The Link between miR-96 Levels and the Developmental Dysplasia of the Hip

miR-96 Seviyeleri ile Gelişimsel Kalça Displazisi Arasındaki Bağlantı

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ABSTRACT

Background: Developmental dysplasia of the hip (DDH) is a sophisticated skeletal disease ranging from subluxation to entire dislocation of the hip as a result of missing growth of the acetabulum and femur. DDH clearly has a multifactorial biological etiology. No doubt, one of them is genetic changes. MicroRNAs (miRNAs) are 21-23 nucleotides RNAs that show biological regulatory functions on a variety of basic physiological or pathological processes including bone formation. Although there have been many studies examining the relationship between bone metabolism and miRNAs, including miR-96, none of these studies, have been directed to the etiology of DDH.
Methods: In our study, we aimed to investigate the relationship between miR-96 expression level and DDH. In this study, peripheral blood samples of 50 DDH and 80 DDH-free individuals under one-year-old were analyzed to investigate the relationship between miR-96 expression level and DDH. Gene ontology (GO) and KEGG pathways enrichment analyses of miR-96 targeting genes were performed using available databases and R environment.
Results: We found that miR-96 level in DDH patients was approximately 3 times lower in comparison to healthy individuals. Gene ontology enrichments of hsa-mir-96 targeting genes underline the involvement of this miRNA in many biological processes including the bone homeostasis. Obtained results indicate that the expression level of miR-96 in the DDH group significantly decreased compared to the control group and this may be linked to the etiology of DDH.
Conclusion: Our work is the first study which aims to correlate the miR-96 level with DDH by investigating the miRNA level in plasma of patients and healthy individuals. In addition to our experimental data and enrichment analyses results for mir-96, considering the previous papers on miR-96 and osteogenesis, here we suggest that miR-96 may a role in the etiology of DDH.

Keywords: miR-96, microRNA, hip dysplasia, DDH, the expression level

ÖZET

Amaç: Gelişimsel kalça displazisi (GKD), asetabulum ve femurun büyüme defektinin bir sonucu olarak subluksasyondan kalça çıkığına kadar değişen karmaşık bir iskelet hastalığıdır. GKD'nin çok faktörlü biyolojik bir etiyolojisi vardır. Şüphesiz bunlardan biri de genetik değişimlerdir. MikroRNA'lar (miRNA'lar), kemik oluşumu da dahil olmak üzere çeşitli temel fizyolojik veya patolojik süreçlerde biyolojik düzenleyici işlevler gösteren 21-23 nükleotidli RNA'lardır. MiR-96 da dahil olmak üzere, kemik metabolizması ile miRNA'lar arasındaki ilişkiyi inceleyen birçok çalışma olmasına rağmen, bu çalışmaların hiçbiri GKD etiyolojisine yönelik değildir.
Yöntem: Çalışmamızda miR-96 ekspresyon düzeyi ile GKD arasındaki ilişkiyi araştırmayı amaçladık. Bu çalışmada, miR-96 ekspresyon düzeyi ile GKD arasındaki ilişkiyi araştırmak için bir yaş altındaki 50 GKD hastası ve 80 GKD'si olmayan bireyden periferik kan örnekleri analiz edildi. Mevcut veri tabanları ve R ortamı kullanılarak miR-96 hedef genlerinin gen ontolojisi (GO) ve KEGG yolları zenginleştirme analizleri yapıldı.
Bulgular: GKD hastalarında miR-96 düzeyinin sağlıklı bireylere göre yaklaşık 3 kat daha düşük olduğunu bulduk. hsa-mir-96'yı hedefleyen genlerin gen ontoloji zenginleştirmeleri, bu miRNA'nın kemik homeostazı dahil birçok biyolojik sürece dahil olduğunu gösterdi. Elde edilen sonuçlar, GKD grubunda miR-96'nın ekspresyon seviyesinin kontrol grubuna göre önemli ölçüde azaldığını ve bunun DDH etiyolojisi ile bağlantılı olabileceğini göstermektedir.
Sonuç: Çalışmamız, hasta ve sağlıklı bireylerin plazmasındaki miRNA düzeyini araştırarak miR-96 düzeyini GKD ile ilişkilendirmeyi amaçlayan ilk çalışmadır. mir-96 için deneysel verilerimiz ve zenginleştirme analiz sonuçlarımıza ek olarak, miR-96 ve osteogenez ile ilgili önceki makaleleri göz önünde bulundurarak, miR-96'nın GKD etiyolojisinde bir rolü olabileceğini düşünüyoruz.

