02
MOLECULAR DIAGNOSTICS
(Circulating Fetal Nucleic Acids)

Principal Investigators

Professor Dennis Lo
Professor Rossa Chiu

Team

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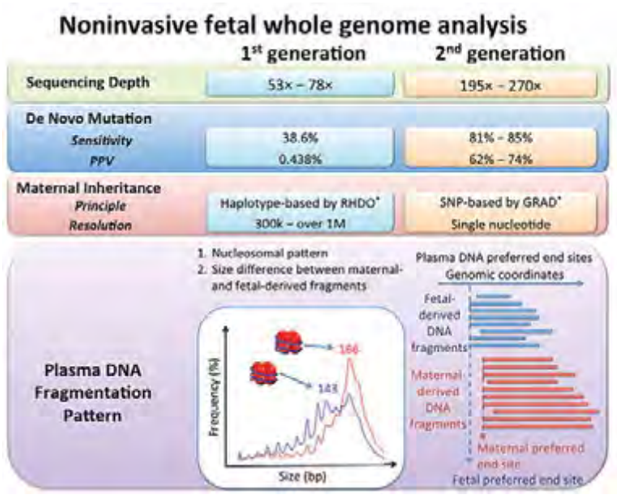
Research Progress Summary

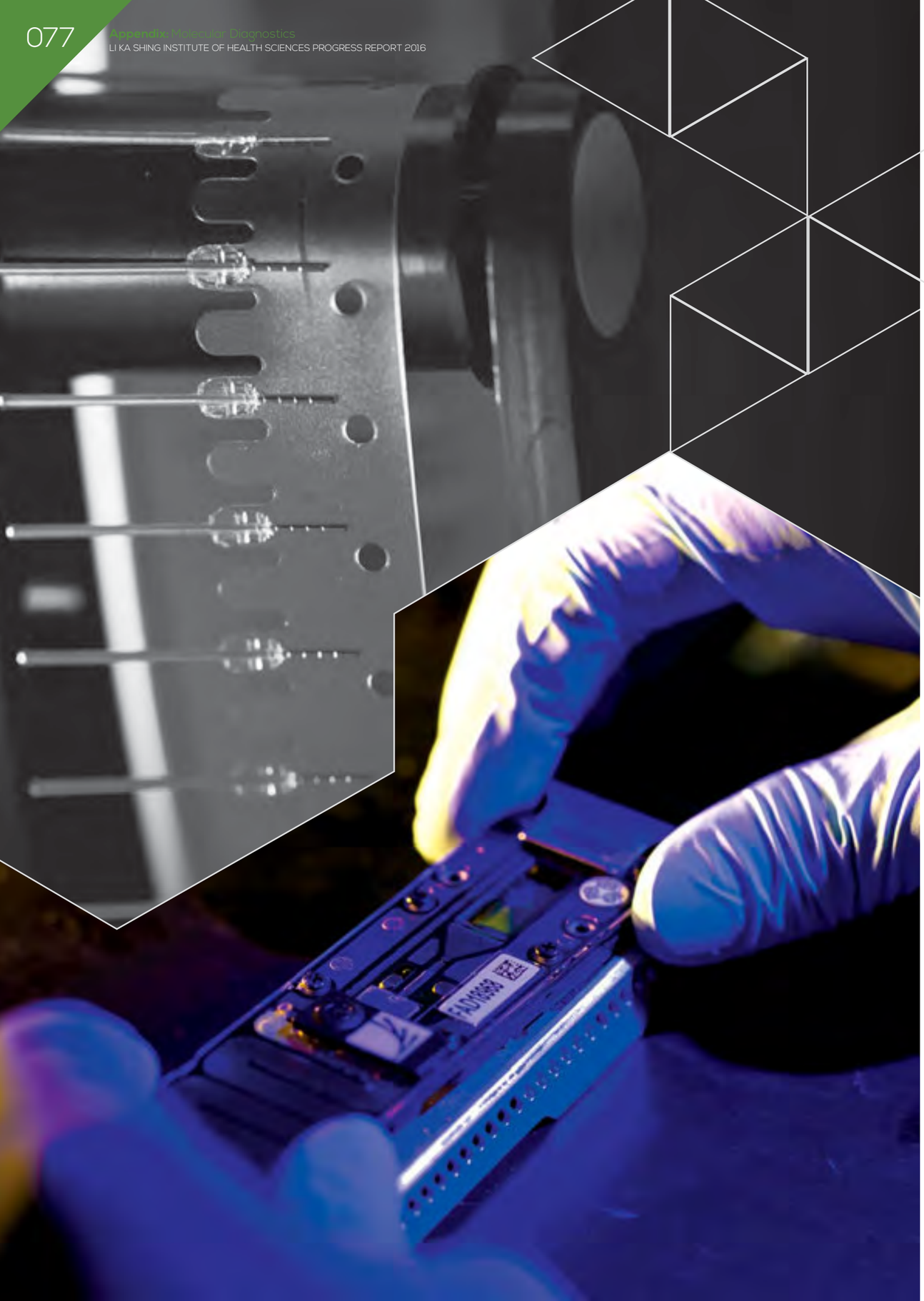
In the reporting year, Professor Dennis Lo and Professor Rossa Chiu successfully developed the second-generation noninvasive fetal genome analysis. This allowed the detection of fetal *de novo* mutations and single-base parental inheritance with the use of maternal plasma. As pioneers in the field, they explored the limit of noninvasive prenatal testing (NIPT) by performing genome-wide sequencing of maternal plasma at 195x and 270x haploid genome coverage. The noninvasive fetal whole genome analysis was first reported by Prof. Lo's group in 2010. In the new generation analysis, the team combined the use of a series of bioinformatics filters, which enabled the detection of fetal *de novo* mutations with a positive predictive value that showed 169-fold improvement over previous attempts. Also, the maternal inheritance of the fetus could be deduced individually on a genome-wide level without using maternal haplotypes. The result represented a 90-fold enhancement in the resolution of the analysis.

