Whole Exome Sequencing in Patients With Ossification of the Spinal Ligaments

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Research Article

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Abstract

Background

The clinical presentations of ossification of spinal ligament (OSL) are majorly myelopathy and/or radiculopathy, with serious neurological pathology resulting in paralysis of extremities and disturbances of motility (motor function) lowering the quality of life. Currently, studies find that missense single nucleotide polymorphisms (SNPs) play a vital role in the susceptibility for complex diseases.

Methods

In the present study, we used whole exome sequencing (WES) method to explore variants in exomes and found out novel potential responsible genes for OSL. Genomic DNA was extracted from ligamentum flavum collected from 5 OSL patients and 5 control patients. Whole-exome sequencing was then performed, while variation forecasts and conservation examination were subsequently assessed.

Results

8 common SNP variants were exhibited in all 5 subjects of OSL, presented in the genes of GRHL2, CUL3, WHAMM, IL17RD, POM121L12, SLC26A8 and PTPN23. After screened with numbers of samples and additional screenings with deleterious Polyphen2_HDIV_score, Polyphen2_HVAR_score and SIFT, 4 common SNP variants were displayed in 4 subjects of OSL, presented in the genes of KRT84, KIF1B, NRAP and CETN1. 7 common SNP variants were existed in 3 subjects of OSL, presented in the genes of CCT3, ANLN, ESRRB, SRBD1, ODF3L1, BRAT1 and RBP3.

Conclusion

We found out novel potential variants in several genes, especially WHAMM, KIF1B and ESRRB, which represented potentially pathogenic mutations in patients with OSL.

Background

Ossification of spinal ligament (OSL) mainly includes ossification of the posterior longitudinal ligament (OPLL), ossification of the ligamentum flavum (OLF) and ossification of supra/interspinous ligament [1]. These conditions sometimes coexist in the same patients, besides, ossification of the spinal ligaments has common pathologic features [1]. OPLL exhibits a hyperostotic disorder in the posterior longitudinal ligaments, described as an ectopic ossification [2]. The clinical performances in OPLL patients are radiculopathy and myelopathy, with severe neurological pathology leading to extremities paralysis and motility disturbances that reduced life quality [2]. Currently, surgical treatment is the standard therapy for OPLL to decompress spinal cord, however, the risk of ossification reprogression still exists [3]. Both environmental (non-genetic) and genetic elements are demonstrated to be involved in OPLL development, more importantly, OPLL displays a remarkable genetic susceptibility [4]. Ossification of the ligamentum flavum (OLF) of the spine is another common disorders of ectopic bone formation in the ligamentum flavum [1]. OLF has various common characteristics with OPLL in epidemiology, etiology and pathology, which regularly coexists with OPLL [5, 6]. Of late, large-scale exome-wide association researches discovery that missense single nucleotide polymorphisms (SNPs) play a crucial role in the susceptibility for complex diseases [7]. Furthermore, the functional relationship between SNPs and the occurrence and progression of OSL should be further illuminated.

Numerous researches have reported the potential genetic loci related to OSL susceptibility. For instance, the intron 33 (+ 20) and promoter (-572) SNPs in COL6A1 are related with OPLL, as well as OLF in Chinese Han population [8]. RUNX2 can be responsible for OPLL and OLF in the spinal ligament in the Chinese Han population [9]. Analysis of genome-wide sib-pair linkage discovered loci with suggestive linkage on 1p21, 7q22, 2p22-2p24, 20p12 and16q24 in OPLL of the spine [10]. A genome-wide association research identifies six susceptibility loci for OPLL: 20p12.3, 8q23.1, 12p11.22, 12p12.2, 8q23.3 and 6p21.1 [11]. COL6A1, in

chromosome 21, is identified as the locus for OPLL in the spine with Genomewide linkage and linkage disequilibrium analyses [12]. TGF-β genes are revealed as crucial candidates that facilitate the susceptibility to OPLL [13, 14]. COL17A1 and PTCH1 genes are implicated in OPLL of cervical spine assessed by whole exome sequencing in Chinese patients [15]. NPP1 polymorphism is associated with postoperative progression of Chinese patients with OPLL [16]. Polymorphism in the gene of bone morphogenetic protein receptor type IA promotes OPLL occurrence in cervical spine [17]. SNPs in the gene of human bone morphogenetic protein-2 influence OPLL susceptibility in spine [18]. Fewer gene analysis researches on OLF alone have been conducted, while some indications propose that hereditary factors may be also implicated in OLF pathogenesis [19, 20]. Although various genes are exhibited to be implicated in OSL occurrence and development, the specific genes that contribute to OSL progression remain to be validated.