Anahtar Sözcükler: miR-96, mikroRNA, kalça displazisi, GKD, ekspresyon düzeyi

INTRODUCTION

Developmental dysplasia of the hip (DDH) (MIM#142700) is a sophisticated skeletal disease ranging from subluxation to entire dislocation of the hip as a result of missing growth of the acetabulum and femur. The percentage of detected DDH varies between societies. DDH is a serious public health problem as it may cause serious complications if left untreated early. DDH clearly has a multifactorial biological etiology. Many risk elements such as breech delivery, female sex, swaddling and oligohydramnios have been identified for DDH to date (1,2). Another etiologic cause that has been studied recently is genetic changes. High rapport observed in twins and family predisposition encourages research on the potential relationship between DDH and genetic etiology. By the same token, a 12-fold risk rise has been noticed among first degree relatives for DDH. As with other complex diseases, many genetic factors in many biological pathways are likely to partake in the pathology of DDH. To date, in literature, by performing several research procedures, many genes, loci, and chromosomes were stated as nominees (3–5).

MicroRNAs (miRNAs) are 21-23 nucleotides RNAs that show biological regulatory functions on a variety of basic physiological or pathological processes including bone formation in many organisms that are targeting messenger RNAs (mRNAs) for degradation or translation suppression (6,7). Bone homeostasis originates from mesenchymal stem cells (MSCs) diversifying into mature osteoblasts and each time of bone generation is inseparable from the sensitive arrangement of various miRNAs. A great number of miRNAs have been reported that have a positive or negative effect on signaling pathways of skeletal homeostasis and osteogenesis (8–11). miR-96 is an oncogenic miRNA known to be involved in many cancer pathways, especially lung and bladder cancer. In addition, many studies have been conducted to date to investigate its relationship with bone homeostasis (12,13).

Although there have been many studies examining the relationship between bone metabolism and miRNAs, including miR-96, none of these studies, have been directed to the etiology of DDH (14–16). In our study, we aimed to investigate the relationship between expression levels of miR-96 and DDH.

MATERIAL and METHODS

Overall, 130 subjects (50 participants in the patient group, 80 participants in the control group) were investigated between March 2018 and September 2019. Patient group is consisted of 27 females and 23 males, and in the control group, there were 45 female and 35 male individuals (p=0.857) (Table 1). All participators were between 0-12 months old. The study was approved by the University Clinical Research Ethics Committee (18/04/38), where the authors were worked, obtained written informed consents from two groups (by parents), and study was conducted in accordance to Declaration of Helsinki.

The participants with following symptoms were deported from the both groups; systematic syndrome, congenital anomaly, hereditary malady, breech presentation, history of oligohydramnios, swaddling and elevated birth weight (>4000 gr). Orthopedist diagnosed the patients with DDH by following the principle of medical anamnesis, clinical tests and pelvic radiographic/ultrasonographic proof. The control group included 80 DDH-free individual who were inscribed from the same department. Peripheral blood samples were taken from all participants and their plasma was rapidly separated and stored at -80°C until they were processed.

miRNA isolation from plasma samples was performed by miRNeasy Serum/Plasma Kit (Qiagen, cat. no: 217184, Hilden, Germany) according to the manufacturer instructions. cDNA synthesis from miRNA was carried out by using Qiagen miScript Reverse Transcription (RT) Kit II (Hilden, Germany, Cat No./ID: 218161) according to the kit procedure. Briefly, we prepared a mixture containing 2 µl 5x miScript HiSpec buffer, 1 µl 10x miScript nucleic acid buffer, 1µl miScript Reverse Transcriptase mix and 6 µl water + miRNA. The PCR conditions were set as follows: 37°C for 60 min and at 95°C for 5 min. The quality and the quantity of cDNA samples were checked by Thermo Scientific Multiscan Spectrophotometer System (Waltham, Massachusetts, USA). cDNA concentration was then adjusted to 200 ng/µl. Reaction compounds of qRT-PCR were prepared by Qiagen miScript SYBR Green PCR kit (Cat No./ID: 218073). For PCR protocol of pre-amplified miRNA-cDNA samples, Corbett rotor-gene® 6000 instrument (Qiagen GmbH, Hilden, Germany) was used. The PCR reaction was performed in 25 µl mix consisting of 12 µl 2x QuantiTect SYBR Green PCR Master Mix, 2.5 µl 10x miScript Universal Primer, 2.5 µl 10x miScript Primer Assay (miR-96 primer assay), 6 µl water and 2 µl pre-Amp-c DNA. The PCR conditions were set as follows: Initial activation step at 95 °C for 15 sec, denaturation at 95 °C for 15 sec, annealing and at 55 °C for 10 sec, and extension at 70 °C for 30 sec for a total of 40 cycles.