By applying the new method, the team analysed a maternal plasma sample from a second trimester case, which showed cystic hygroma and club foot deformity in ultrasound examination. The fetus was later confirmed to have a *de novo* single point mutation in the BRAF gene, which was responsible for cardiofaciocutaneous syndrome. The plasma sample was sequenced to 195x haploid genome coverage. With successive level of filtering, the *de novo* mutation detection rate was 81% with positive predictive value of 62%. More importantly, among the 47 detected mutations, one of them was the BRAF mutation that was responsible for the disease. Also, the fetal genotype deduced among 68% of maternal heterozygous sites at 96.8% accuracy.



Chan et al. PNAS 2016;113:E8159-68

Summary of the key differences between the first and second generation approaches for noninvasive fetal whole-genome analysis. *RHDO represents relative haplotype dosage analysis, and GRAD represents genome-wide relative allelic dosage analysis.



Moreover, the team observed a very interesting phenomenon regarding the biological characteristics of plasma DNA. From the deep sequencing data of maternal plasma DNA prepared using non-PCR (polymerase chain reaction)-amplified libraries, it was found that selected genomic locations were more likely to be at the ends of plasma DNA molecules and a subset of such preferred ends exhibited selectivity for fetal- or maternal-derived DNA in maternal plasma. The ratio of the number of maternal plasma DNA molecules with fetal preferred ends to those with maternal preferred ends showed a correlation with the fetal DNA fraction, which was a crucial parameter and consideration for NIPT.

In molecular diagnostics, pre-analytical factors also play an important role that they would impose significant impact on the performance of a test. Currently, scientists studying circulating nucleic acids may extract DNA from either plasma or serum but actually they are two similar but different media. With regard to that, the team conducted a comprehensive study to compare the quantity, quality and tissue origin of the cell-free DNA in maternal plasma and serum with the use of genomic and epigenomic approaches. Massively parallel sequencing of plasma and serum cell-free DNA showed the presence of longer DNA fragments in serum and methylation deconvolution analysis confirmed that those extra DNA in serum originated from white blood cells. They demonstrated that the final cell-free DNA concentration in serum was a summation of two opposing processes: DNA degradation or removal especially in certain types of serum collection tubes, and contamination of genomic DNA from white blood cells. The result concurred with the current consensus that the use of serum as a source of cell-free DNA should be discouraged because not only endogenous cell-free DNA became contaminated by genomic DNA, it might also be degraded or removed to various extents, thus losing potentially important diagnostic information, such as rare tumour cell-free DNA mutations or fetal mutations present in maternal plasma.

