CRF 8G (Revised Sep 15)

RGC Reference HKU2/CRF/12R
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

To Establish a Metabolic Study Center in Hong Kong: Focusing on the emerging metabolic hormones

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Prof. Karen SL Lam	Department of Medicine, University of Hong Kong	8
Co-Principal investigator(s)	Prof. PS Leung	School of Biomedical Sciences, Chinese University of Hong Kong	5
	Prof. Aimin Xu	Department of Medicine and Department of Pharmacology & Pharmacy, University of Hong Kong	5
	Prof. Bernard MY Cheung	Department of Medicine, University of Hong Kong	5
	Dr. Yu Wang	Department of Pharmacology & Pharmacy, University of Hong Kong	5
	Dr. CM Wong	Department of Medicine, University of Hong Kong	5
Collaborators/ Others			

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	June 1, 2013		
Project Completion Date	May 31, 2016		

Duration (*in month*)	36		
Deadline for Submission of Completion Report	31 May 2017		

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. *To investigate whether FGF21 acts in an autocrine manner in both white and brown adipose tissues to control lipid metabolism and energy expenditure using adipocyte-selective FGF21 knockout mice.*
2. *To elucidate the roles of pancreatic islets-secreted FGF21 in regulating β-cell mass and function in the context of both type 1 and type 2 diabetes.*
3. *To use an integrated functional genomics and proteomics approach to comprehensively elucidate the receptor and postreceptor pathways mediating FGF21 actions in the adipose tissues and pancreatic islets.*
4. *To explore the physiological function of FGF21 and its underlying mechanisms in humans, and to determine whether FGF21 resistance exists in obese individuals.*

5.2 Revised objectives: Not applicable

6. Research Outcome

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

This project enables us to conduct comprehensive basic and clinical studies on FGF21, a metabolic hormone with multiple therapeutic benefits for obesity-related cardiometabolic syndrome:

1) We have discovered a key mechanism to explain how FGF21 exerts multiple therapeutic benefits via its actions in adipose tissues (C8: *Lin Z et al, Cell Metabolism 2013*). FGF21 induces the adipocyte production of adiponectin by enhancing both transcription activation and secretion. Adipocyte-derived adiponectin then travels to skeletal muscle and liver to exert its insulin-sensitizing activities. The anti-diabetic and lipid-lowering effects of FGF21 are abrogated in mice with adiponectin deficiency. Our animal-based findings on FGF21-adiponectin axis have been confirmed in several clinical trials, and our publication has been cited by > 200 times in four years.

2) We have uncovered a novel physiological role of FGF21 in regulating glucose homeostasis during prolonged fasting (C8: *Liang Q et al, Diabetes 2014*). During prolonged fasting, free fatty acids released from lipolysis in adipose tissues promote the secretion of FGF21 from the liver into the bloodstream via activation of PPARalpha. Circulating FGF21 can travel across blood-brain-barrier and acts on the hypothalamic neurons to promote the release of CRH, thereby activating the hypothalamus-pituitary-adrenal axis for production of cortisol, which in turn promotes hepatic gluconeogenesis to maintain glucose homeostasis. This publication (*Diabetes 2014*) was selected for editorial comment in Diabetes.

3) We have identified a hepato-protective function of FGF21 via its autocrine actions in the liver (C8: *Ye D et al, Hepatology 2014*). In response to acetaminophen (APAP) overdose, both hepatic expression and circulating levels of FGF21 in mice are dramatically increased. FGF21 in turns induces hepatic expression of peroxisome proliferator-activated receptor coactivator protein-1α (PGC-1α), thereby increasing the nuclear abundance of nuclear factor erythroid 2-related factor 2 (Nrf2) and subsequent up-regulation of several antioxidant genes. The beneficial effects of recombinant FGF21 on up-regulation of Nrf2 and antioxidant genes and alleviation of APAP-induced oxidative stress and liver injury are largely abolished by

adenovirus-mediated knockdown of hepatic PGC-1α expression, whereas overexpression of PGC-1α is sufficient to counteract the increased susceptibility of FGF21 KO mice to APAP-induced hepatotoxicity. Our publication on these data in Hepatology was selected for editorial comments.