Statistics of obtained data was analyzed by using the specialized analysis program in Qiagen web page (https://www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/). To confirm the statistical analyses of obtained results, GraphPad prism 6 (GraphPad Software, Inc,San Diego,USA) was used. Normality of data was examined by Shapiro-Wilk tests. Non-parametric data of independent groups was examined by Mann-Whitney U test. For all tests p<0.05 was considered as statistically significant.

List of experimentally validated target genes of miR-96 is obtained from Tarbase database (17) and this list is used for enrichment of GO terms. KEGG pathway analysis is provided by using the same set of genes. All statistical calculations, enrichment analyses and visualization of data are implemented in R environment (18), using Bioconductor (19), and 'ClusterProfiler' package (20). False discovery rate (FDR) adjusted p-value is used for statistical significance and it is set as p<0.05.

Table 1. Gender distribution of study groups**.**

Gender	Patient group		Control group		
	Number	Percentage	Number	Percentage	p
Female	27	54	45	56.25	0.857
Male	23	46	35	43.75	

Table 2. MiScript Primer Assays 5' cap

Primer assay	5'cap
miR-96	5'UUUGGCACUAGCACAUUUUUGCU

Table 3. Comparison of miR-96 between groups.

	Avg.ct		Avg ΔCt		$2^{(-\Delta(Ct))}$		p value	
	DDH	Control	DDH	Control	DDH	Control	Fold change	P***
miR-96	20,87	22,42	20,87	22,42	0,000001	0	**2,9252**	0,00005

*** p<0.001

RESULTS

For normalization of expressed miRNA levels, previously defined global values were used. The values of fold change and significance (p) are illustrated in Table 3**.** As the normalized miR-96 values were normally distributed in both groups, we have chosen Mann-Whitney U test in the comparison of miR-96. Emerged results indicate that the expression level of miR-96 in the DDH group significantly decreased compared to the control (p<0.001) (Figure 1).

Regarding the Tarbase database, 872 genes were listed as miR-96 targeting genes (Supplementary Table 1) based on experimental evidences. A total of 145 biological processes (BP), 96 cellular components (CC), and 63 molecular functions (MF) were enriched by the 872 genes targeted by mirR-96 (Supplementary Table 2). Processes such as 'histone modification', 'regulation of autophagy' and 'cell cycle arrest' were found in the top 10 BPs enriched list along with 'adherens junction', 'focal adhesion' and 'cell-substrate adherens junction' as examples of the top 10 CCs (Figure 2). Those genes targeted by miR-96 also enriched MFs, such as 'chromatin binding', 'cell adhesion molecule binding' and 'cadherin binding' as depicted in Figure 2. KEGG enrichment analysis was also carried out on the same list of the genes. A total of 26 KEGG pathways were enriched (Supplementary Table 3) by miR-96 targeting genes including 'Adherens junction', 'p53 signaling pathway', 'FoxO signaling pathway', 'Viral carcinogenesis' and 'PI3K-Akt signaling pathway' pathways (Figure 3).
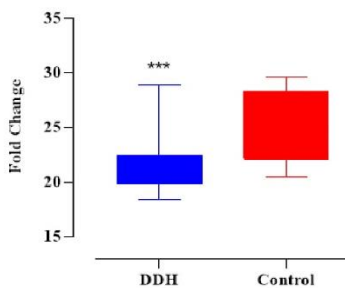

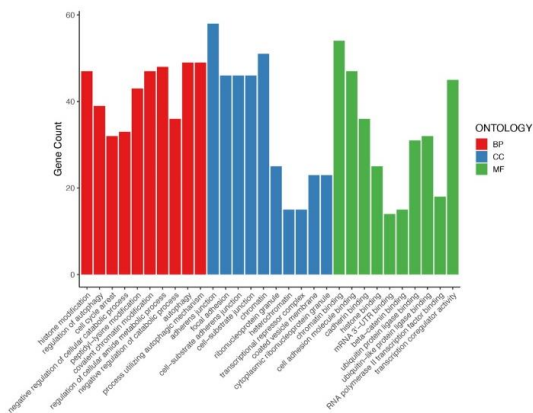**Figure 1.** MiR-96 expression levels between DDH patients and healthy individuals.

