RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *1 January 2016*
Project completion date: *31 December 2020*

1. Project Title: Centre for Research into Circulating Fetal Nucleic Acids

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator (PC)	Lo Dennis Yuk-ming / Li Ka Shing Professor of Medicine	Chemical Pathology/CUHK	5
Co-Principal Investigator(s)	Chiu Rossa Wai-kwun / Choh-Ming Li Professor of Chemical Pathology	Chemical Pathology/CUHK	8
	Leung Tak-yeung / Professor	Obstetrics and Gynaecology/CUHK	3
	Lau Chak-sing / Daniel C K Yu Professor in Rheumatology and Clinical Immunology	Medicine/HKU	3
	Chan Allen Kwan-chee / Professor	Chemical Pathology/CUHK	5
	Sun Hao / Associate Professor	Li Ka Shing Institute of Health Sciences and	5

		Chemical Pathology/CUHK	
	Yi Huso / Assistant Professor[3]	Jockey Club School of Public Health and Primary Care/CUHK	5
	Dong Dong / Research Assistant Professor[5]	Jockey Club School of Public Health and Primary Care/CUHK	5
Co-Investigator(s)	Tam Lai-shan / Professor	Medicine and Therapeutics/CUHK	3
	Jiang Peiyong / Assistant Professor[1]	Chemical Pathology/CUHK	10
Collaborators	Wong Sze-chuen Cesar / Associate Professor	Health Technology and Informatics, The Hong Kong Polytechnic University	N.A.
	Luk Wayne / Professor of Computer engineering	Programming Languages and Systems Section/Imperial College/UK	N.A.
	Oudejans Cees / Professor Experimental Clinical Chemistry	Clinical Chemistry/VU University Medical Center/Netherlands	N.A.
	Faas Brigitte / Clinical Laboratory Geneticist	Human Genetics/ Radboud University Medical Centre/Netherlands	N.A.
	Yakovenko Sergey / General and Embryology Director	Altravita IVF Clinic/Russia	N.A.
	Flood Karen / Maternal Fetal Medicine Fellow	Royal College of Surgeons in Ireland/Ireland	N.A.
	Cheng Yvonne Kwun-yue / Assistant Professor[4]	Obstetrics and Gynaecology/CUHK	N.A.
	Liao Gary Jiawei[2]	Chemical Pathology/CUHK	N.A.

 # Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

[1]Dr Peiyong Jiang has been promoted to Assistant Professor due to his research productivity. His departmental affiliation remains the same and he would continue to contribute to this project in the same role as originally intended. (approved as part of the first progress report)

[2]Dr Gary Liao resigned from his post at CUHK before the project commencement date. Dr Liao's original role was to conduct the experiments related to systemic lupus erythematosus. This role is now assumed by Dr Rebecca Chan who has been hired to fill the project manager of this grant. Dr Chan has a track record in research on systemic lupus erythematosus. (approved as part of the first progress report)

[3]Dr Huso Yi has relocated to Singapore in 2017. Prof TY Leung's group has since continued to pursue Dr Yi's original line of research, namely the social, legal and ethical impact of new prenatal technologies. As a result, the project team has recently published a well-publicised study by Cheng et al on women's preference for non-invasive prenatal DNA testing versus chromosomal microarray

after screening for Down syndrome (see sections 6.1 and 6.2 below). (approved as part of the third progress report)

[4]Dr Yvonne Cheng has resigned from her position in June 2018. Dr Cheng's main role was to recruit patients for the studies. There will be no impact on the team's operation because Prof Tak-yeung Leung would continue to supervise the patient recruitment process. (approved as part of the third progress report)

[5]Application to enlist Dr Dong Dong as Co-PI in place of Dr Huso Yi wa sought from RGC in April 2020.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. To develop new technologies for the analysis of plasma nucleic acids in pregnancies.	1. 100%	1. Completed
2. To elucidate the profile and biology of plasma nucleic acids in pregnancy-associated diseases and normal pregnancies using genomewide molecular approaches.	2. 100%	2. Completed
3. To develop molecular diagnostic approaches for the assessment of pregnancy-associated pathologies including preeclampsia, single gene diseases and complicated twin pregnancies.	3. 100%	3. Completed

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

Over the course of the project, the team has achieved a number of milestones in terms of research achievements which could be grouped under 5 themes. The important publications are highlighted within the description of the 5 themes below.

Fragmentomics as an emerging research field:

This is a term the team has coined to the study of the ending patterns and fragmentation characteristics of cell-free DNA. Cell-free DNA exist in circulation in a naturally fragmented form. By studying the end sequences of cell-free DNA molecules systematically and across the whole genome, the team made the surprising observation that cell-free DNA

