RGC Ref.: N_HKU729/16 NSFC Ref. : 81661168016
(please insert ref. above)

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

Identification of susceptibility genes involved in the pathogenesis and prediction of thyrotoxic periodic paralysis

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Dr. CHEUNG Ching Lung	Prof. SONG Huai Dong
Post	Assistant Professor	Researcher
Unit / Department / Institution	University of Hong Kong	Shanghai Jiao Tong University
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Co-investigator(s) *(with title and institution)*	Prof. Kathryn Tan, University of Hong Kong	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01/01/2017		
Project Completion date	31/12/2020		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31/12/2021		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To identify susceptibility genes of TPP and to determine whether TPP is a specific subtype of GD;
2. To identify the molecular mechanism of TPP;
3. To create an animal model of TPP: a model for studying gene and environmental interaction.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____
_____

1.
2.
3. ....

6. Research Outcome *(maximum 1 page; please make reference to Part C where necessary)*

Major findings and research outcome

Thyrotoxic periodic paralysis (TPP) is a rare and potentially fatal complication of hyperthyroidism characterized by recurrent hypokalemia, episodic muscle weakness and paralysis [1]. In severe attacks, life-threatening cardiopulmonary complications, such as ventricular arrhythmia, total paralysis of respiratory and bulbar muscles, may also occur [1]. We previously identified *KCNJ2* as a susceptibility gene of TPP, and the significant association with *KCNJ2* was consistently observed in other GWAS of TPP [3-5]. However, the susceptibility variant in *KCNJ2* alone could not fully explain the genetic liability of TPP.

In the present study, we first used a two-stage approach in identifying novel variants of TPP. In the first stage, comparing 362 TPP patients with 1089 controls without TPP, 5 SNPs showed significant association with TPP. In the second stage comp 533 TPP patients with 1404 patients with GD and no history of TPP, 3 out of 5 SNPs showing significant association, these are rs1352714 (nearest gene: DCHS2), rs6457617 (nearest genes: HLA-DQB1 and HLA-DQA2), and rs312729 (nearest gene: KCNJ2). The GRS composed using these 3 SNPs had an AUC of 0.74 in discriminating TPP from GD. We also genotyped 34 SNPs in 22 well-known GD susceptibility regions in 533 patients in the TPP cohort. Remarkably, 10 of 34 SNPs in 7 GD susceptibility chromosomal loci showed association with TPP in both discovery and replication cohorts. Given the presence of both susceptibility loci that are and are not associated with GD in TPP patients, our study provided evidence that TPP is a new GD molecular subtype with specific risk genes.

To further dissect the genetics of TPP, we firstly performed an additional GWAS in a southern Chinese cohort in Hong Kong with 92 TPP new cases and 2,077 new controls without TPP, then conducted a meta-analysis with two published GWAS [5 6], totaling 319 TPP cases and 3,516 healthy controls. In the meta-analysis, we identified two novel susceptibility loci of TPP near TRIM2 (4q31.3; rs6827197) and AC140912.1 (16q22.3; rs6420387). To enhance the early identification of the disease, the TPP-associated genetic variants derived from the meta-analysis were employed to develop a weighted genetic risk score (GRS). The weighted GRS had an AUC of 0.827 and 0.682 in the derivation and validation cohorts in Hong Kong. The functionality of these loci were investigated, and expression quantitative trait loci analyses showed the variants altered expression of TRIM2 in nerve and KCNJ2 in skeletal muscle.

Ref:

1. Kung AW. Clinical review: Thyrotoxic periodic paralysis: a diagnostic challenge. *J Clin Endocrinol Metab* 2006;91(7):2490-5. doi: 10.1210/jc.2006-0356 [published Online First: 2006/04/13]
2. Ryan DP, da Silva MR, Soong TW, et al. Mutations in potassium channel Kir2.6 cause susceptibility to thyrotoxic hypokalemic periodic paralysis. *Cell* 2010;140(1):88-98. doi: 10.1016/j.cell.2009.12.024 [published Online First: 2010/01/16]
3. Jongjaroenprasert W, Phusantisampan T, Mahasirimongkol S, et al. A genome-wide association study identifies novel susceptibility genetic variation for thyrotoxic hypokalemic periodic paralysis. *J Hum Genet* 2012;57(5):301-4. doi: 10.1038/jhg.2012.20 [published Online First: 2012/03/09]
4. Song IW, Sung CC, Chen CH, et al. Novel susceptibility gene for nonfamilial hypokalemic periodic paralysis. *Neurology* 2016;86(13):1190-8. doi: 10.1212/WNL.0000000000002524 [published Online First: 2016/03/05]
5. Zhao SX, Liu W, Liang J, et al. Assessment of Molecular Subtypes in Thyrotoxic Periodic Paralysis and Graves Disease Among Chinese Han Adults: A Population-Based Genome-Wide Association Study. *JAMA Netw Open* 2019;2(5):e193348. doi: 10.1001/jamanetworkopen.2019.3348 [published Online First: 2019/05/06]
6. Cheung CL, Lau KS, Ho AY, et al. Genome-wide association study identifies a susceptibility locus for thyrotoxic periodic paralysis at 17q24.3. *Nat Genet* 2012;44(9):1026-9. doi: 10.1038/ng.2367 [published Online First: 2012/08/07]

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

Given that genetic risk score (GRS) in both Hong Kong and Shanghai cohorts showed a promising predictive power of TPP, it is of interest to investigate if the GRS can predict the occurrence of TPP in the clinical setting, i.e. development of precision medicine of Graves' disease (GD). However, the incidence of TPP is very low, and there are other precipitating factors affecting the occurrence of TPP. The most cost-effective way to conduct this study is to establish a prospective cohort of male patients with GD first. After recruiting a significant number of patients with a reasonable follow-up time, we could start evaluating the accuracy of the GRS, with and without other risk factors, in predicting the occurrence of TPP.

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)

Thyrotoxic periodic paralysis (TPP) is a rare and potentially fatal complication of hyperthyroidism. The pathogenesis of TPP is unclear. Our previous study showed that genetic factors affect the risk of TPP. Given the small sample size in the previous study, we aimed to dissect the relationship of genetic factors with TPP. Using the largest sample size in the literature, we showed that TPP is potentially a molecular subgroup of Graves' disease (GD), the most common cause of hyperthyroidism. A total of 4 new genetic variants affecting the risk of TPP have been identified in our studies. The genetic risk score composed using these genetic variants could discriminate GD with TPP from GD without TPP. Moreover, we also showed that these genetic variants may affect TRIM2 and KCNJ2 gene expression in nerve and skeletal muscle and hence affecting the risk of TPP and the associated symptoms. In conclusion, our studies provide robust evidence that genetic factors play an important role in the pathogenesis of TPP.

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) ***(bold** the authors belonging to the project teams and 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 this Joint Research Scheme *(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)*						
2019				Zhao SX, Liu W, Liang J, Gao GQ, Zhang XM, Yao Y, Wang HN, Yuan FF, Xue LQ, Ma YR, Zhang LL, Ye XP, Zhang QY, Sun F, Zhang RJ, Yang SY, Zhan M, Du WH, Liu BL, Chen X, Song ZY, Li XS, Li P, Ru Y, Zuo CL, Li SX, Han B, Zhu H, Qiao J, Xuan M, Su B, Sun F, Ma JH, Chen JL, Tian HM, Chen SJ, **Song HD***; China Consortium for the Genetics of Autoimmune Thyroid Disease.	Assessment of Molecular Subtypes in Thyrotoxic Periodic Paralysis and Graves Disease Among Chinese Han Adults: A Population-Based Genome-Wide Association Study. JAMA Network Open;2(5):e193348.	No	Yes	Yes (81661168016)	No

2020				Li GH, **Cheung CL***, Zhao SX, **Song HD**, Kung AW.	Genome-wide meta-analysis reveals novel susceptibility loci for thyrotoxic periodic paralysis. Eur J Endocrinol.;183(6): 607-617.	No	Yes	Yes (N_HKU729/16)	Yes

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.)*

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)*
10/ 2020/ Seoul	Genome-wide meta-analysis reveals novel susceptibility loci for thyrotoxic periodic paralysis	17th Asia-Oceania Congress of Endocrinology and the 8th Seoul International Congress of Endocrinology and Metabolism	No	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
NA	NA	NA	NA

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

This project was collaborated with Dr. Gloria Li from the Hong Kong Polytechnic University.

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]	2	1 (same paper as the 2nd entry in the journal publications)	0	0	0