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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Expression and Functional Characterization of LBX1 in Adolescent Idiopathic Scoliosis

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof Lam Tsz-ping	Prof Qiu Yong
Post	Associate Professor	Professor
Unit / Department / Institution	Department of Orthopaedics and Traumatology, CUHK	Department of Spine Surgery, Drum Tower Hospital of Nanjing University
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Co-investigator(s) *(with title and institution)*	Prof Tang Leung-sang, Nelson Professor The Chinese University of Hong Kong	Dr Zhu Zezhang Professor Drum Tower Hospital of Nanjing University

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1st Jan 2017		
Project Completion date	31st Dec 2020		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31st Dec 2021		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. to study how mitoflash affects global DNA methylation pattern during the early phase of reprogramming.
2. to study the molecular mechanisms of mitoflash in regulating the demethylation of DNA.
3. to identify the signaling pathway regulated by DNA demethylation under mitoflash changes during reprogramming.

5.2 Revised Objectives

b) Effect of LBX1 on myoblasts-osteoblasts interaction in a co-culture system, and
c) Functional analysis and validation with muscle specific LBX1 conditional knockout model

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Objective 1.

Objective 1 was achieved by Nanjing side and details were described in the Final Report from Nanjing side. mRNA expression of *LBX1* in AIS paraspinal muscles was significantly different between the concave and the convex sides. A similar pattern of difference between two sides was noted at both apex and distal regions although the difference between distal concave and convex sides did not reach statistical significance. Protein expression of *LBX1* in AIS paraspinal muscles was significantly higher in convex side than in concave side. Significantly positive correlation between mRNA level of *LBX1* and myogenic genes such as PAX7, MYOD1 and MYOG was showed in both AIS and non-AIS biopsies. MYF5, ACTA1 and ACTN2 had significant positive correlation with *LBX1* expression only in AIS groups, but not in non-AIS groups. There was no significant correlation between Cobb angle and *LBX1* mRNA/protein level in either concave or convex sides paraspinal muscles in AIS. Risk allele (T) in *LBX1* SNP rs11190870 was significantly associated with AIS. There was no significance between different allele groups (TT, CT, and CC) in skeletal muscle mass, body fat mass, fat free mass, right and left arm lean mass, trunk lean mass, right and left leg lean mass, and handgrip strength at both dominant hand and non-dominant hand in AIS subjects. For SNP rs1322330 that is near *LBX1*, TT has a significantly decreased expression of LBX1 than those with CC. Interactions between the allele TT of rs1322330 and LBX1 were demonstrated in Dual-Luciferase Reporter Assay and Electrophoretic Mobility Shift Assay (EMSA).

Objective 2.

LBX1-knockdown human skeletal muscle myoblast (HSMM) had significantly lower expression of myogenic markers including *PAX7, MYOG, MYF5, ACTN2* and *TNNT3*. *LBX1*-overexpressed HSMM had significantly higher proliferating rate than the HSMM. *LBX1*-overexpressed HSMM had increased expression of muscle myogenic genes such as *MYOD1, MYOG, MYF5, MYF6, ACTA1, TNNT3*, and *DMD* than the control HSMM in cell differentiation. However, there was no noticeable myotubes formation in the *LBX1*-overexpressed HSMM or *LBX1* knocked down LBX1-overexpressed HSMM, while in control group, myotubes formation was apparently observed. There was positive staining of MF20 in control HSMM but not in the *LBX1*-overexpressed or *LBX1* knocked down *LBX1*-overexpressed HSMM. Myokines, such as *FNDC5, MSTN, BDNF, FSTL, osteonectin* (*SPARC*) and *IL6*, had significantly decreased level of expression and secretion in *LBX1*-overexpressed HSMM when compared with control HSMM. In contrast, *FABP3* and *APLN* were up regulated by *LBX1* overexpression. A further knockdown of *LBX1* in *LBX1*-overexpressed cells restored the affected expression or secretion of *FNDC5, BDNF, APLN, FSTL* and *IL6*. The nuclear and total protein level of β-catenin showed no significant differences between *LBX1*-overexpressed HSMM and control group.