It is critical to illustrate the SNPs associated with the ossified ligament. Besides, the functional connections between the associated SNPs and the occurrence and progression of ligament ossification should be also elucidated. We intended to screen the variants and discovered potential responsible genes in 5 patients with ligament ossification by using whole exome sequencing (WES) approach, providing evidence for further study on the potential pathogenesis of ligament ossification.

Methods

Patient specimens

Patient specimens were acquired from ligamentum flavum. Control individuals that visited the orthopedic clinics and patients who were clinically diagnosed with OSL between 2014 and 2016 were enrolled in the study. All procedures were performed in accordance with relevant guidelines. OPLL and OLF were diagnosed according to radiologic features and clinical symptoms. These subjects were symptomatic and needed therapeutic management, and several subjects underwent surgery in spine before or during this research. Besides, subjects with other ossification illnesses in spinal ligament were excluded. Control individuals had a spinal disease (lumbar disc herniation) excluding OLF, OPLL and other ossification illnesses in spinal ligament, while without radiographic performance of spinal ligament ossification. We excluded those subjects who had exposed to pharmacologic drugs affecting calcium or bone metabolism or showed diffuse idiopathic skeletal hyperostosis. All individuals underwent surgery and ligamentum flavum were collected. A QIAamp DNA Midi Kit (QIAGEN, Hilden, Germany) was used to extract genomic DNA from these specimens in accordance with the instruction. The usage of these samples was approved by the Ethics Committee of Peking Union Medical College Hospital and all the patients provided written informed consent.

Whole exome sequencing

SureSelect Human All Exon V5 (Agilent, Santa Clara, CA, USA) was used for exome capture, and an Illumina HiSeq 2000 (San Diego, CA, USA) was applied for sequencing with a coverage depth of 50 ×. Low-quality reads and sequence adaptors were removed, then the reads were aligned to the human reference genome in National Center for Biotechnology Information (NCBI) (GRCh37/ hg19) using Burrows-Wheeler Aligner (BWA). Picard (http://sourceforge.net/projects/picard/) and GATK were used for local realignment, duplicate deletion and base quality recalibration. Following that, SNP calling was executed with SAM toolsand then annotated by ANNOVAR. They were referenced to the 1000 Genomes Project database (http://browser.1000-genomes.org/index.html) and dbSNP (http://www.ncbi.nlm. nih.gov/SNP).

Variation forecasts and conservation examination

Potentially important variants were assessed with online tools. These online tools included PROVEAN (http://provean.jcvi.org/index.php), Align GVGD (http://agvgd.iarc.fr/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), Mutation Assessor (http://mutationassessor.org/) and Mutation Taster (http://mutationtaster.org/). Clustal W was applied for estimating the conservation status of these variants.

Results

Clinical characteristics of the subjects

In this study, a total of 5 control patients and 5 patients with OSL were investigated. In 5 patients with OSL, 3 of them were Han Chinese ethnicity, 1 of them was Hui Chinese ethnicity and 1 of them was Man Chinese ethnicity from China. In 5 control patients, 4 of them were Han Chinese ethnicity and 1 of them was Man Chinese ethnicity from China. In Table 1, we showed the radiographic and demographic data of these patients. The number of ossified ligamentum was also calculated based on radiographic findings .

Table 1
The radiographic and demographic data of 5 patients with OSL and 5 control patients.

