How does genome sequencing help in medical research and enhance quality of healthcare

Journal Club of the Food and Health Bureau
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Dr. Richard CHOY Kwong-wai
Professor,
Deputy Director, Prenatal Genetic Diagnosis Centre
Department of Obstetrics & Gynaecology,
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Outline

- Hearing loss project supported by HMRF
  (Ref No. 01120256; Jan 2014- Dec 2015)
- Introduce genome sequencing technologies and
- How genome sequencing helps research and enhance quality healthcare in Hong Kong

Hearing Loss

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- Definition: partial or complete inability to hear sound in one or both ears*

- Incidence: three per 1000 newborns, one of the most common birth defect.†

- Epidemiological survey‡ :

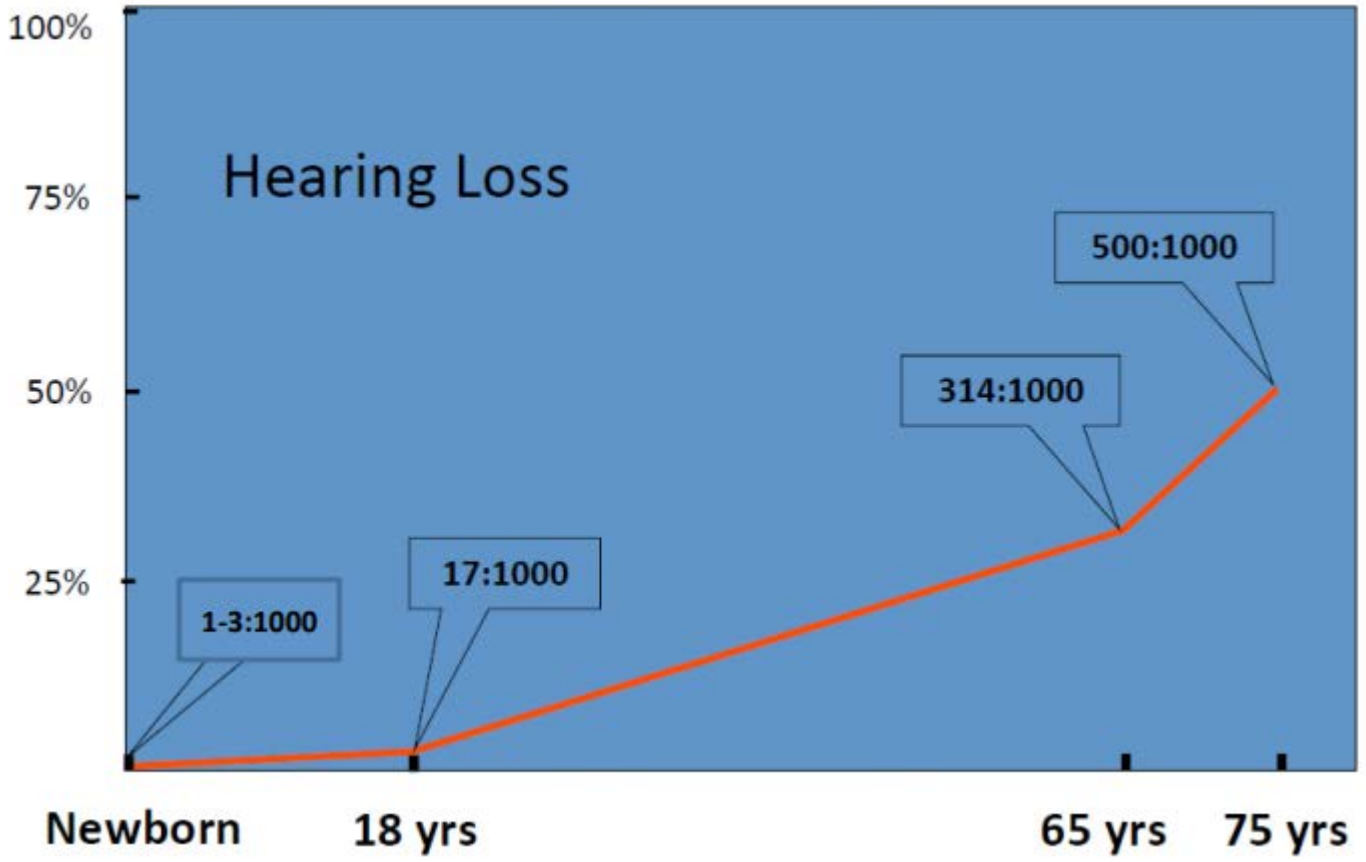
  360 million people worldwide

  20 million in China, 1.5% of total population

* http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0003535/
† Olusanya, B. O., and V. E. Newton, 2007
‡ WHO global estimates on prevalence of hearing loss, 2012.

MORE COMMON than DOWN SYNDROME!!

Prevalence of Hearing Loss Increases With Age



Hearing loss affects children

- Causes delays in speech and language skills

  "s", "sh", "f", "t", "k" and "ed"
- Language deficit results in lower **academic achievement**
- Communication difficulties lead to **social isolation and poor self-concept**
- May have an impact on **vocational choices**

The earlier of intervention, the better performance

"Infants who are diagnosed and received intervention **before six months** of age score 20-40% points higher on the school related measure, e.g. language social adjustment and behavior, compared with hearing-impaired children who receive intervention later on. "

Etiology of Hearing Loss

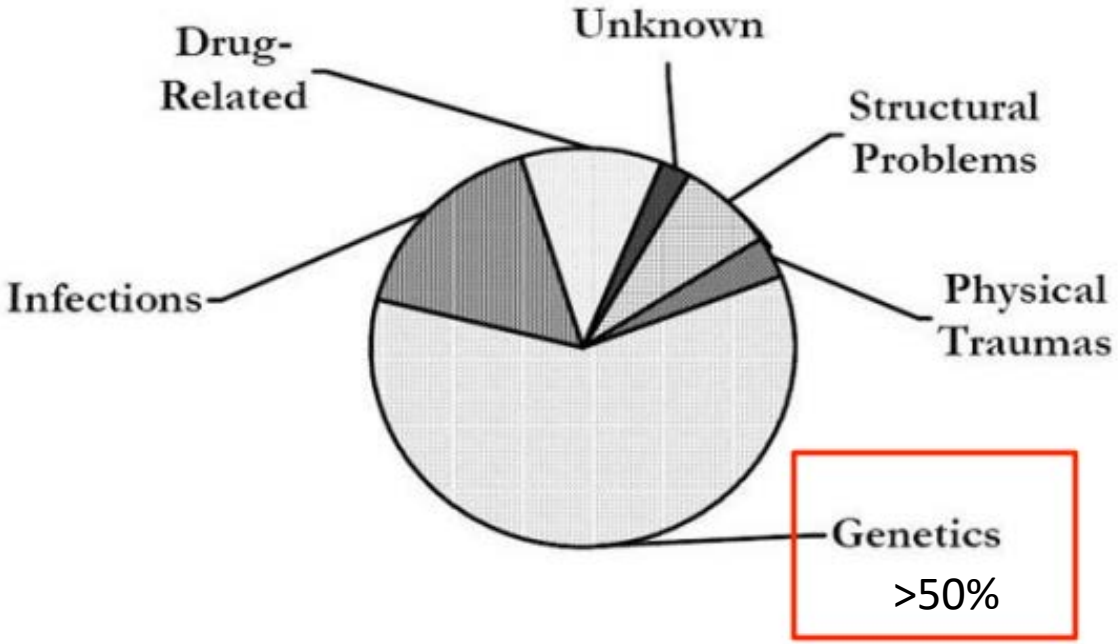
Ototoxic drugs

Antibiotics: aminoglycoside, such as Gentamicin

- patients with specific variants in the mitochondrial genome (mtDNA)