Figure 2. Gene ontology enrichment by miR-96 targetin genes. The top 10 enriched terms (adj P < 0.05) of each GO category (BP, CC, MF) by their corresponding gene count were plotted using barplots.
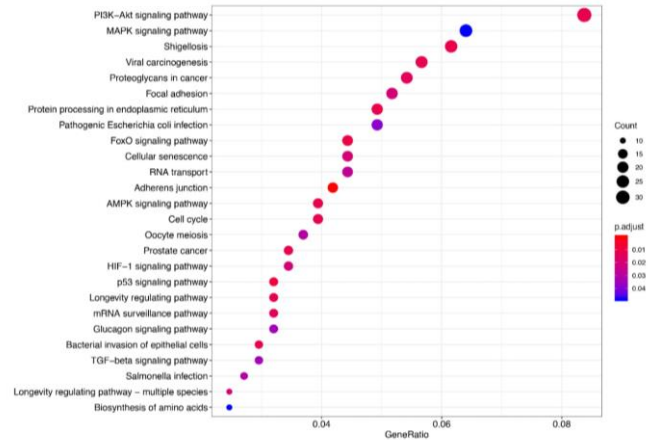

Figure 3. KEGG pathway enrichment by miR-96 targeting genes. All KEGG enriched pathways (adj P < 0.05) by targeted genes were visualized using heatplots.

DISCUSSION

In this case-control study, we aimed to examine miR-96 levels and its association with DDH. Since DDH is a multifactorial complicated disease associated with bone homeostasis of the hip joint, any alteration in osteoclast and osteoblast equilibrium may be responsible for this dysplasia. Although many studies have been conducted on the genetic changes in the etiology of DDH, none of these studies are on miRNAs. miR-96 is encoded by a 78-nucleotide gene located in the long arm of the seventh chromosome. miR-96, like all miRNAs, plays a role in many cellular pathways, especially in the cancer related biological processes, which is also validated by our in-silico analyses (Figure 2-3). Many cancers related biological processes such as 'cell cycle arrest', 'G1/S transition of mitotic cell cycle', 'signal transduction by p53 class mediator', 'G1 DNA damage checkpoint', 'DNA integrity checkpoint' and 'regulation of apoptotic signaling pathway' are enriched by the miR-96 targeting genes list (Supplementary Table 2) indicating an essential role of miR-96 in cancer. Found KEGG pathways such as 'Viral carcinogenesis', 'Prostate cancer', 'Proteoglycans in cancer' and 'p53 signaling pathway' (Supplementary Table 3) provide further support for the participation of miR-96 in cancer pathways. Beside the roles in cancer, in recent years, many studies have been conducted on miR-96 to investigate the relationship between miR-96 and osseous diseases (12,16,21). As a result of these studies, a strong association of miR-96 with this pathway has been found. Currently, it is hypothesized that the Wnt signaling pathway, which plays a role in cancer progression, also plays an important role in bone morphogenesis (13,14). In our enrichment analyses, interestingly, 'bone cell development' biological process is also identified as one of the enriched BPs (Supplementary Table 2). Our analysis suggests that miR-96 function in this pathway by the putative interactions with ANXA2, MED1, FBN1, PTPN6, WASF2 and EP300 genes. Since FBN1 mutations are found in Marfan syndrome, a connective-tissue disorder, it is found intriguing and remarkable to have this gene as a target of miR-96 (Supplementary Table 1-2) which may link the DDH with dysregulation of miR-96 levels (22). DDH, which is one of the most common musculoskeletal findings in patients with Marfan syndrome, and early-onset osteoarthritis due to this, support this view (23,24). Also, Sponseller et al. (25) noticed that Marfan syndrome patients had 2% DDH. This means a 20-fold increased risk compared to the normal population (26). The fact that 4 of the 5 genes responsible for Loeys-Dietz syndrome which show similar characteristics with Marfan syndrome related to the TGF-B family supports this idea. The fact that SMAD3, the gene responsible for Loeys-Dietz syndrome type 3 (Aneurysms-Osteoarthritis syndrome), plays a mediating role in the transition from the TGF-B pathway to the Wnt pathway and its association with the MAPK pathway also supports our idea. The involvement of TGF-B and PI3K-Akt pathway in osteoblast differentiation and MAPK pathway in osteoclast differentiation diversifies the data on the role of miR-96 in the etiology of DDH.