fragmentation is a non-random process. There are genomic locations which featured more regularly at cell-free DNA ends than others and we have termed these the preferred ends (Sun et al *Proc Natl Acad Sci U S A* 2018; 115: E5106-E5114). These findings then prompted us to investigate the ends of cell-free DNA from various angles. We found certain motif sequences to be overrepresented among cell-free DNA molecules (Jiang et al *Cancer Discov* 2020; 10: 664-673). We then showed that the ends may be blunt or jagged in nature (Jiang et al *Genome Res* 2020; 30:1144-1153). These intriguing observations spurred us to study the biological basis by exploring the role of various DNA digesting enzymes, including Deoxyribonuclease 1 Like 3 (DNase1L3) (Serpas et al *Proc Natl Acad Sci U S A* 2019; 116: 641-649); Deoxyribonuclease I (DNase1) (Cheng et al *Clin Chem* 2018; 64: 406-408) and DNA Fragmentation Factor Subunit Beta (Dffb) (Han et al *Am J Hum Genet* 2020; 106: 202-214). During this journey, we uncovered pathologies with altered activity levels of these enzymes would be associated with perturbations in the cell-free DNA profiles (Chan et al *Am J Hum Genet* 2020; 107: 882-894). In other words, by improving our understanding of biological mechanisms, we ultimately may find novel ways to develop diagnostic tests. Interestingly, since we began our exploration of fragmentomics, interests from other groups on the topic have also gathered momentum. In other words, our academic pursuit has led to the emergence of a new research field and the journal, *Science*, even has invited our team to contribute a review on the topic (Lo et al *Science* 2021; 372:eaaw3616).

Expanding non-invasive prenatal diagnostic applications:

Extending from our team's track record, in this project, we continued to push the envelope in non-invasive prenatal diagnostics. Cell-free DNA based screening for fetal whole chromosomal aneuploidies has become an established part of prenatal care worldwide. We then developed improvised methods to provide non-invasive screening of aneuploidies involving a much smaller part of a chromosome, namely microdeletions and microduplications (Yu et al *Clin Chem* 2017; 63: 495-502). We next embarked on the development of approaches to non-invasively tackle the prenatal assessment of single gene disorders. This is a challenging clinical topic to tackle because each single gene disease has relatively rare incidence. Yet collectively, they account for a sizable portion of the clinically significant prenatal and paediatric diseases. Furthermore, even for the same clinical disease, different cases may be caused by different mutations within the same gene. Hence, a one-size-fit-all solution is not readily available. We have previously devised approaches for deducing the fetal genotype based on the parental haplotypes. During this project, we have developed improved and more generic methods for assembling the parental haplotypes (Hui et al *Clin Chem* 2017; 63: 513-524). We have demonstrated that the approaches could be applied to a range of autosomal recessive diseases and X-linked diseases such as the haemophilias (Hudecova et al *Blood* 2017; 130: 340-347). When performed on a genomewide scale, the relative haplotype dosage approaches allowed the deciphering of the sequence of the fetal genome non-invasively by maternal blood analysis (Chan et al *Proc Natl Acad Sci U S A* 2016; 113: E8159-E8168). The study of non-invasive approaches for the prenatal assessment of fetal single gene diseases has now been explored by many other research groups.

Tissue of origin analysis to solve new diagnostic challenges:

Besides aiming to detect sequences associated with diseases, e.g. fetal mutations, the team has also devised a radically different approach to detect pathologies by detecting altered levels of cell-free DNA released by organs. To do so, one needs to be able to differentiate the organ contribution of pools of cell-free DNA. We term such an approach as tissue mapping or tissue-of-origin analysis. To distinguish cell-free DNA molecules released from different organs, we have used DNA methylation (Sun et al *Prenat Diagn* 2018; 38: 196-203) as well as fragmentomic features (Sun et al *Genome Res* 2019; 29: 418-427). We succeeded in distinguishing the placental DNA from maternal DNA in maternal plasma (Sun et al *Prenat*

Diagn 2018; 38: 196-203). We observed that erythroid DNA is present among cell-free DNA in human plasma which was less well known previously (Lam et al *Clin Chem* 2017; 63: 1614-1623). The utility of tissue of origin analysis for the assessment of various pathologies, including cancer, is now an intensely researched topic.

Direct analysis of DNA methylation by single molecule sequencing:
Cytosine methylation of CpG dinucleotides is one important strategy to distinguish tissue origin of cell-free DNA molecules or one that allows the detection of pathological processes associated with aberrant methylation profile via cell-free DNA analysis. Bisulfite sequencing has remained as the key technology to study DNA methylation. However, bisulfite converts all unmethylated cytosines into thymine residues, even when the cytosines are out of the context of CpG sites. Consequently, bisulfite sequencing result in reduced sequence resolution and adds ambiguity. To resolve this, the team has attained a major achievement by successfully developing an approach based on studying the kinetic features of single molecule sequencing data to distinguish methylated cytosine without the need for bisulfite treatment. We believe this method would greatly enhance and facilitate any genetic and genomic studies focused on DNA methylation. These data have been published in Tse et al *Proc Natl Acad Sci U S A* 2021; 118: e2019768118. Since the release of the publication, our team has been approached by a dozens of research groups to provide the bioinformatics code to enable their adoption of the published methodology.

Novel bioinformatics pipelines and algorithms for novel cell-free DNA diagnostics:
All the major achievements described above require the development and support of novel bioinformatics pipelines to uncover the biomarkers, features or diagnostic value of cell-free DNA. The detection of disease-associated signatures among the numerous cell-free DNA molecules in circulation is often described as searching for a needle in a haystack. Thus, one objective of the bioinformatics analysis is to sift through the mountains of cell-free DNA sequence information and amplify the useful signal. Because the team develops novel applications time and time again, the research process needs to be matched by novel bioinformatics approaches. Hence, our projects rarely make use of existing algorithms developed by other groups or companies. Instead, every effort is needed to develop home grown solutions which after publication are highly sought-after by other research groups who would then request for access to our novel pipelines. See also section 6.7 below.