Loop Diuretics: Furosemide

Chemotherapy agents: Cisplatin



https://www.ncbi.nlm.nih.gov/books/NBK1434/

Children with Hearing Impairment: Experience at Child Assessment Service (CAS), Department of Health and in Hong Kong

Lam CC Catherine[1]
[1] Consultant Paediatrician

Figure 1. Protocol and results of universal infant AOAE screening in MCHCs

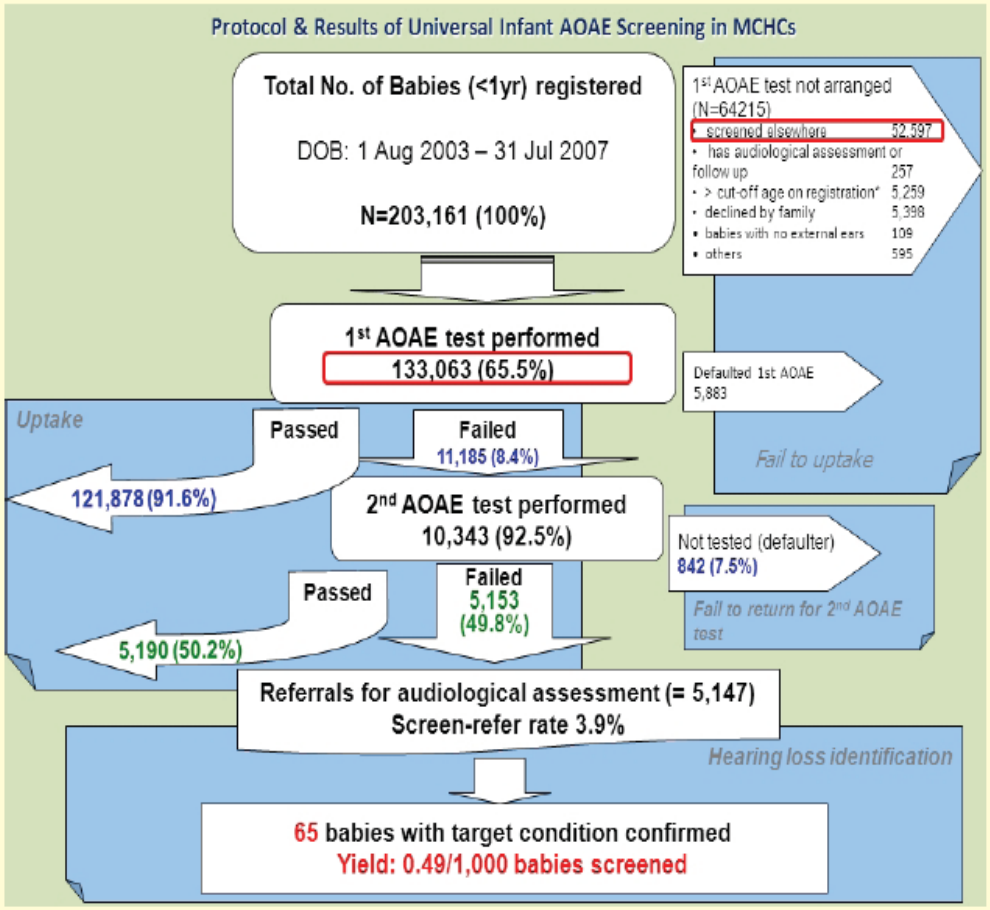
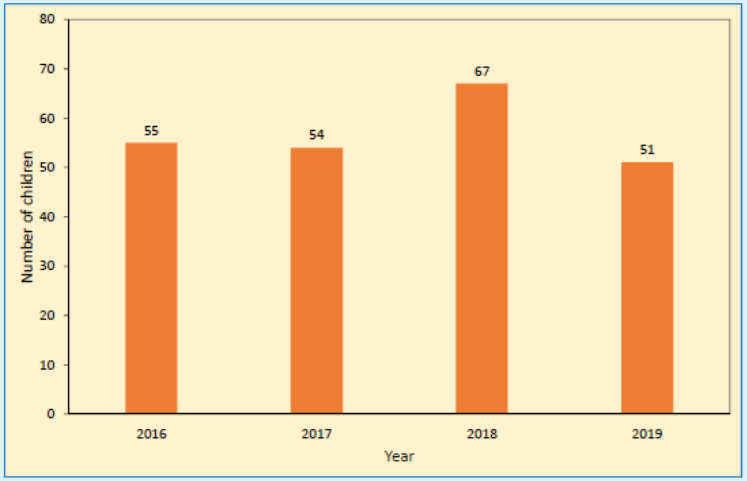


Figure 1. Number of children with significant hearing impairment between 2016 and 2019



Most of the patient was undiagnosed because of the extreme genetic diversity

HEARING LOSS				
Branchio-oto-renal syndrome, type 1	113650	*EYA1*	Deletion / duplication	4 months
Non-syndromic deafness	220290	*GJB2 / GJB6*	point mutation / deletion	**2 months**
	500008	*Mitochondrion*	m.1555A>G point mutation	
Waardenburg syndrome, type 1	193500	*PAX3*	point mutation / deletion	4 months
Deafness, congenital, with inner ear agenesis, microtia, and microdontia	610706	*FGF3*	point mutation	4 months

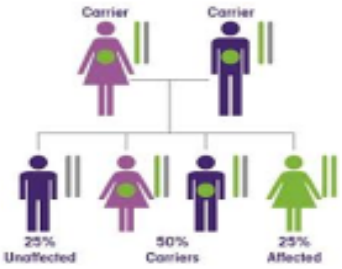
Laboratory Address: 2/F., 2 Kwong Lee Road, Shum Shui Po, Kowloon, Hong Kong SAR, China

DFNB: Deafness nonsyndromic ,(B) autosomal recessive; DFNB1: *GJB2 , GJB2/GJB6*

HMRF funded project challenges current clinical practice paradigms by integrating target sequencing (NGS) into newborn screening for HL, because limited genetic testing is currently performed for newborns with HL, and only ~50% of infants with HL will have an identifiable cause.