Since Dicer deficiency has an notable impact on osteoblasts and osteoclasts pathways, it is suggested that miRNAs may be involved in skeletal development and bone homeostasis as well (27,28). Suomi *et al*. (29) showed that miR-96 suppresses chondrogenic differentiation in favor of osteogenesis which is further validated by another reported study by Laine *et al.* (21) according to their findings, increased miR-96 expression is observed during osteogenesis. Ma *et al*. (30) showed that miR-96 is essential to promote osteoblast differentiation, and it is documented that miR-96 plays this role via the Wnt signaling pathway. Yang *et al.* (12) also demonstrated that miR-96 promotes osteogenic differentiation in osteoblastic cells. These conditions may be one of the components of insufficient osteogenesis observed in DDH patients. To be consistent with these results, in our study, we found that miR-96 levels were approximately 3-fold lower in individuals with DDH compared to DDH-free individuals. However, Liu *et al.* (13) had published that miR-96 inhibits osteogenic differentiation. This outcome is not coherent with our conclusion and other studies. This situation may be due to the fact that the patient group examined in this study was osteoporotic patients older than 70 years.

The authors are aware of the fact that miR-96 expression level evaluation from the hip joint capsule and ligament would have contributed more to elucidate the etiology of the disease and to draw stronger conclusion. Thus, we think that one of the limitations of presented study is investigation of miR-96 level in peripheral blood sample instead of the affected tissue. In addition, the lack of evaluation of the relationship between miR-96 expression level and clinical severity among patients with DDH is another shortcoming of this study. These points will be addressed in further studies.

On the other hand, our work is the first study to investigate the correlation of DDH and miR-96 levels. Considering the crucial roles of miRNAs in different biological processes, and based on our data, we are confident that our findings provide new insights into DDH etiology with respect to altered miR-96 expression level in patients. Support from the follow-up studies, especially the functional studies, may contribute to explain the link between miR-96 and DDH, and consequently miR-96 mimics may be used in the treatment of DDH.

Conflict of interest
No conflict of interest was declared by the authors.

REFERENCES

1.Vaquero-Picado A, González-Morán G, Garay EG, Moraleda L. Developmental dysplasia of the hip: update of management. EFORT open Rev 2019; 4: 548–556.
2.Gyurkovits Z, Sohár G, Baricsa A, Németh G, Orvos H, Dubs B. Early detection of developmental dysplasia of hip by ultrasound. Hip Int 2019; 30: 1120700019879687.
3.Swarup I, Penny CL, Dodwell ER. Developmental dysplasia of the hip: an update on diagnosis and management from birth to 6 months. Curr Opin Pediatr 2018; 30: 84–92.
4.Yan W, Hao Z, Tang S, Dai J, Zheng L, Yu P, Yan W, Han X, Xu X, Shi D, Ikegawa S, Teng H, Jiang Q. A genome-wide association study identifies new genes associated with developmental dysplasia of the hip. Clin Genet 2019; 95: 345–355.
5.Zamborsky R, Kokavec M, Harsanyi S, Attia D, Danisovic L. Developmental Dysplasia of Hip: Perspectives in Genetic Screening. Med Sci 2019; 7: 59.
6.Razmara E, Bitaraf A, Yousefi H, Nguyen TH, Garshasbi M, Cho WC-S, Babashah S. Non-Coding RNAs in Cartilage Development: An Updated Review. Int J Mol Sci 2019; 11: 20.
7.Hosseinpour S, He Y, Nanda A, Ye Q. MicroRNAs Involved in the Regulation of Angiogenesis in Bone Regeneration. Calcif Tissue Int 2019; 105: 223–238.
8.Fang S, Deng Y, Gu P, Fan X. MicroRNAs regulate bone development and regeneration. Int J Mol Sci 2015; 16: 8227–8253.
9.Lian JB, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 2012; 8: 212–227.