	OSL patients	Control patients
Number of patients	5	5
Age (years)	66.40 ± 9.56	39.60 ± 13.05
Sex (M/F)	(3/2)	(3/2)
Number of ossified ligamentum flavum	7.20 ± 4.97	-
Number of ossified posterior longitudinal ligament	2.80 ± 0.84	-

Whole-exome Sequencing, Variation Prediction And Conservation Analysis

Whole-exome sequencing was performed in ligament tissues from 5 OSL patients and 5 control patients. Next, a filtration step was carried out in samples through including all frameshift, nonsense and splicing variants, then the pathogenetic characters were predicted among the missense variants. After screened with number of samples, 8 common SNP variants were presented in all 5 subjects of OSL (Table 2). To investigate whether these variants would induce substantial change in protein and gene expression, certain bioinformatics tools were applied. These variants were presented in the genes of GRHL2, CUL3, WHAMM, IL17RD, POM121L12, SLC26A8 and PTPN23. We discovered two novel variants in WHAMM gene in 5 OSL individuals, that were c.A2207C and c.C2208G. Besides, the functional effects of the missense variants were predicted using in silico analyses (Table 3).

Table 2
Common SNP variants presented in 5 patients of OSL.

Chr	POS	REF	ALT	Gene	Func	NCBI reference	cDNA change	Protein change
chr8	102631911	G	A	GRHL2	exonic	NM_024915	c.G1243A	p. V415I
chr2	225362478	C	T	CUL3	exonic	NM_001257197	c.G1501A	p.V501I
						NM_001257198	c.G1717A	p.V573I
						NM_003590	c.G1699A	p. V567I
chr15	83502065	A	C	WHAMM	exonic	NM_001080435	c.A2207C	p.H736P
chr3	57136585	C	T	IL17RD	exonic	NM_017563	c.G901A	p. V301M
chr7	53103371	G	T	POM121L12	exonic	NM_182595	c.G7T	p.A3S
chr15	83502066	C	G	WHAMM	exonic	NM_001080435	c.C2208G	p.H736Q
chr6	35923246	T	C	SLC26A8	exonic	NM_138718	c.A1600G	p.I534V
						NM_001193476	c.A1915G	p.I639V
						NM_052961	c.A1915G	p. I639V
chr3	47452118	G	A	PTPN23	exonic	NM_015466	c.G2830A	p. A944T

Table 3
The functional effects of the variants predicted.

Chr	POS	Polyphen2_HDIV_score	Polyphen2_HVAR_score	SIFT	Mutation taster	Mutation assessor
chr8	102631911	0.88	0.669	0.27	1	1.89
chr2	225362478	0	0.014	0.16	0	-0.345
chr15	83502065	0.34	0.058	0.33	1	0.895
chr3	57136585	0.777	0.137	0.23	0.031	0
chr7	53103371	0.985	0.785	0.15	1	0
chr15	83502066	0.21	0.041	0.56	1	1.245
chr6	35923246	0.021	0.01	0.65	1	0.955
chr3	47452118	0	0	0.63	1	-0.345

After screened with Number of samples, 56 common SNP variants were presented in 4 subjects of OSL (not been showed). After screened with number of samples, 207 common SNP variants were presented in 3 subjects of OSL (not been showed). We then added additional screenings as following: Polyphen2_HDIV_score: Deleterious, probably damaging, ≥ 0.95; Polyphen2_HVAR_score: Deleterious, probably damaging, ≥ 0.909; SIFT: Deleterious, sift ≤ 0.05. The data showed that 4 common SNP variants were presented in 4 subjects of OSL among 56 common SNP variants (Table 4). These variants were presented in the genes of KRT84, KIF1B, NRAP and CETN1. In addition, 7 common SNP variants were presented in 3 subjects of OSL among 207 common SNP variants (Table 5). These variants were presented in the genes of CCT3, ANLN, ESRRB, SRBD1, ODF3L1, BRAT1 and RBP3. We also exhibited whether these variants would induce substantial change in the expression of gene and protein. In addition, potentially significant variants were further assessed with Mutation Taster and Mutation Assessor (Table 4, Table 5).

Table 4. Four common SNP variants presented in 4 subjects of OSL among 56 common SNP variants.