Hearing loss genetic diagnosis and carrier screening

- Screening for inherited hearing loss conditions ("gene mistakes")
- Identifies couples at risk of passing on genetic conditions to their children



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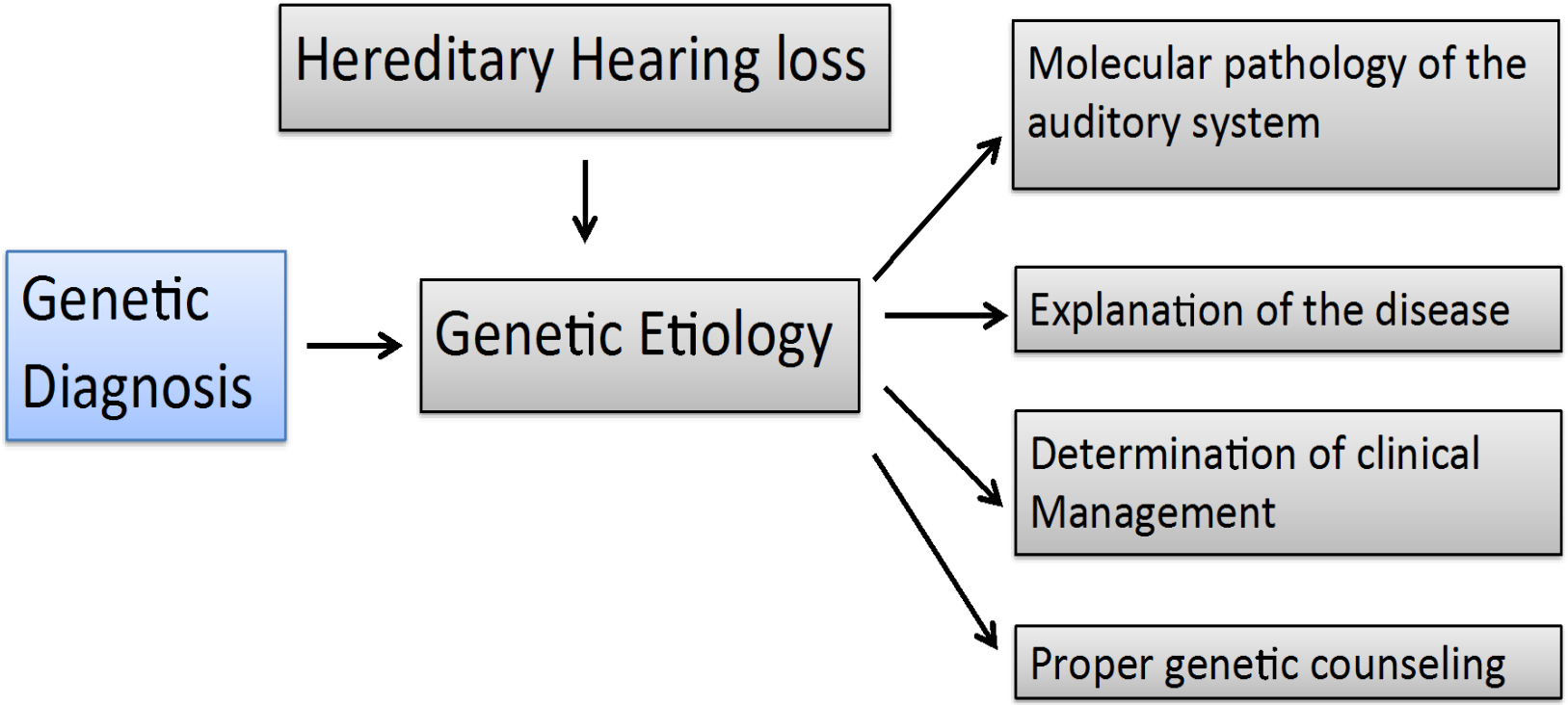
Advantage of early genetics diagnosis:



Main findings

HEALTH AND MEDICAL RESEARCH FUND

Target-enriched massively parallel sequencing for genetic diagnosis of hereditary hearing loss in patients with normal array CGH result

KW Choy*, Y Cao, STS Lam, FM Lo, CC Morton, TY Leung

Hong Kong Med J 2018;24(Suppl 3):S11-4
HMRF project number: 01120256

KEY MESSAGES

1. In our cohort, 15 common hearing-loss mutations with a high carrier frequency (15.9%) were screened; *GJB2* c.109G>A was the most common mutation (10.9%).
2. For patients with hearing loss who were negative for the 15 common mutations, our hearing-loss target capture panel combined with a massively parallel sequencing approach increased detection of pathogenic mutations or likely pathogenic variants by 21%.

Clinical implications

Consensus interpretation in ClinGen Hearing Loss Working Group
p.Met34Thr and p.Val37Ile (c.109G>A) variants in *GJB2* Related Hearing Impairment

Consensus interpretation of the p.Met34Thr and p.Val37Ile variants in *GJB2* by the ClinGen Hearing Loss Expert Panel

Jun Shen, PhD, FACMG[1,2,3], Andrea M. Oza, MS, CGC[3,4], Ignacio del Castillo, PhD[5,6], Hatice Duzkale, MD, PhD[7], Tatsuo Matsunaga, MD, PhD[8], Arti Pandya, MD[9], Hyunseok P. Kang, MD[10], Rebecca Mar-Heyming, PhD[10], Saurav Guha, PhD, FACMG[10,38], Krista Moyer, MS, CGC[10], Christine Lo, MS[10], Margaret Kenna, MD[2,4], John J. Alexander, PhD, FACMG[11,39], Yan Zhang, MD[12], Yoel Hirsch, BS[13], Minjie Luo, PhD, FACMG[14,15], Ye Cao, PhD[16], Kwong Wai Choy, PhD[16], Yen-Fu Cheng, MD, PhD[17,18,19], Karen B. Avraham, PhD[20], Xinhua Hu, PhD[24], Gema Garrido, BS[5,6], Miguel A. Moreno-Pelayo, PhD[5,6], John Greinwald, MD[7], Kejian Zhang, MD, FACMG[7], Yukun Zeng, MD[12], Zippora Brownstein, PhD[20], Lina Basel-Salmon, MD, PhD[20,21,22,23], Bella Davidov, MS[20], Moshe Frydman, MD[20,25], Tzvi Weiden, BS[26], Narasimhan Nagan, PhD, FACMG[27], Alecia Willis, PhD, FACMG[28], Sarah E. Hemphill, BS[3], Andrew R. Grant, BS[3,29], Rebecca K. Siegert, BS[3,29], Marina T. DiStefano, PhD[3], Sami S. Amr, PhD, FACMG[1,2,3], Heidi L. Rehm, PhD, FACMG[1,2,3,29,30] and Ahmad N. Abou Tayoun, PhD, FACMG[31] on behalf of the ClinGen Hearing Loss Working Group

ClinGen
Clinical Genome Resource

Genetics in Medicine (2019) https://doi.org/10.1038/

Conclusion: Resolving controversies in variant classification requires coordinated effort among a panel of international multi-institutional experts to share data, standardize classification guidelines, review evidence, and reach a consensus. We concluded that p.Met34Thr and p.Val37Ile variants in *GJB2* are pathogenic for autosomal recessive nonsyndromic hearing loss with variable expressivity and incomplete penetrance.