Other evidence for the team's research accomplishments:
The project team is primarily engaged in translational diagnostic research. As a result, many of the team's research outputs have commercial value and the intellectual property portfolio associated with the research studies has been licensed to a range of commercial partners include startup companies founded by the project coordinator. See sections 7.4 and 8.1 below for further details. The academic standing of the project team members is also evident by the many research award, honours and accolades bestowed (see section 7.5), the high number of invited talks (see section 7.2) and the roles in professional societies which team members have served (see section 7.3).

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

One key advantage of the TRS funding over standard project grant funding is the much longer project duration of 5 years. In combination with the much larger grant size, these factors enabled the project team to make more sustained efforts to investigation research topics in a

more thorough and persistent manner. More importantly, these factors encouraged our team to undertake riskier and novel projects. The longer duration also allowed the team to forge new collaborative relationships which bore fruit even within the project duration. For example, the project team made a conscious decision to pursue a novel facet of cell-free DNA biology, which the team termed as fragmentomics. This refers to the analysis of the ending patterns of cell-free DNA molecules. This was otherwise an unexplored area. Soon, we observed interesting data showing that cell-free DNA fragmentation is a non-random process with reproducible patterns (Sun et al *Proc Natl Acad Sci U S A* 2018; 115: E5106-E5114). Curious to understand how such reproducible ending patterns were created, the team studied plasma nucleases that have DNA-cleaving properties. Again, soon thereafter, enzymes such as DNase1L3 and Dnase1 have been shown to play some roles in cell-free DNA fragmentation (Cheng et al *Clin Chem* 2018; 64: 406-408; Serpas et al *Proc Natl Acad Sci U S A* 2019; 116: 641-649). After these initial publications, majority of the research output from 2019 onwards involved fragmentomics. Some of these works involved new collaborative partners. Other research groups began to study fragmentomics. Furthermore, this momentum led to the journal, *Science*, to invite the team to contribute a review titled, Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies (Lo et al 2020; 372:eaaw3616).

6.3 If the project has not met its original objectives, why?

Not applicable.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this 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. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance	Under review	Under preparation						
2017				Jiang P, Tong YK, Sun K, Cheng SH, Leung TY, Chan KCA, Chiu RWK, Lo YMD*	Gestational Age Assessment by Methylation and Size Profiling of Maternal Plasma DNA: A Feasibility Study. *Clin Chem* 63: 606-608.	2016	No	Yes	Yes
#2017				Yu SC, Jiang P, Chan KCA, Faas BH, Choy KW, Leung WC, Leung TY, Lo YMD, Chiu RWK*	Combined Count- and Size-Based Analysis of Maternal Plasma DNA for Noninvasive Prenatal Detection of Fetal Subchromosomal Aberrations Facilitates Elucidation of the Fetal and/or Maternal Origin of the Aberrations. *Clin Chem* 63: 495-502.	2016	No	Yes	Yes
#2017				Hui WWI, Jiang P, Tong YK, Lee WS, Cheng YK, New MI, Kadir RA, Chan KCA, Leung TY, Lo YMD, Chiu RWK*	Universal Haplotype-Based Noninvasive Prenatal Testing for Single Gene Diseases. *Clin Chem* 63: 513-524.	2016	No	Yes	Yes

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance	Under review	Under preparation						
2016				Chan KCA, Jiang P, Sun K, Cheng YK, Tong YK, Cheng SH, Wong AI, Hudecova I, Leung TY, Chiu RWK*, Lo YMD*	Second generation noninvasive fetal genome analysis reveals de novo mutations, single-base parental inheritance, and preferred DNA ends. *Proc Natl Acad Sci U S A*; 113: E8159-E8168.	2016	No	Yes	Yes
2016				Wong FC, Sun K, Jiang P, Cheng YK, Chan KCA, Leung TY, Chiu RWK, Lo YMD*	Cell-free DNA in maternal plasma and serum: A comparison of quantity, quality and tissue origin using genomic and epigenomic approaches. *Clin Biochem* 49: 1379-1386.	2016	No	Yes	Yes
2016				Hui WWI and Chiu RWK*	Noninvasive prenatal testing beyond genomic analysis: what the future holds. *Curr Opin Obstet Gynecol* 28: 105-110.	2016	No	Yes	Yes
2016				Lo YMD* and Lam WK	Tracing the tissue of origin of plasma DNA-feasibility and implications. *Ann N Y Acad Sci* 1376:14-17.	2016	No	Yes	Yes
2016				Jiang P and Lo YMD*	The Long and Short of Circulating Cell-Free DNA and the Ins and Outs of Molecular Diagnostics. *Trends Genet* 32: 360-71.	2016	No	Yes	Yes
#2016				Lau JY, Yi H*, Ahmed S	Decision-making for non-invasive prenatal testing for Down syndrome: Hong Kong Chinese women's preferences for individual vs relational autonomy. *Clin Genet* 89: 550–556.	2017	No	Yes	Yes
#2017				Hudecova I, Jiang P, Davies J, Lo YMD, Kadir RA, Chiu RWK*	Noninvasive detection of F8 int22h-related inversions and sequence variants in maternal plasma of hemophilia carriers. *Blood* 130: 340-347.	2017	No	Yes	Yes
2017				Sun K, Chan KCA, Hudecova I, Chiu RWK, Lo YMD, Jiang P*	COFFEE: control-free noninvasive fetal chromosomal examination using maternal plasma DNA. *Prenat Diag* 37: 336-40.	2017	No	Yes	Yes
2017				Vong JSL, Tsang JCH, Jiang P, Lee WS, Leung TY, Chan KCA, Chiu RWK, Lo YMD*	Single-Stranded DNA Library Preparation Preferentially Enriches Short Maternal DNA in Maternal Plasma. *Clin Chem* 63: 1031-7.	2017	No	Yes	Yes
2017				Cheng THT, Jiang P, Tam JCW, Sun X, Lee WS, Yu SCY, Teoh JYC, Chiu PKF, Ng CF, Chow KM, Szeto CC, Chan KCA, Chiu RWK, Lo YMD*	Genomewide bisulfite sequencing reveals the origin and time-dependent fragmentation of urinary cfDNA. *Clin Biochem* 50: 496-501.	2017	No	Yes	Yes
2017				Hudecova I and Chiu RWK*	Non-invasive prenatal diagnosis of thalassemias using maternal plasma cell free DNA. *Best Pract Res Clin Obstet Gynaecol* 39: 63-73.	2017	No	No	Yes
2017				Lam WKJ, Gai W, Sun K, Wong RSM, Chan RWY, Jiang P, Chan NPH, Hui WWI, Chan AWH, Szeto CC, Ng SC, Law MF, Chan KCA, Chiu RWK and Lo YMD*	DNA of Erythroid Origin Is Present in Human Plasma and Informs the Types of Anemia. *Clin Chem* 63: 1614-1623.	2018	No	Yes	Yes