10. Taipaleenmäki H. Regulation of Bone Metabolism by microRNAs. Curr Osteoporos Rep 2018; 16:1–12.
11. Inose H, Ochi H, Kimura A, Fujita K, Xu R, Sato S, Iwasaki M, Sunamura S, Takeuchi Y, Fukumoto S, Saito K, Nakamura T, Siomi H, Ito H, Arai Y, Shinomiya KI, Takeda S. A microRNA regulatory mechanism of osteoblast differentiation. Proc Natl Acad Sci U S A 2009; 106:20794–20799.
12. Yang M, Pan Y, Zhou Y. miR-96 promotes osteogenic differentiation by suppressing HBEGF-EGFR signaling in osteoblastic cells. FEBS Lett 2014 20; 588: 4761–4768.
13. Liu H, Liu Q, Wu X-P, He H-B, Fu L. MiR-96 regulates bone metabolism by targeting osterix. Clin Exp Pharmacol Physiol 2018; 45: 602–613.
14. Kopańska M, Szala D, Czech J, Gabło N, Gargasz K, Trzeciak M, Zawlik I, Snela S. MiRNA expression in the cartilage of patients with osteoarthritis. J Orthop Surg Res 2017; 12: 51.
15. Sui J, Liu Q, Zhang H, Kong Y. Deep integrative analysis of microRNA-mRNA regulatory networks for biomarker and target discovery in chondrosarcoma. J Cell Biochem 2019; 120: 9631–9638.
16. Kim S, Hata A, Kang H. Down-regulation of miR-96 by bone morphogenetic protein signaling is critical for vascular smooth muscle cell phenotype modulation. J Cell Biochem 2014; 115: 889–895.
17. Aragkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos G, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-TarBase v8: A decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res 2018; 46: 239–245.
18.R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
19. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JYH, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004; 5: R80.
20. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: An R package for comparing biological themes among gene clusters. OMICS 2012; 16: 284–287.
21. Laine SK, Alm JJ, Virtanen SP, Aro HT, Laitala-Leinonen TK. MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. J Cell Biochem 2012; 113:2687–2695.
22. Dean JCS. Marfan syndrome: Clinical diagnosis and management. Eur J Hum Genet 2007; 15: 724–733.
23. Al Kaissi A, Zwettler E, Ganger R, Schreiner S, Klaushofer K, Grill F. Musculo-skeletal abnormalities in patients with Marfan syndrome. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders 2013; 6: 1–9.
24. Thakkar SC, Foran JRH, Mears SC, Sponseller PD. Protrusio Acetabuli and Total Hip Arthroplasty in Patients With Marfan Syndrome. J Arthroplasty 2012; 27: 776–782.
25. Sponseller PD, Tomek IM, Pyertiz RE. Developmental dysplasia of the hip in Marfan syndrome. J Pediatr Orthop Part B 1997; 6: 255–259.
26. Hattori T, Inaba Y, Ichinohe S, Kitano T, Kobayashi D, Saisu T, Ozaki T. The epidemiology of developmental dysplasia of the hip in Japan: Findings from a nationwide multi-center survey. J Orthop Sci 2017; 22: 121–126.
27. Gaur T, Hussain S, Mudhasani R, Parulkar I, Colby JL, Frederick D, Kream BE, van Wijnen AJ, Stein JL, Stein GS, Jones SN, Lian JB. Dicer inactivation in osteoprogenitor cells compromises fetal survival and bone formation, while excision in differentiated osteoblasts increases bone mass in the adult mouse. Dev Biol 2011; 340:10–21.
28. Kobayashi T, Lu J, Cobb BS, Rodda SJ, McMahon AP, Schipani E, Merkenschlager M, Kronenberg HM. Dicer-dependent pathways regulate chondrocyte proliferation and differentiation. Proc Natl Acad Sci U S A 2008 12; 105: 1949–1954.
29. Suomi S, Taipaleenmäki H, Seppänen A, Ripatti T, Väänänen K, Hentunen T, Säämänen AM, Laitala-Leinonen T. MicroRNAs regulate osteogenesis and chondrogenesis of mouse bone marrow stromal cells. Gene Regul Syst Bio 2008; 2: 177–191.
30. Ma S, Wang D, Ma C, Zhang Y. MicroRNA-96 promotes osteoblast differentiation and bone formation in ankylosing spondylitis mice through activating the Wnt signaling pathway by binding to SOST. J Cell Biochem 2019; 120: 15429–42.