associated with Autosomal dominant thanatophoric dysplasia type I (OMIM: 187600). Screening of FGFR3 by WES indicated genotype GC for the dead fetus (Heterozygote), and the parents' mutational conformations by Sanger sequencing revealed genotype AA (Homozygote) for both of them. The fetus had musculoskeletal and spinal deformity.

WES was done for the case, and a de novo mutation was observed in exon 5 of the FGFR3 gene. Due to the mutational confirmation in the case, Sanger sequencing was per-formed on the case's parents by Sanger sequencing at the mutation site. Confirmation of mutation on variant revealed that mutation in the FGFR3 gene is de novo and parents are both normal homozygotes and had not the mutation which means the mutation must be occurred in the germ line cells of one of the parents (Figure 3). This mutation follows

the autosomal dominant genetic inheritance. This mutation will cause TD type I, which is consistent with the clinical symptoms of the dead fetus.

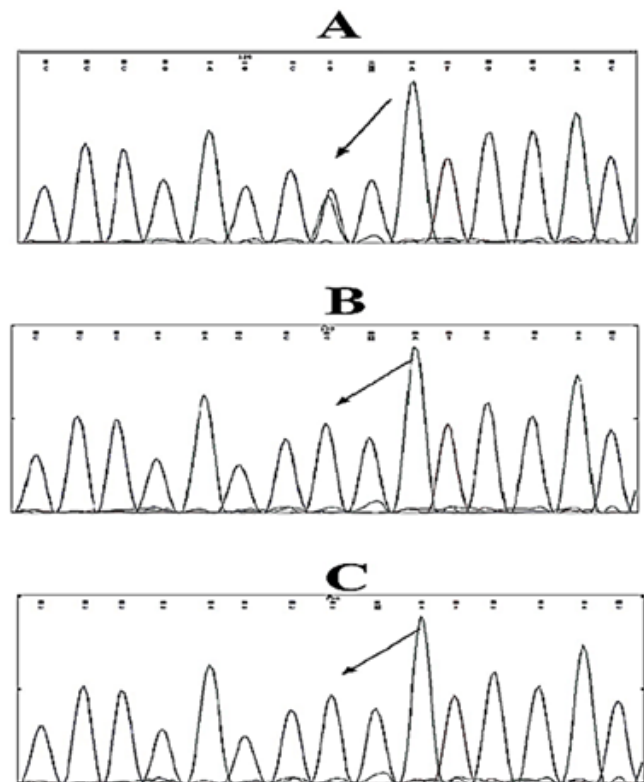


Figure 3: The sequence results of the fetus (A), the Father (B), and the mother (C) represent het-erozygote (GA), homozygote (GG), and homozygote (GG) genotypes, respectively.

5. Discussion

Our small case series shows that new gene mutations of recognized genetic syndromes may produce pain and disability in the musculoskeletal system and the spine, which were hitherto not known to be hallmark symptoms. We report a de novo mutation in exon 5 of the FGFR3 gene by WES and Sanger sequencing tests. A fetus born dead from parents both carrying the normal allele. The mutant allele associated with Autosomal dominant TD type I with manifestations of severe scoliosis with short femur leading to dwarfism. It suggests that FGFR3 genetic mutations with scoliosis and skeletal deformities should be considered for pain management.

Prior reports indicated some mutations of FGFR3 leading to TD. Pannier et al. de-scribed a fetus with lethal TD1 discovered at 24 weeks of pregnancy while the fetus had acute dwarfism lead to death. Radiographs revealed severe rhizomelic shortening of the long bones as well as minor bowing of the femora, radii, and ulnae. The spine was extensively platyspondylated, with H-shaped vertebrae and a shortening of the interpediculate length. Post - mortem investigation demonstrated cerebral cortical abnormalities, temporal lobe polymicrogyria, and significant disruption of long bone density plates. Complementary genetic analysis revealed that two de novo missense mutations in the FGFR3 gene on the same allele were heterozygous (N540K and Q485R). Pannier et al stated that the N540K mutation, when isolated, usually resulted in a milder form of hypochondroplasia [13]. A new insertion mutation (c.742 743insTGT) was discovered by Lindy et al. They explained this mutation as a pathogenic change which results in the clinical manifestation of TD1. Functional analysis of this mutation in zebrafish showed that the FGFR3 protein was catalytically active, causing the FGF pathway to be overexpressed and the signaling in the downstream regions to become overactive [14].