4) We have provided evidence that FGF21 is an obligatory metabolic regulator in pancreatic islets and shed new light onto the role of endogenous FGF21 in the pathogenesis of insulin resistance and islet dysfunction. (C8: *So WY et al, Cell Death Dis 2015*).

5) Our genetic studies have provided support for the role of FGF21 resistance in obesity-related diseases, demonstrating the association of *FGFR1* genetic variants with T2DM and CAD (C9: *Cheung CY et al, International Healthy Aging Symposium 2015*). The association of FGFR1 rs2288696 with T2DM was replicated in 12362 subjects in the Guangzhou Biobank Cohort with a similar direction demonstrated, but the overall association failed to reach genome-wide significance (C8: *Cheung CY et al, Diabetologia 2017*). More importantly, our exome-chip based analysis identified a genome-wide significant association between serum FGF21 levels and a functional missense variant of the glucokinase regulator (*GCKR*) gene, p.Pro446Leu (rs1260326) which likely influences FGF21 expression via increasing glucokinase activity. This novel finding (C8: *Cheung CY et al, Diabetes 2017*) has provided new insight into the genetic regulation of FGF21 expression in humans and also suggests that alterations in hepatic carbohydrate metabolism may impact on FGF21 expression and its circulating levels.

6) We have demonstrated that as a biomarker, FGF21 is superior to other adipokines in predicting incident diabetes, and may actually replace the oral glucose tolerance test (C8: *Woo YC et al, Clin Endocrinol 2017*). Our prospective studies of the HKWDR cohort have provided convincing evidence for the potential clinical application of FGF21 as a biomarker to identify T2DM patients at enhanced risk of cardio-renal complications for targeted preventive measures (C8: *Lee CH et al, JCEM 2015 and JAHA 2017*)

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

This project has provided a series of clinical and animal evidence further reinforcing FGF21 as a potential diagnostic marker and therapeutic target for obesity-related medical complications. Since we have already developed an ultrasensitive assay kit for FGF21, we will coordinate with several clinical centers in Mainland China and Overseas to promote the clinical application of FGF21 as a biomarker for risk prediction and early diagnosis. As our omics-based studies have identified a number of novel signaling pathways in adipose tissues for FGF21, we will apply for additional grants from RGC or other sources to further delineate the mechanistic and functional roles of these pathways in mediating the multiple effects of FGF21. The clinical implication of the novel *GCKR* functional variant p.Pro446Leu (rs1260326) will be further investigated in the HKWDR cohort to determine whether a genetic predisposition to raised FGF21 levels can protect against the development of chronic diabetic complications. Furthermore, the comprehensive research platform established by this project, including a series of tissue-selective knockout models for FGF21 and FGF21 receptors, recombinant proteins and assays, can be further exploited by pharmaceutical companies for development of therapeutics for cardiometabolic diseases by targeting FGF21 and its downstream signaling events.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

The finding of the hepato-protective function of FGF21 and that endogenous FGF21 serves a physiological role in this regard has led to research collaboration with our hepato-biliary surgeons who are internationally renowned for their work in liver transplant. This collaboration has led to the exciting finding that circulating FGF21 can be used as a sensitive biomarker for severe ischemia/reperfusion injury in patients with liver transplantation, a finding of potential major clinical application to enhance the success of liver transplantation (C8: *Science Report 2016*). In our exome-chip based genetic studies, to ensure a sufficient sample size for definitive conclusions to be drawn, we have collaborated with our cardiologists (who provided DNA samples and data from the Chinese CAD cohort) and the investigators of Guangzhou Biobank Cohort Study. These collaborations have led to important findings on the role of the *GCKR gene* in regulating FGF21 expression (C8: *Diabetes 2017*) and the potential role of the *FGFR1* in the pathogenesis of type 2 diabetes via action on FGF21 signaling (C8: *Diabetologia 2017*). These genetic findings will allow further studies to unravel the role of endogenous FGF21 versus FGF21 resistance in the development of other human diseases such as diabetic nephropathy, the information on which is potentially important in guiding the ongoing programs by

various international pharmaceutical companies, on the development of FGF21 analogs for the treatment of human diseases.