In summary, the team had developed a second-generation approach that produced noninvasive fetal genomes at high resolution using maternal plasma DNA sequencing. The work significantly pushed forward the limit of NIPT by showing the feasibility of detecting fetal *de novo* mutations and single-base parental inheritance on a genome-wide level from maternal plasma with substantially enhanced resolution. The preferred-end finding had potential to be used in combination with other fetal fraction estimation approaches for NIPT. Also, it was confirmed that the use of plasma would be the more appropriate medium for noninvasive molecular diagnostics. The team's work not only exerted profound impact on NIPT but also had compelling implications for other areas in molecular diagnostics, for example cancer.

In the reporting period, the team published a total of 8 peer-reviewed articles and reviews in international journals and was invited to give talks in various local and international conferences.

Recognitions

Awards and Fellowships

Member's Full Name	Details
Dennis Lo	The Young Achievers Award by Federation of Hong Kong Chiu Chow Community Organizations (August 2016)
Dennis Lo	AACC's Outstanding Speaker Award 2015 by The American Association for Clinical Chemistry (April 2016)
Dennis Lo	2016 Thomson Reuters Citation Laureate – Chemistry (September 2016)
Dennis Lo	《南方人物周刊》2016魅力中國年度人物 (November 2016)
Rossa Chiu	Croucher Senior Medical Fellowship 2016-2017 by The Croucher Foundation
Rossa Chiu	AACC's Outstanding Speaker Award 2015 by The American Association for Clinical Chemistry (April 2016)

Grants and Consultancy

Full Name of PI	Project Title	Funding Source	Start Date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Amount (HK$)
Dennis Lo	Centre for Research into Circulating Fetal Nucleic Acids (AoE/M-04/06) & Massively Parallel Sequencing Plasma Nucleic Acids for the Molecular Diagnostics of Cancer (T12-404/11)	CUHK Vice-Chancellor's One-off Discretionary Fund	01/01/2015	31/12/2017	3,500,000
Dennis Lo	Centre for Research into Circulating Fetal Nucleic Acids (T12-403/15-N)	CUHK Research Committee – One-off Funding for Research	15/10/2015	30/06/2017	1,000,000
Dennis Lo	Massively Parallel Sequencing Plasma Nucleic Acids for the Molecular Diagnostics of Cancer	Research Grants Council – Theme-based Research Scheme	01/12/2011	30/11/2016	33,342,750
Dennis Lo	Centre for Research into Circulating Fetal Nucleic Acids	CUHK Focus Innovations Scheme – Major Area of Biomedical Sciences	01/07/2012	30/06/2016	7,350,000
Dennis Lo	Centre for Research into Circulating Fetal Nucleic Acids	Research Grants Council – Theme-based Research Scheme	01/01/2016	31/12/2020	48,828,000

Publications

A. Journal Papers

1. Chan KC, Jiang PY, Sun K, Cheng YK, Tong YK, Cheng SH, Wong AI, Hudecova I, Leung TY, Chiu RW, Lo YM. Second generation noninvasive fetal genome analysis reveals de novo mutations, single-base parental inheritance, and preferred DNA ends. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113(50):E8159-68.
2. Chiu RW. Genome-wide plasma DNA aberrations of systemic lupus erythematosus. *Pathology*. 2016; 48:S31. (Abstract)
3. Chiu RW. Hunting for the signatures of cancer by plasma DNA sequencing. *Pathology*. 2016; 48:S30. (Abstract)
4. Chiu RW. Non-invasive prenatal testing: What's next? *Pathology*. 2016; 48: S31. (Abstract)
5. Jiang PY, Lo YM. The long and short of circulating cell-free DNA and the ins and outs of molecular diagnostics. *Trends in Genetics*. 2016; 32(6):360-71.
6. Lo YM, Lam WK. Tracing the tissue of origin of plasma DNA-feasibility and implications. *Annals of the New York Academy of Sciences*. 2016; 1376:14-7.
7. Wong FC, Lo YM. Prenatal diagnosis innovation: Genome sequencing of maternal plasma. *Annual Review of Medicine*. 2016; 67:419-32. (Review)
8. Wong FC, Sun K, Jiang PY, Cheng YK, Chan KC, Leung TY, Chiu RW, Lo YM. Cell-free DNA in maternal plasma and serum: A comparison of quantity, quality and tissue origin using genomic and epigenomic approaches. *Clinical Biochemistry*. 2016; 49(18):1379-86.