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Year of publication	Year of acceptance	Under review	Under preparation						
2017				Tsang JCH, Vong JSL, Ji L, Poon LCY, Jiang P, Lui KO, Ni YB, To KF, Cheng YKY, Chiu RWK and Lo YMD*	Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci U S A* 114: E7786-E7795.	2018	No	Yes	Yes
#2018				Cheng Y, Leung WC, Leung TY, Choy KW, Chiu RWK, Lo TK, Kwok KY, Sahota DS*	Women's preference for non-invasive prenatal DNA testing versus chromosomal microarray after screening for Down syndrome: a prospective study. *BJOG* 125: 451-459.	2018	No	Yes	Yes
2018				Chiu EKL, Hui WWI and Chiu RWK*	cfDNA screening and diagnosis of monogenic disorders - where are we heading? *Prenat Diagn* 38: 52-58.	2018	No	Yes	Yes
2018				Sun K, Lun FMF, Leung TY, Chiu RWK, Lo YMD and Sun H*	Noninvasive reconstruction of placental methylome from maternal plasma DNA: Potential for prenatal testing and monitoring. *Prenat Diagn* 38: 196-203.	2018	No	Yes	Yes
2018				Sun K, Jiang P, Wong AIC, Cheng YKY, Cheng SH, Zhang H, Chan KCA, Leung TY, Chiu RWK and Lo YMD*	Size-tagged preferred ends in maternal plasma DNA shed light on the production mechanism and show utility in noninvasive prenatal testing. *Proc Natl Acad Sci U S A* 115: E5106-E5114.	2018	No	Yes	Yes
2018				Cheng THT, Lui KO, Peng XL, Cheng SH, Jiang P, Chan KCA, Chiu RWK and Lo YMD*	DNase1 Does Not Appear to Play a Major Role in the Fragmentation of Plasma DNA in a Knockout Mouse Model. *Clin Chem* 64: 406-408.	2019	No	Yes	Yes
#2019				Serpas L, Chan RWY, Jiang P, Ni M, Sun K, Rashidfarrokhi A, Soni C, Sisirak V, Lee WS, Cheng SH, Peng W, Chan KCA, Chiu RWK, Reizis B* and Lo YMD*	Dnase1l3 deletion causes aberrations in length and end-motif frequencies in plasma DNA. *Proc Natl Acad Sci U S A* 116: 641-649.	2019	No	Yes	Yes
2019				Sun K, Jiang P, Cheng SH, Cheng THT, Wong J, Wong VWS, Ng SSM, Ma BBY, Leung TY, Chan SL, Mok TSK, Lai PBS, Chan HLY, Sun H, Chan KCA, Chiu RWK and Lo YMD*	Orientation-aware plasma cell-free DNA fragmentation analysis in open chromatin regions informs tissue of origin. *Genome Res* 29: 418-427.	2019	No	Yes	Yes
2019				Vong JSL, Jiang P, Cheng SH, Lee WS, Tsang JCH, Leung TY, Chan KCA, Chiu RWK and Lo YMD*	Enrichment of fetal and maternal long cell-free DNA fragments from maternal plasma following DNA repair. *Prenat Diagn* 39: 88-99.	2019	No	Yes	Yes