Chr	POS	REF	ALT	Gene	Func	NCBI reference	cDNA change	Protein change	Mutation Taster	Mutation Assessor
chr12	52777578	C	T	KRT84	exonic	NM_033045	c.G551A	p. R184Q	0	2.865
chr1	10397567	A	G	KIF1B	exonic	NM_015074	c.A3260G	p. Y1087C	1	2.415
chr10	115410234	T	C	NRAP	exonic	NM_001261463	c.A746G	p.Y249C	0	2.44
						NM_006175	c.A746G	p.Y249C		
						NM_198060	c.A746G	p. Y249C		
chr18	580623	T	C	CETN1	exonic	NM_004066	c.T215C	p.M72T	0	2.52

(Additional screenings were added as following: Polyphen2_HDIV_score: Deleterious, probably damaging, ≥0.95; Polyphen2_HVAR_score: Deleterious, probably damaging, ≥0.909; SIFT: Deleterious, sift ≤0.05)

Table 5. Seven common SNP variants presented in 3 subjects of OSL among 207 common SNP variants.

Chr	POS	REF	ALT	Gene	Func	NCBI reference	cDNA change	Protein change	Mutation Taster	Mutation Assessor
Chr1	156280971	G	A	CCT3	exonic	NM_001008800	c.C1057T	p.L353F	0	2.985
						NM_005998	c.C1171T	p. L391F		
Chr7	36438709	C	G	ANLN	exonic	NM_001284301	c.C194G	p.S65W	0	2.255
						NM_001284302	c.C194G	p.S65W		
						NM_018685	c.C194G	p. S65W		
Chr14	76905712	A	G	ESRRB	exonic	NM_004452	c.A16G	p.R6G	1	2.095
Chr2	45640334	T	C	SRBD1	exonic	NM_018079	c.A2432G	p. K811R	0.237	0.805
Chr15	76017452	C	T	ODF3L1	exonic	NM_175881	c.C122T	p. P41L	0	3.15
Chr7	2578371	G	A	BRAT1	exonic	NM_152743	c.C1798T	p. L600F	0.002	2.255
Chr10	48388228	C	T	RBP3	exonic	NM_002900	c.G2650A	p. V884M	0.991	2.305

(Additional screenings were added as following: Polyphen2_HDIV_score: Deleterious, probably damaging, ≥0.95; Polyphen2_HVAR_score: Deleterious, probably damaging, ≥0.909; SIFT: Deleterious, sift ≤0.05)

Discussion

About 3% of the whole genome, the basis for protein-coding genes, are targeted by WES, while exome and targeted sequencing offer a balance between cost and benefit, compared with whole genome sequencing (WGS) [21]. However, the molecular etiology is still limited to confirm the genetic heterogeneity in the disease of OSL. Whole exome sequencing implicates PTCH1 and COL17A1 genes in OPLL of the cervical spine in Chinese patients [15]. A whole-exome sequencing approach defines the molecular signatures of anterior cruciate ligament ruptures and Achilles tendinopathy [22]. In the present study, we selected 5 patients with typical radiological findings of OSL and 5 control patients to allow description of genetic variants related with OSL through WES approach.

We detected 8 common SNP variants in all 5 subjects of OSL. These variants were generated in the genes of GRHL2, CUL3, WHAMM, IL17RD, POM121L12, SLC26A8 and PTPN23. Furthermore, among that, two novel variants were identified in WHAMM, that were c.A2207C and c.C2208G, while their corresponding protein changes were p.H736P and p.H736Q. These recognized genes may participate in pathologic progression of OSL. WHAMM (WAS Protein Homolog Associated with Actin, Golgi Membranes, and Microtubules) is one member of Wiskott-Aldrich syndrome protein (WASP) family, which functions for the ubiquitously expressed Arp2/3 complex as nucleation-promoting factors [23]. WHAMM links actin assembly to autophagy through the Arp2/3 complex [24]. WHAMM promotes actin polymerization to initiate autolysosome tubulation on autolysosomes [25]. An actin WHAMM interaction links autophagy and SETD2 [26]. Rab1 recruits WHAMM but limits actin nucleation during membrane remodeling [27]. Autophagy is involved in the osteogenic differentiation of ligament fibroblasts and promotes the development of OPLL [28]. Therefore, it is possible that novel variants in WHAMM gene can regulate the action of autophagy to affect osteogenic differentiation in the osteogenic process. Our data, together with these previous studies, imply that WHAMM may be one of important genes attributed to underlying genetic factors in the disease of OSL, whereas more studies need to be further investigated.