Key Impacts

HEALTH AND MEDICAL RESEARCH FUND

Target-enriched massively parallel sequencing for genetic diagnosis of hereditary hearing loss in patients with normal array CGH result

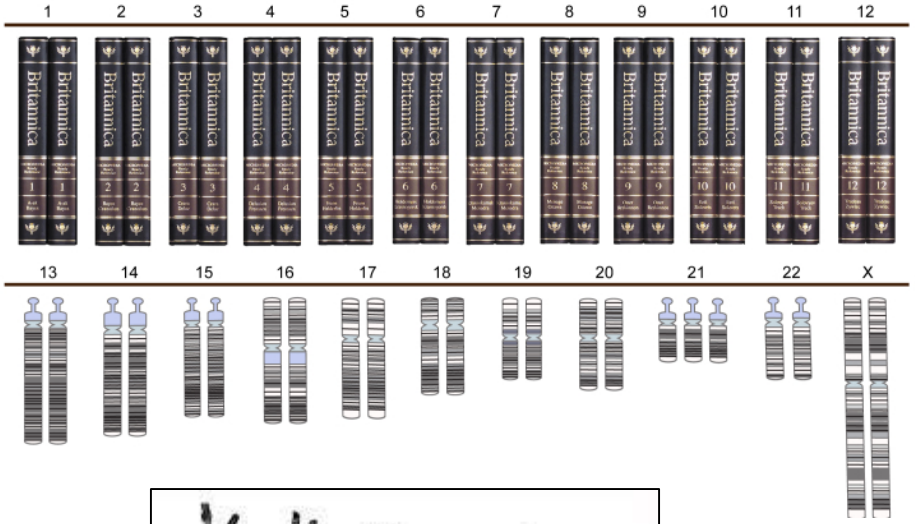
KW Choy*, Y Cao, STS Lam, FM Lo, CC Morton, TY Leung

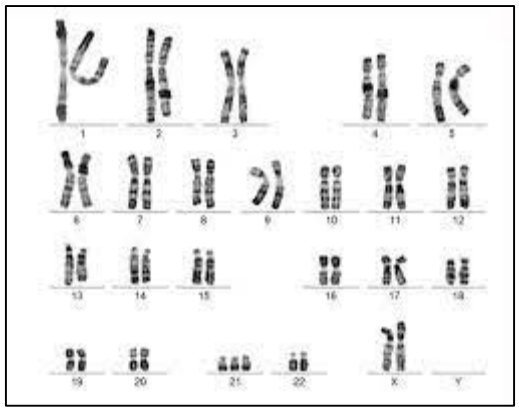
1. *On deaf individual*
   - Enabling diagnosis through data sharing
   - Guiding management for optimal outcome
2. *On family*
   - Counseling for recurrence risk
   - Informing pre-implantation/prenatal diagnosis
3. *On healthcare system*
   - Reducing costs of unnecessary clinical testing
   - Becoming referral center for diagnostic testing
   - Demonstrating value of integrating genomic sequencing into newborn screening

Lessons learned

Requires different methods to study the full spectrum of genome variants

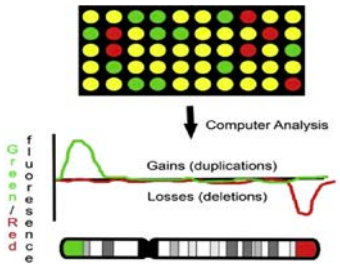
The human genome – a '*diploid encyclopedia*' of the information required to sustain biological life

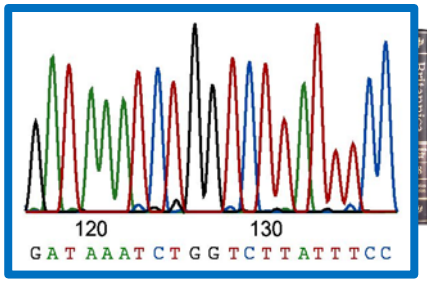




Karyotyping (70`)

chromosomal microarray analysis (CMA)[1]



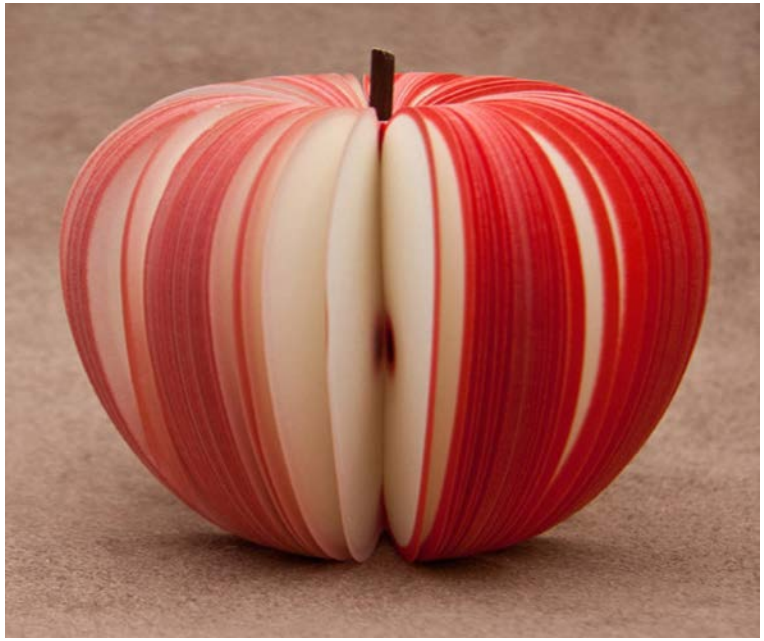


SNV – changes in 'letters of the alphabet ; **CNV** - changes in paragraphs & pages of book
Trisomy 21 associated with Down Syndrome = copy number variation, no mutant gene

Lupski. American Journal of Human Genetics 2019

Genome sequencing vs. CMA (Exome)

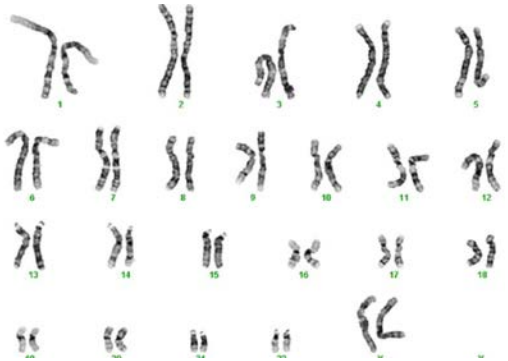
Genome sequencing even coverage down to single bp resolution



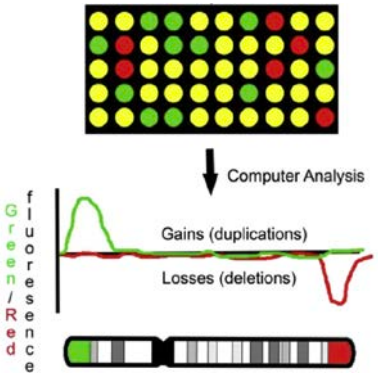
Exome: corresponds 1.5%-2% of the genome
CMA: limited and uneven genome coverage