The Fibroblast Growth Factor Receptor 3 (FGFR3) gene contains a limited number of mutations which have been linked to TD1. There are five most frequently discovered mutations accounting for 90% of all TD1 cases [15]. c.742C>T (p.R248C) and c.1118A>G (p.Y373C), two of the most prevalent TD1 mutations, have been found to activate FGFR3 via promoting the formation of covalently bound dimers which are kept together by a di-sulfide bond between the free cysteine residues inserted to the mutated protein [16-18]. Interestingly, Xie et al demonstrated spinal astrocytic FGFR3 activation leads to mechanical hypersensitivity by amplified TNF- α in spared nerve injury (SNI). The spinal cords of SNI rats had elevated GFAP expression as well as mechanical hypersensitivity in the hind paws. In accordance with higher GFAP expression, increased FGFR3 expression was seen in the spinal dorsal horn of SNI models. In vivo and in vitro demonstrated that increased FGFR3 upregulates the expression of GFAP and TNF in astrocytes. In considerations underlying biomechanics, FGFR3-TBX3 axis activation increased TNF-expression in

primary spinal astrocytes in culture. Mechanical hypersensitivity was brought on by spinal TNF-synthesis in SNI rat models. They concluded that FGFR3 is implicated in neuropathic pain (NPP) maintenance through activation of the FGFR3-TBX3 axis, which causes the synthesis of TNF. Based on their reports, the FGFR3 receptor and associated astrocyte signaling pathways are potential molecular targets for NPP administration [19].

Our study also found a new mutation in the first exon of the KLHL40 gene (Leu19Arg) in a 14-year-old female with NM signs. Because of consanguineous marriage and confirming the cause of the disease, mutation conformations revealed the mutation in both of her parents as the carriers. This mutation follows an autosomal recessive model of inheritance. More than 80% of mothers with affected pregnancies have fetal akinesia/hypokinesia and/or polyhydramnios. The c.1582G>A p.(Glu528Lys) mutation is commonly found in Turkish and Japanese populations signifying a founder mutation, but there is no common haplotype in either Japanese or Turkish patients, yet. [20] The Thr506Pro (c.1516A>C) variant in KLHL40 has been suggested as a hot-spot mutation in Chinese patients. [21, 22] There noticeable reports about the pains resulted in patients with NM. The major concern from a pulmonary perspective is respiratory muscle and diaphragm weakening, which restricts the use of benzodiazepines, opioids, and neuro-muscular blocking drugs. [23, 24] This in turn makes managing the ventilatory system and discomfort under general anesthesia more challenging according to the Bang et al's study. [25] They further suggested that regional anesthetic may eliminate these respiratory system concerns depending on the kind of sedation employed [6]. Along with physical and occupational treatment problems, there have been cardiologic and respiratory abnormalities described in the literature which must be taken into consideration by pain management before surgery. [26]

Previous reports showed that KLHL40 binds to NEB, a thin filament protein often re-lated to Nemaline myopathy (NM), as well as leiomodin 3 (LMOD3), a new muscle protein highly similar to leiomodin 2 (LMOD2), which regulates actin at the thin filaments [8-10]. Similar to LMOD2, it was found that LMOD3 also localizes to the sarcomere thin filaments. Unlike most other BBK proteins that mediate substrate degradation, KLHL40 exclusively elevates NEB and LMOD3 stability and inhibits LMOD3 ubiquitination. Consequently, lack of KLHL40 decreases NEB and LMOD3 protein in mice as well as in patients with NM. By founding a new pro-stability role of KLHL40 protein for the thin filament proteins NEB and LMOD3, Garg et al.'s study revealed a molecular basis for NM in patients with KLHL40 dysfunction and suggested an unrecognized role of LMOD3 in the maintenance of sarcomere function and NM. [27]

Present study demonstrated the that the amino acid changes, for example from Leucine to Proline can have important impacts on protein structure and resultant new clinical syndromes. Leucine

is one of the amino acids with hydrophobic side chain and amino acid Proline, which changes the direction of the protein backbone and causes a turn in the secondary structure and finally the final structure of the protein. Therefore, it might be suggested that the amino acid change from Leucine to Proline can make a notable difference in protein folding. Also, because this mutation occurs approximately in the middle of the protein sequence, it may alter functionally sensitive regions. New clinical pain syndromes with myopathy, decreased function, and accelerated degeneration of the musculoskeletal and spinal system.