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Year of publication	Year of acceptance	Under review	Under preparation						
2020				Jiang P, Xie T, Ding SC, Zhou Z, Cheng SH, Chan RWY, Lee WS, Peng W, Wong J, Wong VWS, Chan HLY, Chan SL, Poon LCY, Leung TY, Chan KCA, Chiu RWK and Lo YMD*	Detection and characterization of jagged ends of double-stranded DNA in plasma. *Genome Res* 30:1144-1153.	2021	Yes	Yes	Yes
2020				Jiang P, Sun K, Peng W, Cheng SH, Ni M, Yeung PC, Heung MMS, Xie T, Shang H, Zhou Z, Chan RWY, Wong J, Wong VWS, Poon LC, Leung TY, Lam WKJ, Chan JYK, Chan HLY, Chan KCA, Chiu RWK and Lo YMD*	Plasma DNA End-Motif Profiling as a Fragmentomic Marker in Cancer, Pregnancy, and Transplantation. *Cancer Discov* 10: 664-673.	2021	Yes	Yes	Yes
2020				Han DSC, Ni M, Chan RWY, Chan VWH, Lui KO, Chiu RWK and Lo YMD*	The Biology of Cell-free DNA Fragmentation and the Roles of DNASE1, DNASE1L3, and DFFB. *Am J Hum Genet* 106: 202-214.	2021	Yes	Yes	Yes
2020				Sin STK, Jiang P, Deng J, Ji L, Cheng SH, Dutta A, Leung TY, Chan KCA, Chiu RWK and Lo YMD*	Identification and characterization of extrachromosomal circular DNA in maternal plasma. *Proc Natl Acad Sci U S A* 117: 1658-1665.	2021	Yes	Yes	Yes
2020				Chiu RWK*, Dutta A, Hensson AG, Lo YMD, Mischel P and Regenberg B	What Is Extrachromosomal Circular DNA and What Does It Do? *Clin Chem* 66: 754-759.	2021	Yes	Yes	Yes
#2020				Chan RWY, Serpas L, Ni M, Volpi S, Hiraki LT, Tam LS, Rashidfarrokhi A, Wong PCH, Tam LHP, Wang Y, Jiang P, Cheng ASH, Peng W, Han DSC, Tse PPP, Lau PK, Lee WS, Magnasco A, Buti E, Sisirak V, AlMutairi N, Chan KCA, Chiu RWK, Reizis B and Lo YMD*	Plasma DNA Profile Associated with DNASE1L3 Gene Mutations: Clinical Observations, Relationships to Nuclease Substrate Preference, and In Vivo Correction. *Am J Hum Genet* 107: 882-894.	2021	Yes	Yes	Yes
2020				Chiu RWK*, Heitzner E, Lo YMD, Mouliere F and Tsui DWY	Cell-free DNA fragmentomics: the new "Omics" on the block. *Clin Chem* 66:1480-1484.	2021	Yes	Yes	Yes
#2021				Dong D, Ahmed S, Nichini E, Yi H, Jafri H, Rashid Y, Ahmed M, Zhu J	Decision making on antenatal screening results: A comparative Q-method study of women from two Chinese cities. *Health Expect* 24: 363-376.	2021	Yes	Yes	Yes

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Year of publication	Year of acceptance	Under review	Under preparation						
#2021				Ma ML, Yakovenko S, Zhang H, Cheng SH, Apryshko V, Zhavoronkov A, Jiang P, Chan KCA, Chiu RWK and Lo YMD*	Fetal mitochondrial DNA in maternal plasma in surrogate pregnancies: Detection and topology. *Prenat Diagn* 41: 368-375.	2021	Yes	Yes	Yes
2021				Sin STK, Ji L, Deng J, Jiang P, Cheng SH, Heung MMS, Lau CSL, Leung TY, Chan KCA, Chiu RWK and Lo YMD*	Characteristics of fetal extrachromosomal circular DNA in maternal plasma: methylation status and clearance. *Clin Chem* 67: 788-796.	2021	Yes	Yes	Yes
2021				Tse OYO, Jiang P, Cheng SH, Peng W, Shang H, Wong J, Chan SL, Poon LCY, Leung TY, Chan KCA, Chiu RWK and Lo YMD*	Genome-wide detection of cytosine methylation by single molecule real-time sequencing. *Proc Natl Acad Sci U S A* 118: e2019768118.	2021	Yes	Yes	Yes
2021				Lo YMD*, Han DSC, Jiang P, Chiu RWK	Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies. *Science* 372:eaaw3616.	2021	Yes	Yes	Yes

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered:
(Please attach a copy of each conference abstract)

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
July/ 2017/ San Diego	Prospective analysis of 20,000 cases reveals that the combined use of cell-free DNA counting and size measurement improves specificity of NIPT	ISPD 21st International Conference on Prenatal Diagnosis and Therapy	2017	No	Yes (during the oral presentation)	No
Oct/ 2017/ Orlando	Noninvasive reconstruction of placental methylome by genomewide bisulfite sequencing and size analysis of maternal plasma DNA	American Society of Human Genetics meeting	2019	No	Yes	No
Mar/ 2018/ Cambridge, UK	Non-invasive cellular monitoring by integrative single-cell and cell-free plasma RNA transcriptomics analysis	Wellcome Genome Campus	2019	No	Yes	No

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Sep/2019/Jerusalem, Israel	Prologed persistence of fetal cfDNA after *in utero* fetal demise: cases involving a demised anomalous twin during pregnancy	Circulating nucleic acids in plasma and serum 2019	2021	Yes	Yes	No
Sep/2019/Singapore	New approach for mining the fetal preferred ends	ISPD 23rd International Conference on Prenatal Diagnosis and Therapy	2021	Yes	Yes	No
Sep/2019/Singapore	Potential application of circulating cell-free DNA analysis for noninvasive monitoring of lupus pregnancy	ISPD 23rd International Conference on Prenatal Diagnosis and Therapy	2021	Yes	Yes	No

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

Only the review article by Hudecova and Chiu (*Best Pract Res Clin Obstet Gynaecol* 2017) did not acknowledge RGC funding because this article presented an overview of the field with no original data.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

Because of the 5-year project duration, the project team was able to establish collaborations for projects which require a longer time horizon.