Superiority of Whole Genome Sequencing (WGS)

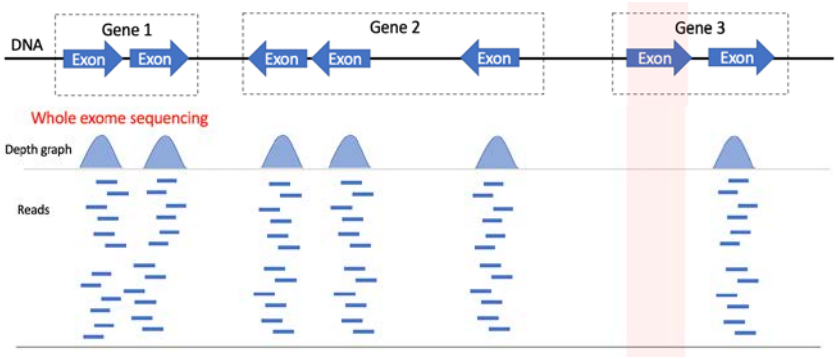
G banded karyotype



chromosomal microarray analysis (CMA)[1]



Next generation sequencing



	Karyotype	CMA	WES
Resolution	5-10Mb	50-100Kb	1bp
Number of loci	~500	~0.05-2 million	~50 million
Variants detected	Variants>5Mb	Copy number variants	SNVs/InDels (mainly) in coding regions
Variants per person	0 or 1	10-100s	~20,000
Diagnostic yield	5-15%	12-20%	20-37%

WGS??

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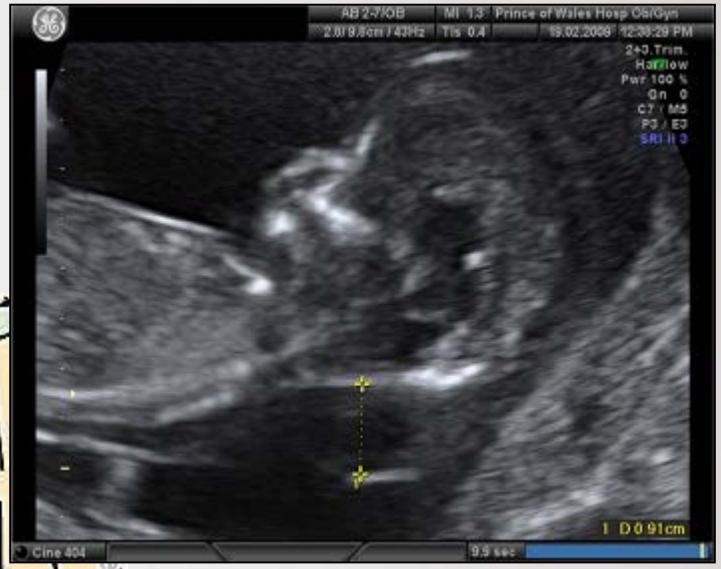
1. SMFM. *et al. Am J Obstet Gynecol*. 2016
2. Wright CF, *et al. Nat Rev Genet*. 2018.
3. Chau & Choy *Curr Opin Obstet Gynecol* 2021

Not uncommon in prenatal diagnosis

11 wk; NT =9.1mm

CVS: Karyotype and CMA = normal

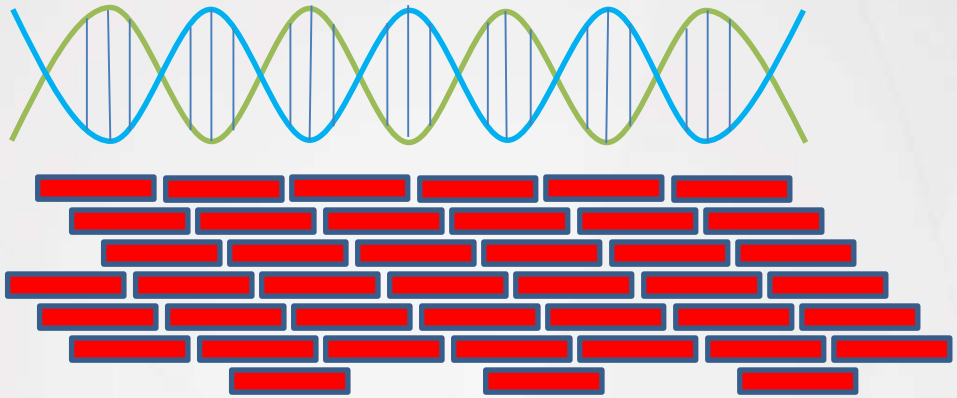


Whole Genome Sequencing (x30)

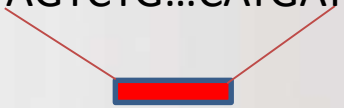
Expensive and many VUS

gene

reads



AGTCTG…CATGAT



Project No. :	04152666
Project Title :	Whole genome sequencing analysis of genetically undiagnosed euploid fetuses with increased nuchal translucency
Principal Applicant (PA) :	Prof Richard CHOY Kwong-wai

Prenatal Diagnosis of Fetuses With Increased Nuchal Translucency by Genome Sequencing Analysis

Kwong Wai Choy[1,2,3†], Huilin Wang[4†], Mengmeng Shi[1†], Jingsi Chen[5†], Zhenjun Yang[1], Rui Zhang[4], Huanchen Yan[5], Yanfang Wang[4], Shaoyun Chen[4], Matthew Hoi Kin Chau[1], Ye Cao[1,6], Olivia Y.M. Chan[1], Yvonne K. Kwok[1], Yuanfang Zhu[4], Min Chen[5], Tak Yeung Leung[1,2,3] and Zirui Dong[1,2,5]*

frontiers in Genetics

ORIGINAL RESEARCH
published: 16 August 2019
doi: 10.3389/fgene.2019.00761

CU Medicine HONG KONG

Genome Sequencing

TABLE 1 | Prenatal detection rates of the fetuses with increased NT by CMA/K

Clinical indications	Number of cases	CMA with/w	GS		*P* value
		Diagnostic yield	Diagnostic yield	95% C.I. (%)#	
Isolated (increased NT with/without other soft markers)	34 (68%)	5/34 (14.7%)	10/34 (29.4%)	15.1–47.5	0.144$
Syndromic (increased NT with other fetal structural malformations)	16 (32%)	3/16 (18.8%)	6/16 (37.5%)	15.2–64.6	0.433*
Overall	50	8/50 (16%)	16/50 (32%)	19.5–46.7	0.061$

#95% confidence interval was calculated by binomial exact calculation.
$Pearson chi-square.
*Fisher's exact test.