In addition, we found 4 common SNP variants were presented in 4 subjects of OSL among 56 common SNP variants when added additional screenings with deleterious Polyphen2_HDIV_score, Polyphen2_HVAR_score and SIFT. The genes are KRT84, KIF1B, NRAP and CETN. The novel variant in the gene of KIF1B was identified as risk factors for disease by the five prediction tools. KIF1B, the kinesin motor protein, has previously been involved in the axonal transport of synaptic vesicles and mitochondria and has been related with susceptibility to multiple sclerosis (MS) [29]. Elevated levels of KIF2A and KIF1B proteins in mature Dendritic

cells (mDCs) inhibit lamins degradation, likely by hampering autophagosome-lysosome fusion [30]. IGF1R, the receptor tyrosine kinase, is a new direct binding partner of KIF1Bβ, and its binding and transport is impaired specifically by the Y1087C mutation in KIF1Bβ [31]. IGF1R signaling plays essential roles in self-renewal, pluripotency (or multipotency), and therapeutic efficacy of multiple stem cells [32].MiR-376c-3p reduces IGF1R/Akt signaling in bone marrow-derived stromal cells (BMSC) and is one mechanism by which osteogenesis may be inhibited [33]. Let-7c targets IGF-1R to inhibit the osteo/odontogenic differentiation of dental pulp-derived mesenchymal stem cells (DPMSCs) treated with IGF-1 [34]. Mesenchymal stem cells (MSCs) in human spinal ligaments localize to collagenous matrix and perivascular area, which have a high propensity toward osteogenesis [35]. Based on the previous findings, we hypothesize that novel variants in KIF1B gene may alter osteogenesis progression of mesenchymal stem cells (MSCs) through IGF1R signaling in spinal ligaments. These observations suggest that the novel variants in the gene of KIF1B may contribute to the pathophysiology of OSL.

Besides, we found 7 common SNP variants were presented in 3 subjects of OSL among 207 common SNP variants when added additional screenings with deleterious Polyphen2_HDIV_score, Polyphen2_HVAR_score and SIFT. The genes are CCT3, ANLN, ESRRB, SRBD1, ODF3L1, BRAT1 and RBP3. The novel variants in the genes of ESRRB and RBP3 were assessed as risk factors for disease in all five prediction tools. During the reprogramming of epiblast stem cells, Esrrb (estrogen-related receptor β), the orphan nuclear receptor, plays a pioneering role in recruiting the core pluripotency factors Sox2, Oct4 and Nanog to inactive enhancers in closed chromatin [36]. Ncoa3 sustains embryonic stem cell self-renewal and reprogramming through functioning as an essential Esrrb coactivator [37]. Esrrb mediates reprogramming of mouse embryonic fibroblasts (MEFs) to induced pluripotent stem (iPS) cells by conjunction with Oct4 and Sox2 [38]. Mutations of ESRRB encoding Esrrb causes autosomal-recessive nonsyndromic hearing impairment DFNB35 [39]. Mesenchymal stem cells (MSCs) in OPLL have a high propensity toward osteogenesis [40]. Osteogenic differentiation is suppressed by a histamine-2-receptor antagonist in MSCs from OPLL patients [41]. MiR-615-3p suppresses osteogenic regulators GDF5 and FOXO1 to inhibit osteogenic differentiation of human lumbar ligamentum flavum cells [42]. Therefore, we speculate that whether ESRRB participates in OSL through regulates the osteogenic differentiation in MSCs, which needs to be validated in the future study. To our knowledge, there are limited studies on the RBP3 gene. These results suggested that protein change induced by a single-nucleotide substitution in the gene of ESRRB could be one potential mechanism affecting OSL in patients.

Our data exhibited that several common SNP variants were only presented in 3 or 4 subjects among the 5 OSL patients. Although ascertaining the exact causes for the discrepancies among these patients is hard, potential contributors to the inconsistent consequences may include study design, sample size, genetic heterogeneity between populations, levels of gene-environment interactions and environmental factors such as mechanical stress [13]. Inclusion standards with more serious phenotypes can promote the genomic research of disease-susceptibility genes through organizing genetically more homogeneous individuals [43].