One example relates to studying the ethical and social impact of non-invasive prenatal diagnostics. Non-invasive prenatal diagnostics is a fairly new phenomenon. Hence, there is much to explore in the ethical and social context. Yet, there aren't many established experts on the topic. Therefore, the core team had to develop and grow the expertise while engaging new research partners to pool ideas and patient access to tackle such a new field.

Lau et al *Clin Genet* 2016 was a study led by a project team member Prof Huso Yi in collaboration with Leeds Institute of Health Sciences which qualitatively assessed the decision-making process surrounding non-invasive prenatal testing for Down syndrome among local Hong Kong Chinese women. Through the initial experience, the team was then able to expand the research to a quantitative survey involving many more Hong Kong pregnant women.

This has led to the study by Cheng et al *BJOG* 2018 which quantified women's preferences when offered a choice between non-invasive prenatal screening for a limited set of chromosomal aneuploidies and invasive diagnostic testing for a much wider range of chromosomal aneuploidies. To attain the needed sample size for a quantitative survey, this study involved collaborations between obstetricians at three public hospitals in Hong Kong (Prince of Wales Hospital, Kwong Wah Hospital, Princess Margaret Hospital).

Another example of the inter-institutional collaborations established and strengthened because of this project relates to those that provided access to human samples of rare diseases. Again, this was due to the longer duration of this project which meant we were able to afford the time needed to recruit families with the very rare disease of DNase1L3 deficiency. The project team aimed to study the enzymatic mechanisms governing the natural fragmentation of cell-free DNA. Through the collaboration with New York University, we investigated mice with DNase1L3 knockout to study the plasma DNA end features influenced by the presence and absence of DNase1L3. The data were published in Serpas et al *Proc Natl Acad Sci U S A* 2019.

Further collaborations were then established with Università degli Studi di Genova, Genova, Italy and The Hospital for Sick Children, Toronto, Canada, which provided access to samples of human individuals with DNase1L3 deficiency and enabled us to confirm the mouse findings being relevant to humans (Chan et al *Am J Hum Genet* 2020).

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
HUI Wai I	MPhil	2015	August 2017
YU Cheuk Yin Stephanie	Global physician scheme of MBChB	2014	June 2018
WONG Felix	Hong Kong College of Pathologists	2014	November 2020
ZHANG Haiqiang	PhD	2016	August 2019
JIAO Yinming	PhD	2016	July 2020

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

The key bioinformatics algorithms and databases that have been developed during this reporting period are those that have been featured in the following publications:

Chan et al Second generation noninvasive fetal genome analysis reveals de novo mutations, single-base parental inheritance, and preferred DNA ends. *Proc Natl Acad Sci U S A*; 113: E8159-E8168.

Tsang et al Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci U S A* 2017; 114: E7786-E7795.

Sun et al Noninvasive reconstruction of placental methylome from maternal plasma DNA: Potential for prenatal testing and monitoring. *Prenat Diagn* 2018; 38: 196-203.

Sun et al Size-tagged preferred ends in maternal plasma DNA shed light on the production mechanism and show utility in noninvasive prenatal testing. *Proc Natl Acad Sci U S A* 2018; 115: E5106-E5114.

Sun et al Orientation-aware plasma cell-free DNA fragmentation analysis in open chromatin regions informs tissue of origin. *Genome Res* 2019; 29: 418-427.

Tse et al Genome-wide detection of cytosine methylation by single molecule real-time sequencing. *Proc Natl Acad Sci U S A* 118: e2019768118.

Since these manuscripts have been published, we have received requests from researchers all over the world to access the dataset or pipeline.

6.8 Other education activities and/or training programmes developed:

Date	Event	Talk title / Programme
21 July 2016	Seminar by Dr Charles Cantor, SAB member, Executive Director, Retrotope, Inc, USA	The oxygen paradox: a novel approach to treating neurodegenerative diseases
25 July 2016	Seminar by Dr Diana Bianchi, SAB member, Executive Director, Mother Infant Research Institute Vice Chair for Research & Academic Affairs Department of Pediatrics Floating Hospital for Children	Amniotic Fluid Transcriptomics: A Window into Fetal Development
29 May 2017	Seminar by Prof Lyn Chitty, Professor of Genetics and Fetal Medicine, Chair, Institute of Child Health, University College London	Extending the scope of a prenatal diagnostic service for monogenic disorders
24 June 2017	Hong Kong Down Syndrome Association, 30th Anniversary Symposium (see poster)	A series of talks: *Prof Dennis Lo*: Non-invasive Prenatal Testing of Down Syndrome: from Dream to Reality *Dr Brian Chung*, Clinical Associate Professor, Department of Paediatrics and Adolescent Medicine, The University of Hong Kong: Insights on Clinical Genetics and Down Syndrome *Prof Kenneth Fung*, Director, Centre for Special Educational Needs and Inclusive Education, The Hong Kong Institute of Education: Supporting the learning of students with intellectual disabilities in mainstreaming schools *Ms Maggie Yeung*, Chairlady, Parents Committee, The Hong Kong Down Syndrome Association: "Life Voyage" Let's Set Sail!
20 Dec 2017	Life at the single molecule level	Professor Sunney Xie, Mallinckrodt Professor of Chemistry and Chemical Biology, Department of Chemistry and Chemical Biology, Harvard University
20 Sep 2018	Human Biomed Applications of PacBio Long-Read Sequencing	Dr Meredith Ashby, Staff Scientist, Pacific Biosciences
6 Dec 2018	Prenatal Genetic Testing: What patients need to know	Dr Mary Norton, Professor, Obstetrics, Gynecology & Reproductive Sciences, David E. Thorburn, M.D. and Kate McKee Thorburn Endowed Chair in Perinatal Medicine and Genetics, University of California, San Francisco
13 Dec 2020	TRS Public Symposium 2020: Centre for Research into Circulating Fetal Nucleic Acids	Professor Dennis Lo, Li Ka Shing Professor of Medicine, The Chinese University of Hong Kong

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

All deliverables have been addressed.