Enhancement of Prenatal Diagnosis for Special Cases with the Introduction of Public funded Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS) in 2021

Existing service & current situation

- Publicly funded 1st tier Down screening was introduced in 2010; 2nd tier with Non-invasive prenatal testing (NIPT) in 2019 and Chromosomal Microarray (CMA) for high risk cases in 2019

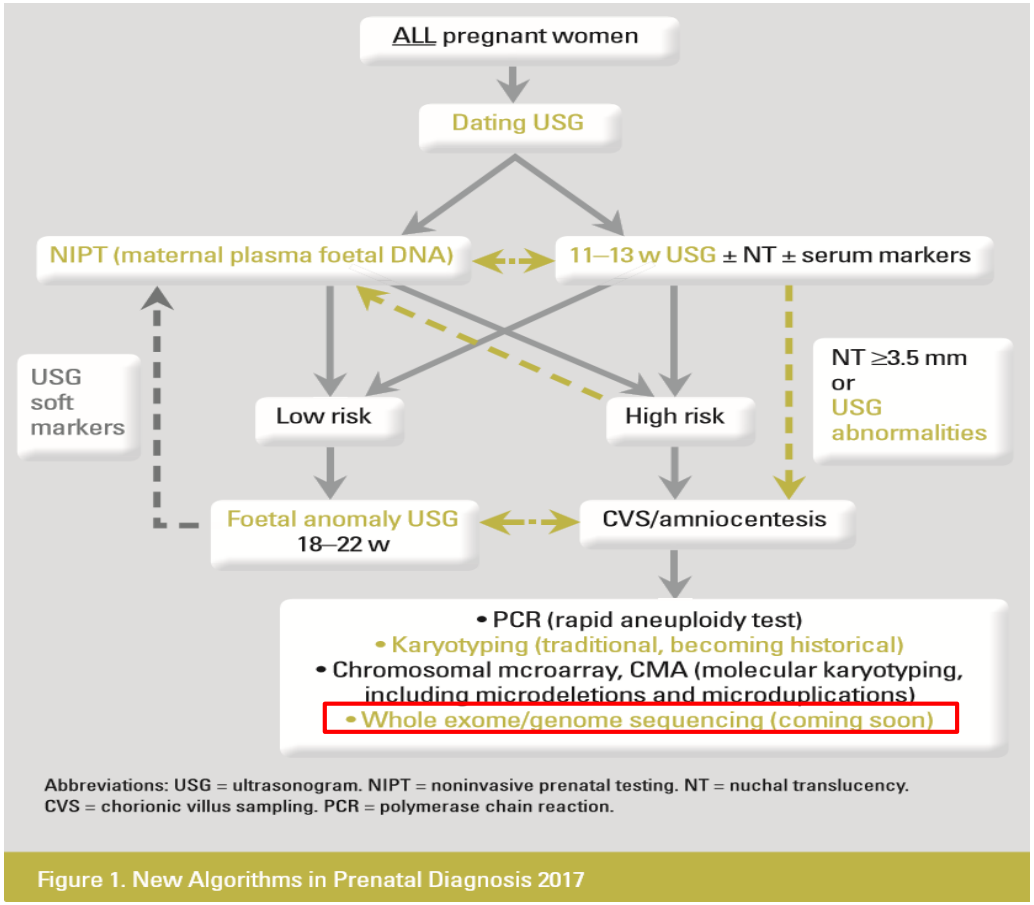


Figure 1. New Algorithms in Prenatal Diagnosis 2017

CC (Genetic Service)

Service gap and size of problem (Jan 2019 to Mar 2020):

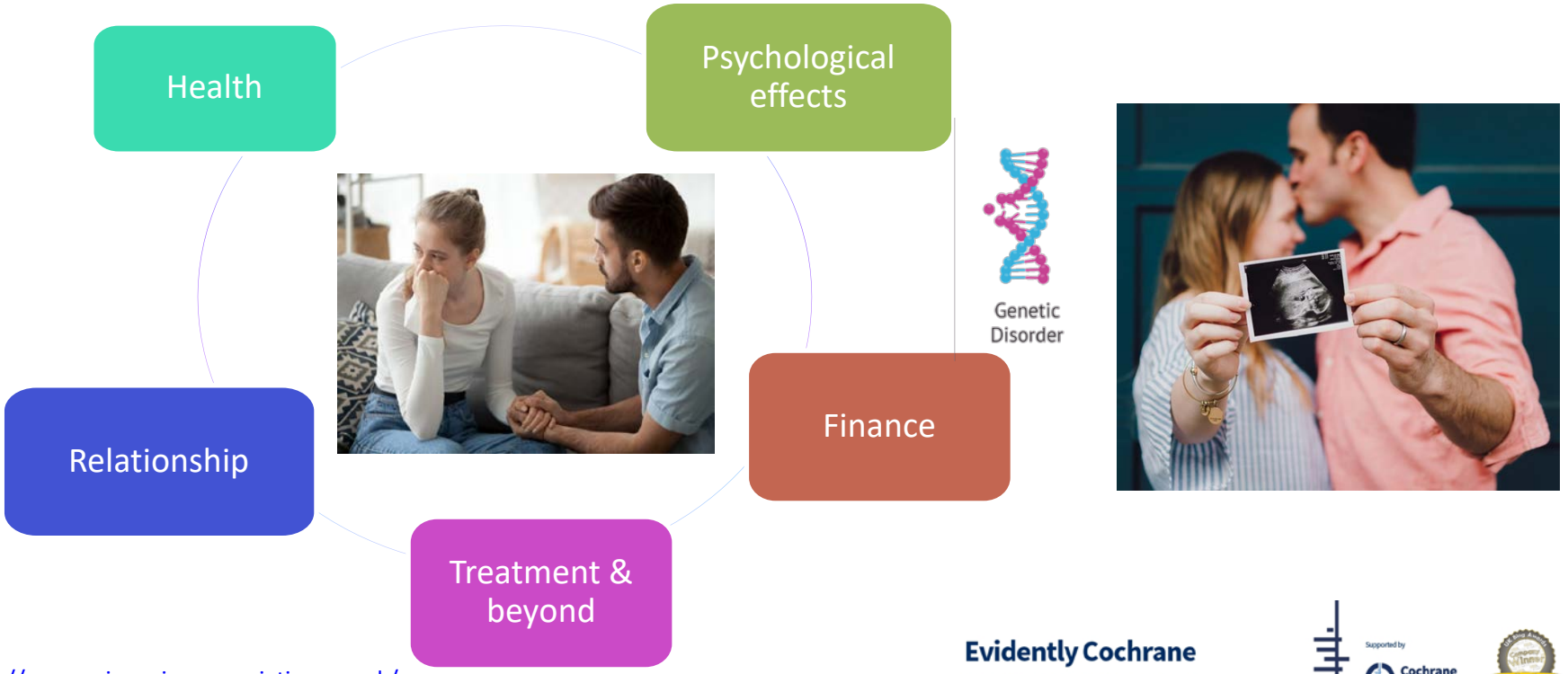
- Special prenatal cases with the presence of ultrasound fetal anomalies, but CVS/amniocentesis → PCR, karyotyping & CMA could not give a diagnosis in >50% of these special cases, which require WES or WGS to further improve prenatal diagnosis
- **Offer special prenatal cases (HA) indicating for WES or WGS per year to start with from 2021/22 for 3 years**