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)

In this project, we investigated extensively how FGF21, a metabolic hormone with multiple therapeutic benefits for obesity-related medical conditions, carries out its functions in the body. We demonstrated that FGF21 from the liver regulates lipid and glucose metabolism, and protects the liver against drug-induced toxicity. An inter-talk between the liver and the brain, mediated by FGF21, protects against the danger of low blood sugar during fasting, by increasing cortisol release from the adrenal. FGF21 from the fat tissue, on the other hand, contributes to energy homeostasis and, through influencing the production of adiponectin from the fat cells, enhances insulin sensitivity. In the pancreas, FGF21 interacts with growth hormone in regulating insulin secretion. We also demonstrated, for the first time, that FGF21 levels in the blood can be regulated by changes in a gene involved in liver glucose metabolism. We further showed that the measurement of blood FGF21 levels may predict the success of liver transplant, and in our diabetic patients, helps to identify which individuals would develop kidney and heart diseases. The new information from this project will be important in managing diabetes and liver diseases, and in ongoing international programs on developing drugs similar to FGF21 as novel therapy.

CRF 8G (Revised Sep 15)

Part C: Research Output

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

(Please attach a copy of the 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) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)						
	2017			Lee CH, Woo YC, Chow WS, Cheung CYY, Fong CHY, Yuen MMA, **Xu A**, Tse HF*, **Lam KSL***	Role of circulating fibroblast growth factor 21 measurement in primary prevention of coronary heart disease among Chinese patients with type 2 diabetes *J Am Heart Assoc (In Press)*	No	Yes	Yes	Yes
2017				Cheung CYY, Tang CS, Xu A, Lee CH, Au KW, Xu L, Fong CHY, Kwok KHM, Chow WS, Woo YC, Yuen MMA, Cherny SS, Hai J, **Cheung BMY**, Tan KCB, Lam TH, Tse HF*, Sham PC*, **Lam KSL***	An exome-chip association analysis in Chinese reveals a functional missense variant of GCKR that regulates FGF21 levels *Diabetes 2017 Apr 6 [Epub ahead of print]*	No	Yes	Yes	Yes

2017				Cheung CYY, Tang CS, **Xu A**, Lee CH, Au KW, Xu L, Fong CHY, Kwok KHM, Chow WS, Woo YC, Yuen MMY, Hai JSH, Jin YL, **Cheung BMY**, Tan KCB, Cherny SS, Zhu F, Zhu T, Thomas GN, Cheng KK, Jiang CQ, Lam TH*, Tse HF*, Sham PC*, **Lam KSL***	Exome-chip association analysis reveals an Asian-specific missense variant in PAX4 associated with type 2 diabetes in Chinese individuals *Diabetologia 2017;60(1):107-115*	No	Yes	Yes	Yes
2017				YC Woo, CH Lee, CHY Fong, **A Xu**, AWK Tso, **BMY Cheung***, **KSL Lam***	Serum fibroblast growth factor 21 is a superior biomarker to other adipokines in predicting incident diabetes *Clin Endocr (Oxf)* 2017; 86:37-43	No	Yes	Yes	Yes
	2017			Kwok KHM, **Lam KSL***	FGF21 mimetics for treating atherosclerosis (Review) *Endocr Metab (In Press)*	No	Yes	Yes	Yes
2017				Huang Z, **Xu A*, Cheung BMY***	The Potential Role of Fibroblast Growth Factor 21 in Lipid Metabolism and Hypertension	No	Yes	No	Yes