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The PC led the project team, determined the project directions and set the priorities. The PC was active in disseminating the research findings by participating in many conferences and symposia around the world (see list of invited talks provided in the attachment associated with section 7.2 below). The PC supervised postgraduate students and held regular data review and progress meetings. The PC was actively involved in the conceptualisation of new methodologies, patent filing and patent prosecution.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Yes, in support of the Government's initiative to strengthen the capability, infrastructure and industry base for innovation in Hong Kong, the team has applied for and successfully awarded an InnoHK grant. The proposal submitted by the team was titled, Centre for Novostics. "Novostics" represent novel diagnostics. The team plans to further develop the research in aspects of molecular diagnostics within the Centre. The Centre would also proactively seize the opportunities to translate the research output into impact. As part of the scheme, project teams have been granted laboratory space at the Hong Kong Science Park. We believe the InnoHK grant has provided a smooth transition for continuing the research of this recently completed Theme-based Research grant.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Yes, both the PC and Deputy PC have been invited to speak at many international conferences. Please refer to the list appended to this report.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Yes, Prof Lo is a Director of Academy of Sciences of Hong Kong and a Founding Board Director of the International Society of Reproductive Genetics. Prof Lo is an Associate Editor of the Nature Partner Journal, *Genomic Medicine*. Prof Dennis Lo and Prof Rossa Chiu are Associate Editors of *Clinical Chemistry*, the No. 1 ranked journal in laboratory medicine. Prof Lo is senior Editor of *eLife*. Prof Lo is also an Editorial Board member of *Cancer Communications*, *Journal of Pathology*, *Philosophical Transactions of the Royal Society B*, *Disease Markers*, *Prenatal Diagnosis*, *Journal of genomes and Exomes*, *Marrow* and *American Journal of Hematology*. Prof Chiu is an Associate Editor of *Human Genetics and Genomics Advances* and is an Editorial Board member of *Clinical Biochemistry*, *Critical*

Reviews in Clinical Laboratory Sciences and *The Clinical Biochemist Reviews*. Prof Chiu is Secretary to the board of directors of as well as Chair of the Global Education and Outreach Committee of the International Society for Prenatal Diagnosis.

7.4 Any documentary proof of the application of technologies arising directly from this project?

Yes, the non-invasive fetal chromosomal aneuploidy screening test by maternal plasma DNA sequencing has been implemented worldwide, including Hong Kong, at the Hong Kong Children's Hospital and in the private sector. Between 2011 to 2012, professional societies recommended the implementation of the non-invasive maternal plasma DNA screening test for women whose pregnancy was deemed to be at high risk for fetal chromosomal aneuploidies. However, with the availability of new evidence, including those generated by our group, from 2015 onwards, the professional guidelines, such as those from the American College of Obstetricians and Gynecologists and American College of Medical Genetics and Genomics have been amended to state that the non-invasive maternal plasma DNA screening tests were applicable to women of all risk profiles. Studies around the world (e.g. Robson and Hui. National decline in invasive prenatal diagnostic procedures in association with uptake of combined first trimester and cell-free DNA aneuploidy screening. *Aust N Z J Obstet Gynaecol* 2015;55:507-10) have shown that the rates of invasive prenatal diagnosis, such as amniocentesis, have been reduced 30% to 40% as a result of the implementation of the non-invasive maternal plasma DNA test. Additional details are described in section 8.1 below.

One of the licensees of the technology portfolio, Illumina, gained CE mark for two of its non-invasive prenatal Down syndrome screening kits that are related to the team's work (https://www.genomeweb.com/molecular-diagnostics/illumina-secures-ce-mark-veriseq-nipt; https://www.illumina.com/company/news-center/press-releases/2019/2400554.html).

On the other hand, the approaches developed by the project team for the non-invasive detection of fetal single gene diseases have been implemented in England (Drury et al. Implementing Non-Invasive Prenatal Diagnosis (NIPD) in a National Health Service Laboratory; From Dominant to Recessive Disorders. Adv Exp Med Biol 2016;924:71-75) and parts of US (https://www.genomeweb.com/molecular-diagnostics/noninvasive-prenatal-testing-moves-beyond-chromosomal-abnormalities-single).

7.5 Other awards and recognitions as a result of this project (please specify):

A list has been appended to this report.

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

The project team has been adopting a proactive approach to technology transfer. First, as new technologies are developed, the team files new patent applications. We would then observe if there are commercial opportunities to derive practical utilities from the research results. The team's technology has become widely accessible through the establishment of a pool of relevant patents by Illumina and Sequenom, licensees of the project team's intellectual

property on non-invasive prenatal diagnostics, in 2014 (https://www.genomeweb.com/business-news/illumina-sequenom-pool-nipt-patents-settling-ip-disputes). The patent pool has been widely sublicensed to many parties around the world. These licenses are bringing royalty income back to Hong Kong and contributing to the sustainability of the team's research and further technology transfer activities in Hong Kong.