Choy et al. Front Genet. 2019

Couples with recurrent miscarriages are never easy

Definition- ≥ 2 times consecutive miscarriages (<24 weeks)

- Common in Hong Kong, recurrent miscarriage (RM) affects
- approximately **1 in 100** women[1,2](≥3 times) and becomes **1 in 20** (≥2 times)

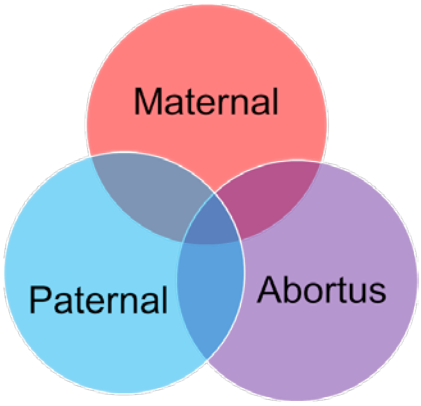


https://www.miscarriageassociation.org.uk/
https://babygest.com/en/recurrent-miscarriage/

Evidently Cochrane
Sharing health evidence you can trust

Supported by Cochrane UK

Genetic factors: most common causes of RM

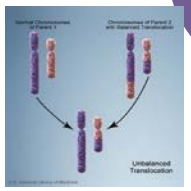


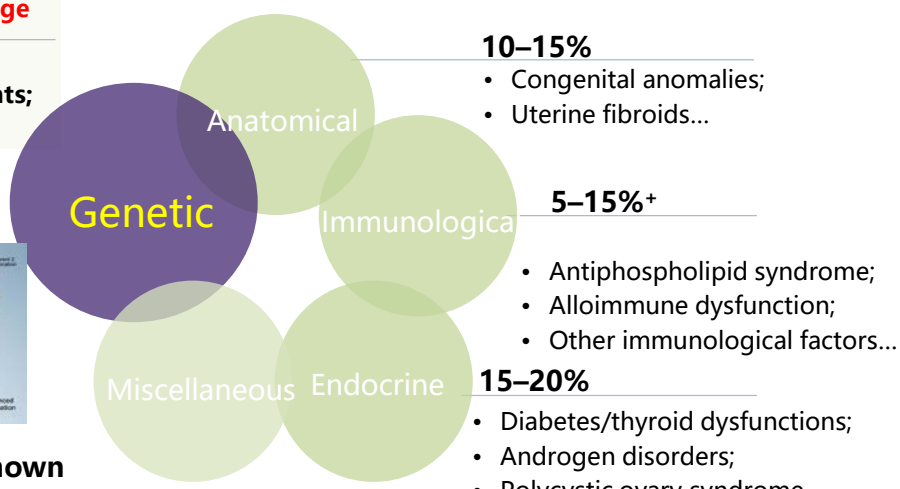
Whose problem?

50–60%

- **The most common cause of early spontaneous miscarriage**
- **~~Fetal aneuploidy;~~**
- **Parental balanced chromosomal rearrangements;**
- **Other genetic disorders…**

Up to 5% of RM is associated with a parental balanced chromosomal rearrangement





10–15%

- Congenital anomalies;
- Uterine fibroids…

5–15%+

- Antiphospholipid syndrome;
- Alloimmune dysfunction;
- Other immunological factors…

15–20%

- Diabetes/thyroid dysfunctions;
- Androgen disorders;
- Polycystic ovary syndrome…

Unknown

- Infectious;
- Environmental/exercise/stress;
- Toxic habits;
- **Other unknown factors**…

Garrido-Gimenez C, et al., 2015;91:151-162.

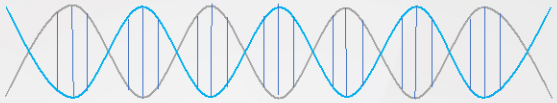
A modified genome sequencing approach

Low-pass GS: cost-effective

High read-depth (30-X)

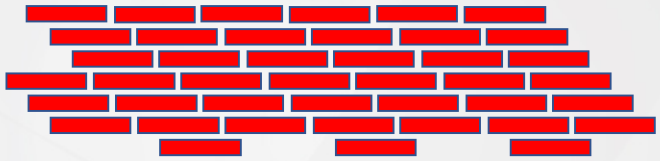
High cost, detects more variants of uncertain significance

Genome



30X

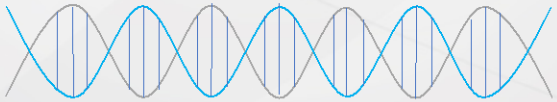
Sequencing data



Low-pass: low-coverage and high-pass

Cost-effective, high throughput

Genome



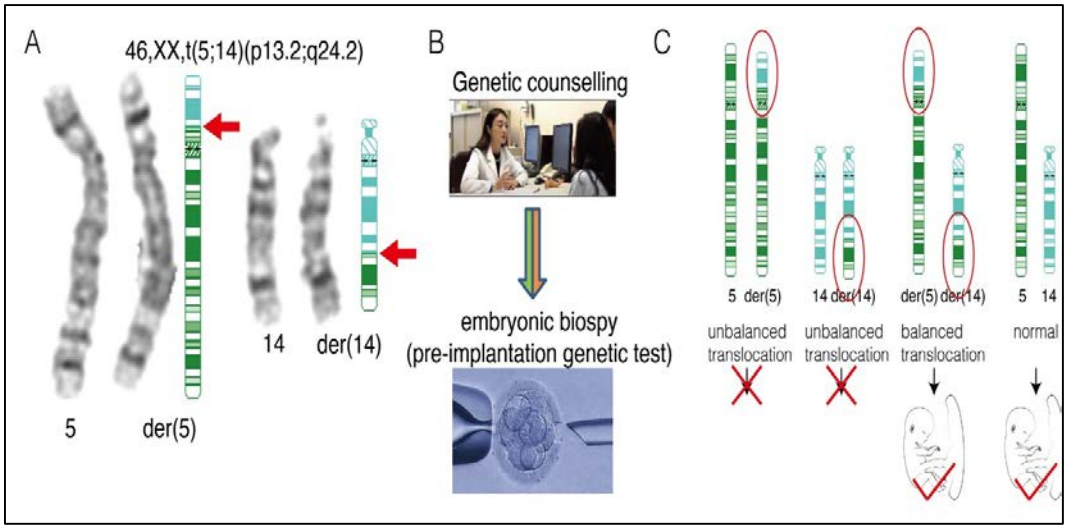
<10X

Sequencing data

Dong et al Human Mutation; Dong et al Genet Med 2016, 2018

Pilot Genome Seqencing data: Recurrent Miscarriage couple

- Low-pass genome sequencing in RM couples
  - : SV & CNV defects common: 1 in 9 couples
- Doubled diagnostic yield to 11.7% (instead of 5%)
- Addressed limitation of current methods (karyotyping)



ARTICLE

Genome Sequencing Explores Complexity of Chromosomal Abnormalities in Recurrent Miscarriage