					(Review) *Curr Hypertens Rep 2017;19(4):28*				
2016				Ye D, Li H, Wang Y, Jia W, Zhou J, Fan J, Man K, Lo C, Wong C, **Wang Y**, **Lam KSL, Xu A***	Circulating Fibroblast Growth Factor 21 Is A Sensitive Biomarker for Severe Ischemia/reperfusion Injury in Patients with Liver Transplantation *Sci Rep. 2016 Jan 25;6:19776*	No	Yes	Yes	Yes
2015				Lee CH, Hui EYL, Woo YC, Yeung CY, Chow WS, Yuen MMY, Fong CHY, **Xu A*, Lam KSL***	Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria *J Clin Endocrinol Metab 2015 Apr;100(4): 1368-75*	Yes	Yes	Yes	Yes
2015				So WY, Cheng Q, **Xu A, Lam KSL, Leung PS***	Loss of fibroblast growth factor 21 action induces insulin resistance, pancreatic islet hyperplasia and dysfunction in mice *Cell Death*	No	Yes	Yes	Yes

					Dis 2015; 26;6:e1707				
2014				Liang Q, Zhong L, Zhang J, Wang Y, Bornstein SR, Triggle CR, Ding H, **Lam KSL*, Xu A***	FGF21 maintains glucose homeostasis by mediating the cross talk between liver and brain during prolonged fasting *Diabetes 2014 Dec;63(12):4064-75*	Yes	Yes	Yes	Yes
2014				Ye D, Wang Y, Li H, Jia W, Man K, Lo CM, **Wang Y**, **Lam KSL*, Xu A***	Fibroblast growth factor 21 protects against acetaminophen-induced hepatotoxicity by potentiating peroxisome proliferator-activated receptor coactivator protein-1α-mediated antioxidant capacity in mice *Hepatology. 2014 Sep;60(3):977-89*	No	Yes	Yes	Yes
2014				**Wong CM***, **Wang Y**, Lee JT, Huang Z, Wu D, **Xu A***, **Lam KSL**	Adropin is a brain membrane-bound protein regulating physical activity via the NB-3/Notch signaling pathway in mice *J Biol Chem. 2014*	Yes	No	Yes	Yes

					12;289(37):25976-86				
2014				Hui E, Yeung CY, Lee PC, Woo YC, Fong CHY, Chow WS, **Xu A*, Lam KSL***	Elevated Circulating Pigment Epithelium-Derived Factor Predicts the Progression of Diabetic Nephropathy in Patients With Type 2 Diabetes *J Clin Endocrinol Metab 2014*;99(11): E2169-77	Yes	No	Yes	Yes
2014				**Cheung BMY***, Deng HB	Fibroblast growth factor 21: a promising therapeutic target in obesity-related diseases *Expert Rev Cardiovasc Ther 2014;12(6):659-66*	No	Yes	Yes	Yes
2013				Lin Z, Tian H, **Lam KSL,** Lin S, Hoo RC, Konishi M, Itoh N, **Wang Y,** Bornstein SR, **Xu A***, Li X*.	Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice *Cell Metab 2013;17(5):779-89*	Yes	No	Yes	Yes
2013				Woo YC, **Xu A, Wang Y, Lam KSL*.**	Fibroblast growth factor 21 as an emerging metabolic regulator: clinical	Yes	No	Yes	Yes