On the other hand, Prof Dennis Lo, Rossa Chiu and Allen Chan founded Xcelom as an attempt to directly participate in the development of the biotechnology sector in Hong Kong. Xcelom became a market leader in Hong Kong in the delivery of non-invasive prenatal screening for fetal chromosomal aneuploidies. Prof Lo, Chiu and Chan recently founded another company, named DRA (which represents a combination of the first initial of the first names of the three founders), which aimed to provide high quality non-invasive prenatal screening for fetal chromosomal aneuploidies to women in Mainland China. DRA has partnered with KingMed Diagnostics, the largest laboratory diagnostics company in China that has established a network of 37 medical laboratories across China. Furthermore, the project team worked closely with the Hong Kong Hospital Authority so that the maternal plasma DNA screening test became available as part of public healthcare service with the opening of the Hong Kong Children's Hospital.

8.2 Others (please specify):

None else.

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Yes, fragmentomics of maternal plasma DNA, tissue-of-origin analysis of maternal plasma DNA and direct methylation analysis by single molecule sequencing are new ideas evolved directly from this project. Please refer to section 6.1 above for details.

9.2 Whether there are new projects evolved directly from this project?

Yes, there were many new projects related to each of the new ideas mentioned above that evolved directly from this project. We have provided a brief summary of these projects under section 6.1.

9.3 Whether there are new collaborations developed directly from this project?

Yes, the new collaborations developed directly from this project are:

Lam et al *Clin Chem* 2017 describes the development of a set of novel epigenetic markers for erythroid DNA based on cell-free DNA detection. The plasma level of these markers reflect the bone marrow activity and have potential for the management of anaemias and other blood cell disorders. This work was achievable due to collaboration with haematologists.

Cheng et al *BJOG* 2018 reports the results of women's preferences when offered a choice between non-invasive prenatal screening for a limited set of chromosomal aneuploidies and invasive diagnostic testing for a much wider range of chromosomal aneuploidies. This study involved collaborations between obstetricians at three public hospitals in Hong Kong (Prince of Wales Hospital, Kwong Wah Hospital, Princess Margaret Hospital).

Serpas et al *Proc Natl Acad Sci U S A* 2019 investigated the biological basis of cell-free DNA fragmentation. We collaborated with New York University and gained access to mice with DNase1L3 knockout. The data revealed that DNase1L3 does play a role in the fragmentation of plasma DNA but other mechanisms are at play.

Further collaborations were then established with Università degli Studi di Genova, Genova, Italy and The Hospital for Sick Children, Toronto, Canada, which provided access to samples of human individuals with DNase1L3 deficiency and enabled us to confirm the mouse findings being relevant to humans (Chan et al *Am J Hum Genet* 2020).

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

Under section 7.1 above, we mentioned our team's success in securing funding from the InnoHK grant scheme to support Centre for Novostics in further expanding our molecular diagnostics research. Part of the project funding is devoted to prenatal diagnostics research which carries on from this Theme-based research project.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections 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	36	6	N/A	5	Type	No.
					Patent licences	2

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons
N/A	

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Prenatal diagnosis is an integral part of obstetrics. Non-invasive prenatal testing based on circulating fetal DNA analysis has resulted in a paradigm shift in antenatal care. In 1997, the project coordinator first discovered the presence of cell-free fetal DNA in the circulation of pregnant women. Within a decade, the team made non-invasive DNA-based prenatal testing a clinical reality by developing a Down syndrome test that has subsequently been adopted in many countries. In the present project, we brought together the same multi-disciplinary team of pathologists, laboratory scientists, obstetricians, bioinformaticians and public health specialists, to solve the key unmet diagnostic needs in prenatal medicine. Specifically, we have been developing the next generation tools for the analysis of cell-free nucleic acids and to study the biology and pathological characteristics of cell-free fetal nucleic acids that have not been unravelled to date. The novel tools and new biological insights will be directed towards the overall goal of developing approaches for the assessment of pregnancy-associated pathologies, such as single gene diseases, fetal demise and preeclampsia. The implications and benefits of non-invasive prenatal testing will be explored by an ethical, legal and social arm of our team. Knowledge and know-how have been disseminated through training of postgraduate students and research personnel, as well as through educational workshops for the obstetrics and laboratory medicine professions.

In terms of research, the team has continued to push the envelope of the field. The team has achieved the non-invasive decoding of the fetal genome at much higher resolution which resulted in substantial improvement in one's ability to non-invasively detect fetal de novo mutations. The team has successfully developed several approaches for the non-invasive detection of fetal single gene diseases, thus expanding the clinical applications of non-invasive prenatal testing beyond the assessment of fetal chromosomal aneuploidies. The team has uncovered interesting biological phenomenon about circulating DNA that was not appreciated previously. The team found that there are specific genomic locations that serve as preferential fragmentation sites of cell-free DNA. These preferred sites on the ends of cell-free DNA fragments aid with the identification of which organ released the DNA molecules. The team has transferred the non-invasive prenatal test for fetal chromosomal aneuploidy screening to the Hospital Authority where the test is delivered to the public through Hong Kong Children's Hospital.