Zirui Dong,[1,2,3,4,22] Junhao Yan,[1,5,6,22] Fengping Xu,[2,7,8,22] Jianying Yuan,[2,7,22] Hui Jiang,[2,7,22] Huilin Wang,[3,4,9] Haixiao Chen,[2,7] Lei Zhang,[1,5,6] Lingfei Ye,[2,7] Jinjin Xu,[2,7] Yuhua Shi,[1,5,6] Zhenjun Yang,[2,3,7] Ye Cao,[3,4] Lingyun Chen,[2,7] Qiaoling Li,[2,7] Xia Zhao,[2,7] Jiguang Li,[2,7] Ao Chen,[2,7] Wenwei Zhang,[2,7] Hoi Gin Wong,[3,4] Yingying Qin,[1,5,6] Han Zhao,[1,5,6] Yuan Chen,[2,7] Pei Li,[2] Tao Ma,[2,7] Wen-Jing Wang,[2,7] Yvonne K. Kwok,[3,4] Yuan Jiang,[2,10] Amber N. Pursley,[11] Jacqueline P.W. Chung,[3] Yan Hong,[13,14] Karsten Kristiansen,[2,8] Huanming Yang,[2,7,12] Raul E. Piña-Aguilar,[15,16] Tak Yeung Leung,[3,4,17,18] Sau Wai Cheung,[11,17] Cynthia C. Morton,[15,16,19,20,21] Kwong Wai Choy,[3,4,17,18,*] and Zi-Jiang Chen[1,5,6,13,14,18,*]

Potential long-term impact of genome sequencing in reproductive medicine?

126 couples with balanced chromosomal rearrangements identified by *Genome Seq*

Detected by both methods (n=76)

Additional detection by *Genome Seq* (n=50)

IVF+PGT (n=26)

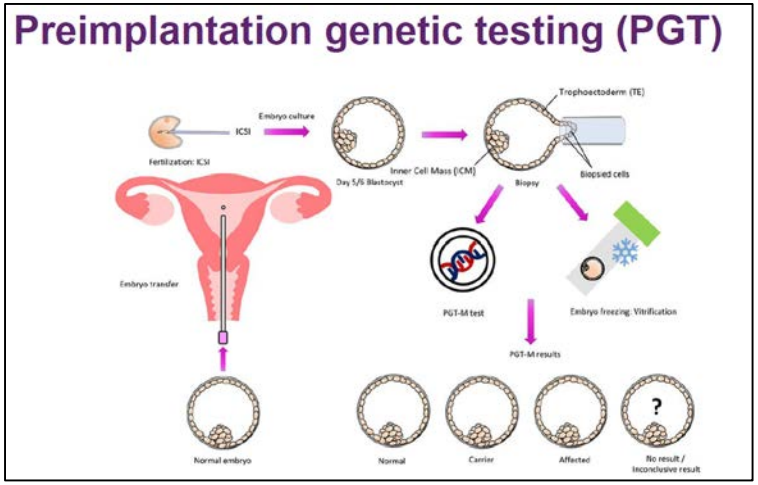
Pregnancy (n=19; 73%)

Miscarriage (n=1; 5.2%)

Natural pregnancy (n=10; 20%)

Miscarriage or fetal anomalies (n=5; 50%)

PGT helps RM couples prevent subsequent miscarriage



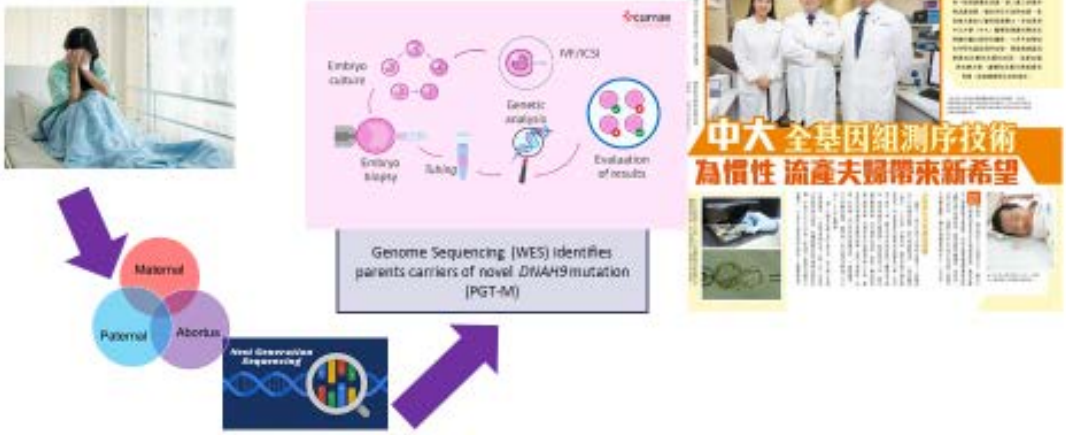
CRF funded infertility study

Home > Funding Opportunities > Collaborative Research Fund > Funded Research > Collaborative Research Fund (CRF) 2021/22

C4062-21GF	Recurrent First Trimester Miscarriage: Genetic Etiology, Diagnosis and Prevention	CUHK / PolyU, HKU	48	8,021,650

Mission of our CRF grant:
To identify the genetic cause of infertility and offer international accredited PGT services to help patients

RM Patient: consecutive miscarriage X3
Normal karyotype and CMA results



Method: low-pass genome sequencing and long-read sequencing
Comprehensive analysis of CNV, SV and AOH in WGS Trios (N=900)

HMRF project summary

Etiology	• Providing a genetic diagnosis for patient with syndromal and non-syndromal hearing loss
Novelty	• Identifying genetic factors including previously unknown SVs, CNVs and SNVs among the abortuses and infertile couples contributory to RM
Translational	• Making GS technology translational into clinical diagnostics locally in Hong Kong and globally
Training and Education	• Training staff for the HK strategic service framework in genetic and genomic services and global needs

Currently, we provide four different DNA-based NGS genetic diagnosis services in Hong Kong

(3) FetalExome

(4) GenomSeq

Dong et al...Choy KW Genet Med. 2018; 2021
Wang et al....Choy KW Genet Med. 2020

香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

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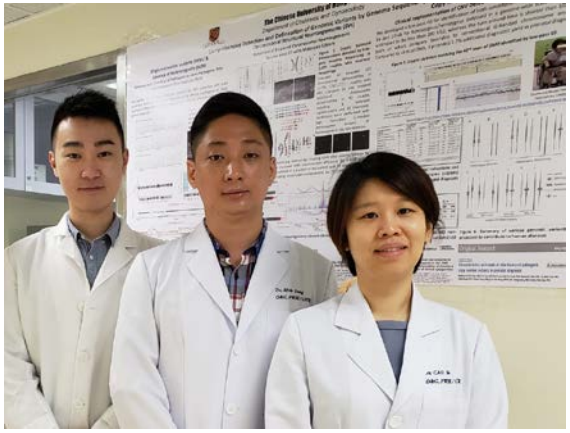

Contact information: richardchoy@cuhk.edu.hk