6. Conclusion

In conclusion, the present study, in consistent with other recent studies related to re-reports of new mutations in the FGFR3 gene, is adding new clinical data to TD. This has raised concerns about the prevalence of TD, and highlights the importance of genetic screening for the disease in prenatal testing. New genetic mutations in NM in patients with KLHL40 dysfunction may also produce new musculoskeletal and spinal symptoms that may be misinterpreted. Nowadays, genetic factors must be taken into account when working patients up for complaints that are unresponsive to traditional care measures. Misdirected therapies could lead to unnecessary interventions and surgeons.

7. Funding

No funding.

8. Institutional Review Board Statement

The study was approved by the Institutional Review Board of Col-sanitas University (protocol code CEIFUS 106-19 approved on February 12, 2019, for studies involving humans).

9. Informed Consent Statement

Patients volunteered for the study and consented in writing according to the declaration of Helsinki, last amended by the World Medical Association General Assembly in Fortaleza, Brazil, in October 2013. Study patients were adequately informed of the aims, methods, and the lack of funding for the study, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study, and the discomfort it may entail.

10. Acknowledgments

None

11. Conflict of Interest

None

References

1. Kaartinen S, Aaltonen S, Korhonen T, Latvala A, Mikkelsen M, Kujala UM, et al. Is diversity of leisure-time sport activities associated with low back and neck-shoulder region pain? A Finnish twin cohort study. *Prev Med Rep.* 2019; 15: 100933.
2. Liechty A, Tsang S, Turkheimer E, Duncan GE. Association between low back pain and body mass index in adult twins: an analysis of monozygotic and dizygotic twins of the Washington State Twin Registry. *Spine J.* 2020; 20(11): 1805-15.
3. McLean D, Pearce N, Walls CB, Wigley RD. The "Twin Study" and the misunderstanding of epidemiology that clouds occupational associations and low back disorder. *NZ Med J.* 2011; 124(1339): 109-11.
4. Nyman T, Mulder M, Iliadou A, Svartengren M, Wiktorin C. High heritability for concurrent low back and neck-shoulder pain: a study of twins. *Spine (Phila Pa 1976).* 2011; 36(22): E1469-76.
5. Patterson TG, Carvalho ESAP, Aquino D, Ferreira M, Ferreira P. Factors associated with care-seeking for low back pain when genetics and the familial environment are considered. *Musculoskelet Sci Pract.* 2021; 53: 102365.
6. Pietikäinen S, Silventoinen K, Svedberg P, Alexanderson K, Hunan-Seppälä A, Koskenvuo K, et al. Health-related and sociodemographic risk factors for disability pension due to low back disorders: a 30-year prospective Finnish Twin Cohort Study. *J Occup Environ Med.* 2011; 53(5): 488-96.
7. Livshits G, Popham M, Malkin I, Sambrook PN, MacGregor AJ, Spector T, et al. Lumbar disc degeneration and genetic factors are the main risk factors for low back pain in women: the UK Twin Spine Study. *Annals of the Rheumatic Diseases.* 2011; 70(10): 1740-5.
8. Biczko A, Berezcki F, Koch K, Varga PP, Lazary A. Genetic variants of interleukin 1B and 6 are associated with clinical outcome of surgically treated lumbar degenerative disc disease. *BMC Musculoskelet Disord.* 2022; 23(1): 774.
9. Xue J, Song Y, Liu H, Liu L, Li T, Gong Q. Vitamin D receptor gene polymorphisms and risk of intervertebral disc degeneration: An updated meta-analysis based on 23 studies. *Medicine (Baltimore).* 2021; 100(20): e25922.
10. Chen X, Xie Y, Wan S, Xu J, Cai B, Zhang Y, et al. A novel heterozygous mutation c.680A>G (p. N227S) in SLC34A1 gene leading to autosomal dominant hypophosphatemia: A case report. *Medicine (Baltimore).* 2019; 98(20): e15617.
11. Novais EJ, Diekman BO, Shapiro IM, Risbud MV. p16(Ink4a) deletion in cells of the intervertebral disc affects their matrix homeostasis and senescence associated secretory phenotype without altering onset of senescence. *Matrix Biol.* 2019; 82: 54-70.
12. Zorina-Lichtenwalter K, Parisien M, Diatchenko L. Genetic studies of human neuropathic pain conditions: a review. *Pain.* 2018; 159(3): 583-94.
13. Pannier S, Martinovic J, Heuertz S, Delezoide AL, Munnich A, Schibler L, et al. Thanatophoric dysplasia caused by double missense FGFR3 mutations. *American Journal of Medical Genetics Part A.* 2009; 149(6): 1296-301.
14. Lindy AS, Basehore MJ, Munisha M, Williams AL, Friez MJ, Writzl K, et al. Identification of a novel insertion mutation in FGFR3 that causes thanatophoric dysplasia type 1. *American Journal of Medical Genetics Part A.* 2016; 170(6): 1573-9.
15. Passos-Bueno MR, Wilcox WR, Jabs EW, Sertie AL, Alonso LG, Kitoh H, et al. Clinical spectrum of fibroblast growth factor receptor