Conclusions

In conclusion, we recognized potential novel variants in several genes, especially WHAMM, KIF1B and ESRRB in patients with OSL among Chinese population. We exhibit the characteristics of SNPs which may be involved in the pathogenesis of ligament ossification and assess the variants in new genes associated with ligament ossification. Further functional studies should be performed to provide evidence that these variants regulate osteogenic differentiation and may be susceptibility genetic factors in the pathogenesis of OSL. Our discoveries are crucial for complete insight to the genetics of ligament ossification. To evaluate their latent functions as molecular drivers in ligament ossification, further studies on the variants are necessary.

This work also has several limitations: (1) a relatively small sample size; (2) lack of functional description for the novel variants; (3) some of the genes may not represent phenocopies, particularly when they are only identified in three individuals; (4) some candidate genes and variants of significance may have been missed by WES analysis. In addition, the evident difference in age between OSL patients and control individuals showed in the present study is also reported in previous researches, which have demonstrated that these differences have no impact to the outcomes [8]. For excluding patients from the controls and reducing the confounding bias, age criteria of recruiting the control individuals should be adopted in our future studies.

Abbreviations

OSL
Ossification of spinal ligament; SNPs:Single nucleotide polymorphisms; WES:Whole exome sequencing; OPLL:Ossification of the posterior longitudinal ligament; OLF:Ossification of the ligamentum flavum; NCBI:National Center for Biotechnology Information; BWA:Burrows-Wheeler Aligner; WGS:Whole genome sequencing; WASP:Wiskott-Aldrich syndrome protein; MS:multiple sclerosis; mDCs:mature Dendritic cells; BMSC:bone marrow-derived stromal cells; DPMSCs:dental pulp-derived mesenchymal stem cells; MSCs:mesenchymal stem cells; MEFs:mouse embryonic fibroblasts; iPS:induced pluripotent stem

Declarations

Ethics approval and consent to participate

The usage of samples was approved by the Ethics Committee of Peking Union Medical College Hospital and all the patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yu Zhao and Xing Wei conceived, designed and supervised the study; Jiahao Li led the statistical analysis of the data; The first draft of the manuscript was written by Jiliang Zhai and Jiahao Li. All authors read and approved the final manuscript before submission.

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References

1. Ohara Y. Ossification of the Ligaments in the Cervical Spine, Including Ossification of the Anterior Longitudinal Ligament, Ossification of the Posterior Longitudinal Ligament, and Ossification of the Ligamentum Flavum. Neurosurg Clin N Am. 2018; 29(1):63-68.
2. Nam DC, Lee HJ, Lee CJ, Hwang SC. Molecular Pathophysiology of Ossification of the Posterior Longitudinal Ligament (OPLL). Biomol Ther (Seoul). 2019; 27(4):342-348.
3. Abiola R, Rubery P, Mesfin A. Ossification of the Posterior Longitudinal Ligament: Etiology, Diagnosis, and Outcomes of Nonoperative and Operative Management. Global Spine J. 2016; 6(2):195-204.
4. Yan L, Gao R, Liu Y, He B, Lv S, Hao D. The Pathogenesis of Ossification of the Posterior Longitudinal Ligament. Aging Dis. 2017; 8(5):570-582.
5. Lang N, Yuan HS, Wang HL, et al. Epidemiological survey of ossification of the ligamentum flavum in thoracic spine: CT imaging observation of 993 cases. Eur Spine J. 2013; 22(4):857-862.