					perspectives (Review) *Clin Endocrinol (Oxf). 2013;78 (4):489-96*				

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

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 RGC (Yes or No)	Accessible from the institutional repository (Yes or No)
09/2016 Seoul, Korea	Plasma level of fibroblast growth factor 21 is independently related to blood pressure. Late breaking abstract.	The 26th Scientific Meeting of the International Society of Hypertension	No	Yes	Yes	No
10/2016 Beijing, China	Blood level of fibroblast growth factor 21 and risk of future hypertension	27th Great Wall International Congress of Cardiology, 21st Annual Scientific Meeting of the International Society of Cardiovascular Pharmacotherapy and the World Heart Failure Congress 2016	No	Yes	Yes	No
09/2015 Stockholm, Sweden	Hepatic FGF21 protects mice against dietinduced lipid dysregulation and insulin resistance	European Association for the Study of Diabetes Annual Meeting 2015	No	Yes	Yes	No
06/2015 Boston, USA	Adipose tissue FGF21 resistance contributes to hypoadiponectinemia and insulin resistance in obesity: Role of miR-34a	The 75th Scientific Sessions of the American Diabetes Association	No	Yes	Yes	No

03/2015 HK	Genetic polymorphisms of genes involved in the FGF21 signalling pathway are associated with obesity and its related cardiometabolic traits	The 19th International Symposium on Healthy Aging	No	Yes	Yes	No
07/2014 Melbourne, Australia	FGF21 maintains glucose homeostasis by mediating the crosstalk between liver and brain during prolonged fasting	5th FIP Pharmaceutical Sciences World Congress 2014 (Oral presentation)	Yes	No	Yes	No
06/2014 Chicago, USA	Circulating Fibroblast Growth Factor 21 Level Predicts the Progression of Diabetic Nephropathy in Patients with Type 2 Diabetes	16th International Congress of Endocrinology & the Endocrine Society 96th Annual Meeting & Expo (Oral presentation)	Yes	No	Yes	No
05/2014 Osaka, Japan	Loss of fibroblast growth factor 21 action induces pancreatic islet hyperplasia and dysfunction	57th annual meeting of the Japan Diabetes Society (Oral presentation)	Yes	No	Yes	No
09/2013 Barcelona, Spain	High glucose represses fibroblast growth factor 21 (FGF21) action through peroxisome proliferator-activated receptor gamma in mouse pancreatic islets	49th annual meeting, European Association for the Study of Diabetes (Oral presentation)	Yes	No	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
Wang Yu-Dong	PhD	June 01, 2011	May 31, 2014
So Wing-Yan	PhD	September 01, 2009	August 31, 2014
Liang Qing-Ning	PhD	September 01, 2009	August 31, 2014
Zhong Ling	PhD	September 01, 2011	August 31, 2015
Lee Tsz-Hang Jimmy	M.Phil	September 01, 2013	August 31, 2015
Huang Zhe	PhD	September 01, 2012	August 31, 2016
Pan Yang	PhD	September 01, 2013	August 31, 2017
Kwok Kelvin	PhD	September 01, 2013	August 31, 2017
Cheong Lai-Yee	PhD	September 01, 2014	August 31, 2018

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

1) The PhD thesis by Dr. Liang Qingning, received the Li Ka Shing Best Thesis Award at HKU in 2015 (only one best thesis was selected each year).
2) Dr Chloe YY Cheung received the Young Investigator Award at the 12th International Symposium on Healthy Aging for her abstract on "A functional missense variant of GCKR is associated with increased circulating FGF21 levels in an exome-chip association study among Chinese".
3) Our major research findings resulting from this project have been well cited and led to the invitation for review articles on FGF21 by various international journals (C8: *Woo YC, et al, Clin Endocr 2013; Cheung BM, et al, Expert Rev Cardiovasc Ther 2014; Kwok KH & Lam KS, Endocr Metab 2017; Huang Z, et al, Curr Hypertens Rep 2017*).
4) The impact of our work on FGF21 as a biomarker is recognized internationally and has led to invitation for collaboration from Professor Anthony Keech (lead investigator of the FIELD Study), NHMRC Clinical Trials Centre, the University of Sydney.

Project Coordinator

Contact Information: ___2255 4783_____