In a myoblasts-osteoblasts co-culture system, conditional medium was collected from *LBX1* gain-of-function cellular model and subjected to treatment of osteoblast cell line hFOB. There was a lower level of osteogenic marker *SPP1* in *LBX1*-overexpressed conditional medium treated group than control group after two- or four-days osteogenic differentiation. However, the hFOB showed comparable ALP activities in two treatment groups after 6 days osteogenic differentiation.*Lbx1* expression was knocked down in gastrocnemius in mice model followed by induction of acute muscle injury. *Lbx1* knockdown inhibits myogenic markers mRNA expression at day 4, 7, 10 and 14 post injury. H&E staining at day 14 post injury showed that *Lbx1* knockdown significantly decreased fiber cross-sectional area. Besides, *ex vivo* mechanical test indicated that *Lbx1* knockdown significantly reduced muscle twitch after muscle regeneration.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

1 Missing link between *LBX1* SNP and *LBX1* expression and/or function
There is still lack of experimental evidence showing how the risk allele affect the LBX1 expression and/or function. One strategy is to compare the expression of LBX1 in muscles collected from subject groups with different genotypes of rs11190870. In the AIS cohort in this study, the proportion of rs11190870 genotype TT, CT and CC were 35%, 50%, 15%, respectively. Number of subjects for genotype CC was not sufficient from statistical point of view. Therefore, further study with a much larger samples size of muscle biopsies is required to address this issue. Another strategy is to establish cellular model with TT and CC genotypes respectively at rs11190870 with genome editing technique, for example CRISPR-Cas9, which will cost less time than accumulating enough sample size for the strategy mentioned previously. For this purpose, future work is needed to edit the SNP rs11190870 in HSMM model and evaluate the expression of *LBX1* and cell activities including myogenic differentiation, proliferation, metabolomic profile.

2 Biological function of *LBX1*
As a transcript factor, the downstream genes that regulated by *LBX1* have not been reported before. Although our *in vitro* study showed that myogenic genes could be modulated by overexpressing and knocking down of *LBX1*, is *LBX1* binding to their promoter/enhancer regions directly or is *LBX1* regulating their upstream genes remained to be unknown. Hence, molecular biological techniques, such as RNA sequencing or CHIP-seq, are needed to identify the direct downstream targets of *LBX1* and to reveal its novel functions besides myogenesis as well.

3 Imbalanced LBX1 expression in AIS paraspinal muscles: Primary or secondary?
Whether the imbalanced expression of LBX1 in AIS concave and convex sides paraspinal muscles is primary or secondary is an important question to answer in exploring the role of LBX1 in AIS etiopathogenesis. Since it is not possible to collect muscle biopsies repeatedly from patients to check LBX1 expression longitudinally due to ethic issue, suitable mice models are needed to address this question. However. in the literature, animal models mimicking progressive spinal deformity in scoliosis are limited. Therefore, a novel and reliable animal model for this purpose is warranted.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

To the best of my knowledge, this is the first series of studies to demonstrate the link between AIS predisposing gene, namely LBX1, and various muscle phenotypes at molecular, cellular and tissue levels in AIS with appropriate control subjects in order to minimize the confounding effects due to age and spinal deformity. Our study demonstrated abnormal muscle phenotypes in patients with AIS and highlighted their potential causative effect on AIS etiopathogenesis. The effects of LBX1 on myogenic differentiation in human myoblasts suggested a possible novel pathological mechanism underlying the abnormal muscle phenotypes in AIS.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