6. Guo JJ, Luk KD, Karppinen J, Yang H, Cheung KM. Prevalence, distribution, and morphology of ossification of the ligamentum flavum: a population study of one thousand seven hundred thirty-six magnetic resonance imaging scans. Spine (Phila Pa 1976). 2010; 35(1):51-56.
7. Yang Y, Peng X, Ying P, et al. AWESOME: a database of SNPs that affect protein post-translational modifications. Nucleic Acids Res. 2019; 47(D1):D874-D880.
8. Kong Q, Ma X, Li F, et al. COL6A1 polymorphisms associated with ossification of the ligamentum flavum and ossification of the posterior longitudinal ligament. Spine (Phila Pa 1976). 2007; 32(25):2834-2838.
9. Liu Y, Zhao Y, Chen Y, Shi G, Yuan W. RUNX2 polymorphisms associated with OPLL and OLF in the Han population. Clin Orthop Relat Res. 2010; 468(12):3333-3341.
10. Karasugi T, Nakajima M, Ikari K, et al. A genome-wide sib-pair linkage analysis of ossification of the posterior longitudinal ligament of the spine. J Bone Miner Metab. 2013; 31(2):136-143.
11. Nakajima M, Takahashi A, Tsuji T, et al. A genome-wide association study identifies susceptibility loci for ossification of the posterior longitudinal ligament of the spine. Nat Genet. 2014; 46(9):1012-1016.
12. Tanaka T, Ikari K, Furushima K, et al. Genomewide linkage and linkage disequilibrium analyses identify COL6A1, on chromosome 21, as the locus for ossification of the posterior longitudinal ligament of the spine. Am J Hum Genet. 2003; 73(4):812-822.
13. Han IB, Ropper AE, Jeon YJ, et al. Association of transforming growth factor-beta 1 gene polymorphism with genetic susceptibility to ossification of the posterior longitudinal ligament in Korean patients. Genet Mol Res. 2013; 12(4):4807-4816.
14. Jekarl DW, Paek CM, An YJ, et al. TGFBR2 gene polymorphism is associated with ossification of the posterior longitudinal ligament. J Clin Neurosci. 2013; 20(3):453-456.
15. Wei W, He HL, Chen CY, et al. Whole exome sequencing implicates PTCH1 and COL17A1 genes in ossification of the posterior longitudinal ligament of the cervical spine in Chinese patients. Genet Mol Res. 2014; 13(1):1794-1804.
16. He Z, Zhu H, Ding L, Xiao H, Chen D, Xue F. Association of NPP1 polymorphism with postoperative progression of ossification of the posterior longitudinal ligament in Chinese patients. Genet Mol Res. 2013; 12(4):4648-4655.
17. Wang H, Jin W, Li H. Genetic polymorphisms in bone morphogenetic protein receptor type IA gene predisposes individuals to ossification of the posterior longitudinal ligament of the cervical spine via the smad signaling pathway. BMC Musculoskelet Disord. 2018; 19(1):61.
18. Yan L, Chang Z, Liu Y, Li YB, He BR, Hao DJ. A single nucleotide polymorphism in the human bone morphogenetic protein-2 gene (109T > G) affects the Smad signaling pathway and the predisposition to ossification of the posterior longitudinal ligament of the spine. Chin Med J (Engl). 2013; 126(6):1112-1118.
19. Qu X, Chen Z, Fan D, et al. Two novel BMP-2 variants identified in patients with thoracic ossification of the ligamentum flavum. Eur J Hum Genet. 2017; 25(5):565-571.
20. Fan T, Meng X, Sun C, et al. Genome-wide DNA methylation profile analysis in thoracic ossification of the ligamentum flavum. J Cell Mol Med. 2020; 24(15):8753-8762.
21. Suwinski P, Ong C, Ling MHT, Poh YM, Khan AM, Ong HS. Advancing Personalized Medicine Through the Application of Whole Exome Sequencing and Big Data Analytics. Front Genet. 2019; 10(49.
22. Gibbon A, Saunders CJ, Collins M, Gamieldien J, September AV. Defining the molecular signatures of Achilles tendinopathy and anterior cruciate ligament ruptures: A whole-exome sequencing approach. PLoS One. 2018; 13(10):e0205860.
23. Rottner K, Hanisch J, Campellone KG. WASH, WHAMM and JMY: regulation of Arp2/3 complex and beyond. Trends Cell Biol. 2010; 20(11):650-661.
24. Kast DJ, Dominguez R. WHAMM links actin assembly via the Arp2/3 complex to autophagy. Autophagy. 2015; 11(9):1702-1704.
25. Dai A, Yu L, Wang HW. WHAMM initiates autolysosome tubulation by promoting actin polymerization on autolysosomes. Nat Commun. 2019; 10(1):3699.
26. Seervai RNH, Grimm SL, Jangid RK, Tripathi DN, Coarfa C, Walker CL. An actin-WHAMM interaction linking SETD2 and autophagy. Biochem Biophys Res Commun. 2020;