- mutations. *Human mutation*. 1999; 14(2): 115-25.
16. Tavormina PL, Shiang R, Thompson LM, Zhu YZ, Wilkin DJ, Lachman RS, et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nature genetics*. 1995; 9(3): 321-8.
 17. Naski MC, Wang Q, Xu J, Ornitz DM. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. *Nature genetics*. 1996; 13(2): 233-7.
 18. D'Avis PY, Robertson SC, Meyer AN, Bardwell WM, Webster MK, Donoghue DJ, et al. Constitutive activation of fibroblast growth factor receptor 3 by mutations responsible for the lethal skeletal dysplasia thanatophoric dysplasia type I. *Cell growth & differentiation: the molecular biology journal of the American Association for Cancer Research*. 1998; 9(1): 71-8.
 19. Xie KY, Wang Q, Cao DJ, Liu J, Xie XF. Spinal astrocytic FGFR3 activation leads to mechanical hypersensitivity by increased TNF- α in spared nerve injury. *International Journal of Clinical and Experimental Pathology*. 2019; 12(8): 2898.
 20. Ravenscroft G, Miyatake S, Lehtokari VL, Todd EJ, Vornanen P, Yau KS, et al. Mutations in KLHL40 are a frequent cause of severe autosomal-recessive nemaline myopathy. *Am J Hum Genet*. 2013; 93(1): 6-18.
 21. Yi S, Zhang Y, Qin Z, Yi S, Zheng H, Luo J, et al. A novel and recurrent KLHL40 pathogenic variants in a Chinese family of multiple affected neonates with nemaline myopathy 8. *Mol Genet Genomic Med*. 2021; 9(6): e1683.
 22. Yuan H, Wang Q, Zeng X, He P, Xu W, Guo H, et al. Clinical and molecular analysis of four unrelated Chinese families with pathogenic KLHL40 variants causing nemaline myopathy 8. *Orphanet J Rare Dis*. 2022; 17(1): 149.
 23. Ramakrishnan P, Siddiqui S. Myasthenia Gravis as a Cause of Failed Extubation Diagnosed by Diaphragmatic Ultrasound. *J Coll Physicians Surg Pak*. 2019; 29(5): 474-5.
 24. Grignaschi S, Mongodi S, Alfonsi E, Mojoli F, Vertui V, Zanframundo G, et al. Respiratory failure due to concomitant interstitial lung disease and diaphragmatic involvement in a patient with anti-MDA5 dermatomyositis: a case report. *Clin Exp Rheumatol*. 2022; 40 Suppl 134(5): 121-3.
 25. Bang SU, Kim YS, Kwon WJ, Lee SM, Kim SH. Peripheral nerve blocks as the sole anesthetic technique in a patient with severe Duchenne muscular dystrophy. *J Anesth*. 2016; 30(2): 320-3.
 26. Flynn JM, Luedtke LM, Ganley TJ, Dawson J, Davidson RS, Dormans JP, et al. Comparison of titanium elastic nails with traction and a spica cast to treat femoral fractures in children. *J Bone Joint Surg Am*. 2004; 86(4): 770-7.
 27. Garg A, O'Rourke J, Long C, Doering J, Ravenscroft G, Bezprozvannaya S, et al. KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. *J Clin Invest*. 2014; 124(8): 3529-39.