	The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Number	Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	*(bold the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*
1	2021	N/A	N/A	N/A	**Xu L, Feng Z, Dai Z, Lee WYW, Wu Z, Liu Z, Sun X, Tang N, Cheng JC, Qiu Y, Zhu Z***	A Functional SNP in the Promoter of LBX1 Is Associated With the Development of Adolescent Idiopathic Scoliosis Through Involvement in the Myogenesis of Paraspinal Muscles. Frontiers in Cell and Developmental Biology. 2021 Nov 30;9:777890. doi: 10.3389/fcell.2021.777890.	N/A	Yes	Yes	No
2	2021	N/A	N/A	N/A	**Wang Y, Chen H, Zhang J, Lam TP, Hung ALH, Cheng JCY, Lee WYW***	Potential Muscle-Related Biomarkers in Predicting Curve Progression to the Surgical Threshold in Adolescent Idiopathic Scoliosis—A Pilot Proteomic Study Comparing Four Non-Progressive vs. Four Progressive Patients vs. A Control Cohort. Journal of Clinical Medicine.10(21):4927.	N/A	Yes	Yes	No
3	2021	N/A	N/A	N/A	**Zhang J, Wang Y, Cheng KL, Cheuk K, Lam TP, Hung ALH, Cheng JCY, Qiu Y,** Müller R, Christen P*, **Lee WYW***	Association of higher bone turnover with risk of curve progression in adolescent idiopathic scoliosis. Bone. 143:115655.	N/A	Yes	Yes	No
4	2020	N/A	N/A	N/A	**Xu L, Dai Z, Xia C, Wu Z, Feng Z, Sun X, Liu Z, Qiu Y, Cheng JC, Zhu Z*.**	Asymmetric Expression of Wnt/B-catenin Pathway in AIS: Primary or Secondary to the Curve? Spine (Phila Pa 1976). 2020 Jun 15;45(12):E677-E683.	N/A	Yes	Yes	No

5	2020	N/A	N/A	N/A	**Zhang J, Cheuk KY, Xu L, Wang Y, Feng Z, Sit T, Cheng KL,** Nepotchatykh E, **Lam TP, Liu Z, Hung ALH, Zhu Z,** Moreau A, **Cheng JCY, Qiu Y, Lee WYW***	A validated composite model to predict risk of curve progression in adolescent idiopathic scoliosis. EClinicalMedicine. 18:100236. doi: 10.1016/j.eclinm.2019.12.006.	N/A	Yes	Yes	No
6	2020	N/A	N/A	N/A	**Xu L, Wang Y, Wu Z, Dai Z, Liu Z, Qiu Y, Cheng JC, Zhu Z*.**	A Novel Coding Variant in SLC39A8 Is Associated With Adolescent Idiopathic Scoliosis in Chinese Han Population. Spine (Phila Pa 1976). 2020 Feb 15;45(4):226-233.	N/A	Yes	Yes	No
7	2019	N/A	N/A	N/A	**Chen H, Zhang J, Wang Y, Cheuk KY, Hung ALH, Lam TP, Qiu Y, Feng JQ*, Lee WYW*, Cheng JCY.**	Abnormal lacuno-canalicular network and negative correlation between serum osteocalcin and Cobb angle indicate abnormal osteocyte function in adolescent idiopathic scoliosis. FASEB J. 33(12):13882-13892.	N/A	Yes	Yes	No
8	2019	N/A	N/A	N/A	**Man GC, Tang NL, Chan TF, Lam TP, Li JW, Ng BK, Zhu Z, Qiu Y, Cheng JC*.**	Replication Study for the Association of GWAS-associated Loci With Adolescent Idiopathic Scoliosis Susceptibility and Curve Progression in a Chinese Population. Spine (Phila Pa 1976). 2019 Apr 1;44(7):464-471.	N/A	Yes	Yes	No
9	2019	N/A	N/A	N/A	**Xu L, Wu Z, Xia C, Tang N, Cheng JCY, Qiu Y, Zhu Z***	A Genetic Predictive Model Estimating the Risk of Developing Adolescent Idiopathic Scoliosis. Current Genomics. 2019 May;20(4):246-251.	N/A	Yes	Yes	No
10	2019	N/A	N/A	N/A	**Wu Z, Wang Y, Dai Z, Qiu Y, Xu L*, Zhu Z.**	Genetic Variants of ABO and SOX6 are Associated With Adolescent Idiopathic Scoliosis in Chinese Han Population. Spine (Phila Pa 1976). 2019 Sep;44(18):E1063-E1067.	N/A	Yes	Yes	No
11	2019	N/A	N/A	N/A	**Xia C, Xue B, Wang Y, Qin X, Qiu Y, Zhu Z, Xu L***	Investigating Role of IRX Family in Development of Female Adolescent Idiopathic Scoliosis: Which One Is Real Cause? World Neurosurg. 2019 Jul;127:e132-e136.	N/A	Yes	Yes	No