27. Russo AJ, Mathiowetz AJ, Hong S, Welch MD, Campellone KG. Rab1 recruits WHAMM during membrane remodeling but limits actin nucleation. Mol Biol Cell. 2016; 27(6):967-978.
28. Yang Y, Lin Z, Chen J, et al. Autophagy in spinal ligament fibroblasts: evidence and possible implications for ossification of the posterior longitudinal ligament. J Orthop Surg Res. 2020; 15(1):490.
29. Lyons DA, Naylor SG, Scholze A, Talbot WS. Kif1b is essential for mRNA localization in oligodendrocytes and development of myelinated axons. Nat Genet. 2009; 41(7):854-858.
30. Turan A, Grosche L, Krawczyk A, et al. Autophagic degradation of lamins facilitates the nuclear egress of herpes simplex virus type 1. J Cell Biol. 2019; 218(2):508-523.
31. Xu F, Takahashi H, Tanaka Y, et al. KIF1Bbeta mutations detected in hereditary neuropathy impair IGF1R transport and axon growth. J Cell Biol. 2018; 217(10):3480-3496.
32. Teng CF, Jeng LB, Shyu WC. Role of Insulin-like Growth Factor 1 Receptor Signaling in Stem Cell Stemness and Therapeutic Efficacy. Cell Transplant. 2018; 27(9):1313-1319.
33. Camp E, Pribadi C, Anderson PJ, Zannettino ACW, Gronthos S. miRNA-376c-3p Mediates TWIST-1 Inhibition of Bone Marrow-Derived Stromal Cell Osteogenesis and Can Reduce Aberrant Bone Formation of TWIST-1 Haploinsufficient Calvarial Cells. Stem Cells Dev. 2018; 27(23):1621-1633.
34. Liu GX, Ma S, Li Y, et al. Hsa-let-7c controls the committed differentiation of IGF-1-treated mesenchymal stem cells derived from dental pulps by targeting IGF-1R via the MAPK pathways. Exp Mol Med. 2018; 50(4):25.
35. Chin S, Furukawa K, Ono A, et al. Immunohistochemical localization of mesenchymal stem cells in ossified human spinal ligaments. Biochem Biophys Res Commun. 2013; 436(4):698-704.
36. Adachi K, Kopp W, Wu G, et al. Esrrb Unlocks Silenced Enhancers for Reprogramming to Naive Pluripotency. Cell Stem Cell. 2018; 23(2):266-275 e266.
37. Percharde M, Lavial F, Ng JH, et al. Ncoa3 functions as an essential Esrrb coactivator to sustain embryonic stem cell self-renewal and reprogramming. Genes Dev. 2012; 26(20):2286-2298.
38. Feng B, Jiang J, Kraus P, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nat Cell Biol. 2009; 11(2):197-203.
39. Collin RW, Kalay E, Tariq M, et al. Mutations of ESRRB encoding estrogen-related receptor beta cause autosomal-recessive nonsyndromic hearing impairment DFNB35. Am J Hum Genet. 2008; 82(1):125-138.
40. Harada Y, Furukawa K, Asari T, et al. Osteogenic lineage commitment of mesenchymal stem cells from patients with ossification of the posterior longitudinal ligament. Biochem Biophys Res Commun. 2014; 443(3):1014-1020.
41. Liu X, Kumagai G, Wada K, et al. Suppression of osteogenic differentiation in mesenchymal stem cells from patients with ossification of the posterior longitudinal ligament by a histamine-2-receptor antagonist. Eur J Pharmacol. 2017; 810(156-162.
42. Yin J, Zhuang G, Zhu Y, et al. MiR-615-3p inhibits the osteogenic differentiation of human lumbar ligamentum flavum cells via suppression of osteogenic regulators GDF5 and FOXO1. Cell Biol Int. 2017; 41(7):779-786.
43. Buxbaum JD, Silverman JM, Smith CJ, et al. Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. Am J Hum Genet. 2001; 68(6):1514-1520.