12	2018	N/A	N/A	N/A	**Zhang J, Chen H,** Leung RKK, Choy KW, **Lam TP,** Ng BKW, **Qiu Y,** Feng JQ, **Cheng JCY, Lee WYW***	Aberrant miR-145-5p/bcatenin signal impairs osteocyte function in adolescent idiopathic scoliosis The FASEB Journal doi: 10.1096/fj.201 800281 Vol. 32	2018	Yes	Yes	No
13	2017	N/A	N/A	N/A	**Zhu Z, Xu L, Leung-Sang Tang N, Qin X, Feng Z, Sun W, Zhu W, Shi B, Liu P, Mao S, Qiao J, Liu Z, Sun X, Li F, Chun-Yiu Cheng J, Qiu Y***	Genome-wide association study identifies novel susceptible loci and highlights Wnt/beta-caten in pathway in the development of adolescent idiopathic scoliosis Human Molecular Genetics 26(8):1577-15 83	2018	Yes	Yes	No
14	2017	N/A	N/A	N/A	**Xu L, Xia C, Qin X, Sun W, Tang NL, Qiu Y, Cheng JC, Zhu Z***	Genetic variant of BNC2 gene is functionally associated with adolescent idiopathic scoliosis in Chinese population Molecular Genetics and Genomics 292(4):789-79 4	2018	Yes	Yes	No
15		N/A	N/A	N/A	**Xu L, Xia C, Zhu W, Feng Z, Qin X, Sun W, Qiu Y, Zhu Z.***	Lack of association between AKAP2 and the susceptibility of adolescent idiopathic scoliosis in the Chinese population BMC Musculoskelet al Disorders 18(1):368	2018	Yes	Yes	No

9. **Recognized international conference(s) in which paper(s) related to this research project was/were delivered** *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Number	Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
1	Jan/ 2021/ Virtual	Role of differentially expressed LBX1 in adolescent idiopathic scoliosis (AIS) paraspinal muscle phenotypes and muscle-bone crosstalk through modulating myoblasts	International Research Society of Spinal Deformities (IRSSD) 2020 Congress	N/A	Yes	Yes	No
2	Aug/ 2020/ Virtual	(Poster) How does LBX1 function in the differentiated paraspinal muscle phenotypes and muscle-bone crosstalk in Adolescent Idiopathic Scoliosis (AIS)	Orthopaedic Research Society (ORS) 2020 Annual Meeting	N/A	Yes	Yes	No
3	Sep/ 2019/ USA	(Poster) Is LBX1 Playing a Role in the Differentiated Paraspinal Muscle Phenotypes and Muscle-bone Interaction in Adolescent Idiopathic Scoliosis (AIS)	The American Society for Bone and Mineral Research (ASBMR) 2019 Annual Meeting	N/A	Yes	Yes	No
4	Aug/ 2019/ China	(Poster) Is Lbx1 Playing a Role in the Differentiated Paraspinal Muscle Phenotypes and Muscle-Bone Interaction in AIS	International Chinese Musculoskeletal Research Society (ICMRC) The 4th International Chinese Musculoskeletal Research Conference (ICMRC-2019)	N/A	Yes	Yes	No
5	Sep/ 2018/ Canada	(Oral and 2018 ASBMR Young Investigator Award) Lower CYP27B1 expression impairs osteoblasts activity in adolescent idiopathic scoliosis – a new insight to improve bone quality by vitamin D supplementation	American Society for Bone and Mineral Research 2018 Annual Meeting	2018	Yes	Yes	No

6	Sep/ 2018/ Canada	(Poster) Plasma microRNA as novel biomarker for Curve Progression in Adolescent Idiopathic Scoliosis – a 6 years longitudinal follow up study	Pre-meeting of American Society for Bone and Mineral Research 2018 Annual Meeting	2018	Yes	Yes	No
7	Sep/ 2018/ Canada	(Poster) Lower CYP27B1 expression impairs osteoblasts activity in adolescent idiopathic scoliosis – a new insight to improve bone quality by vitamin D supplementation	Pre-meeting of American Society for Bone and Mineral Research 2018 Annual Meeting	2018	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Dr Wang Yujia	PhD	1st Aug 2016	6th Aug 2020

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	15	7	0	0	