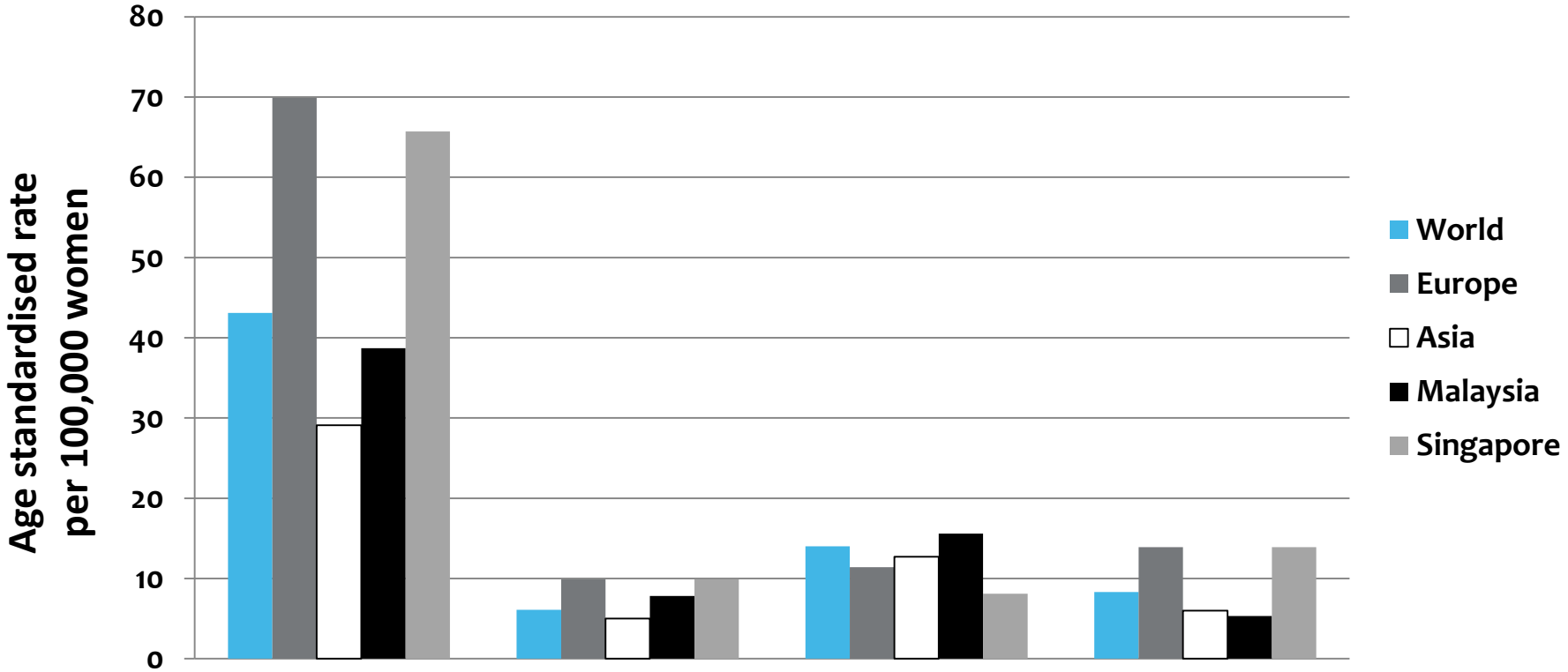
Hi-Plex for High-Throughput Mutation Screening of *BRCA1*, *BRCA2*, *TP53*, and *PALB2* in Breast and Ovarian Cancer Patients

Sean Wen, Lai Kah Nyin, Daniel J. Park, Tu Nguyen-Dumont, Fleur Hammet, Melissa Southey, Woo Yin Ling, Yip Cheng Har, Nur Aishah Mohd Taib, Teo Soo Hwang

Cancer Research Malaysia

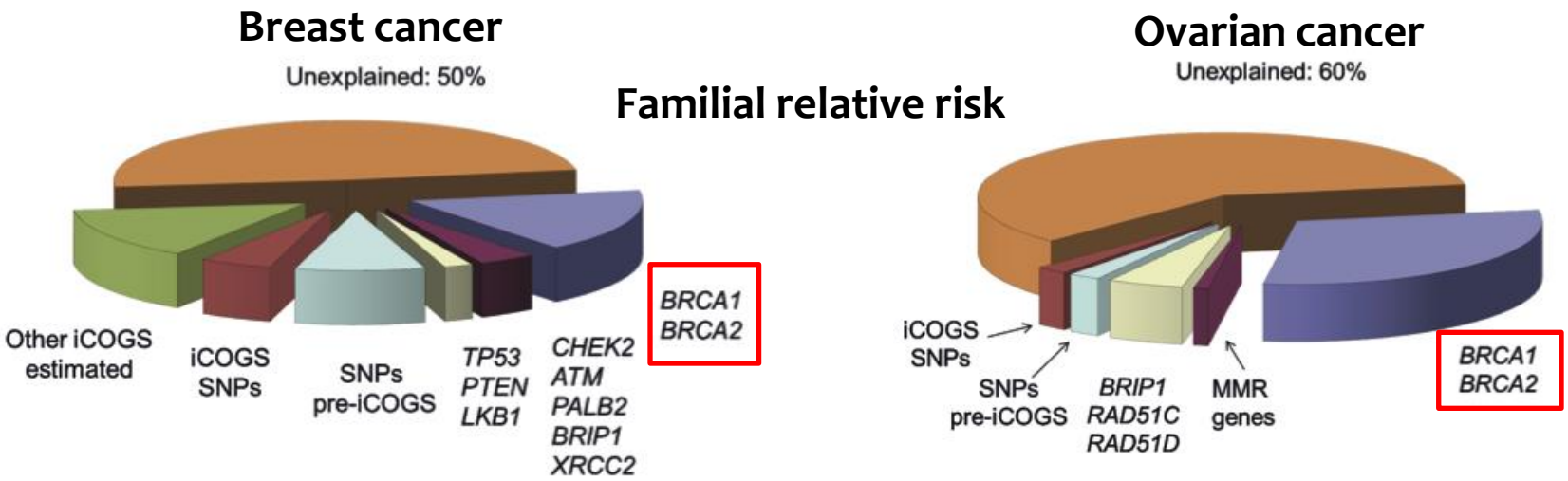
Worldwide distribution of female cancers



Cancer type	Breast	Ovary	Cervix	Endometrium
Incidence	**1,671,149**	238,719	527,624	319,605
Mortality	521,907	151,917	265,672	76,160
M:I	31%	**64%**	50%	24%

Source: GLOBOCAN 2012

Breast and ovarian predisposition genes

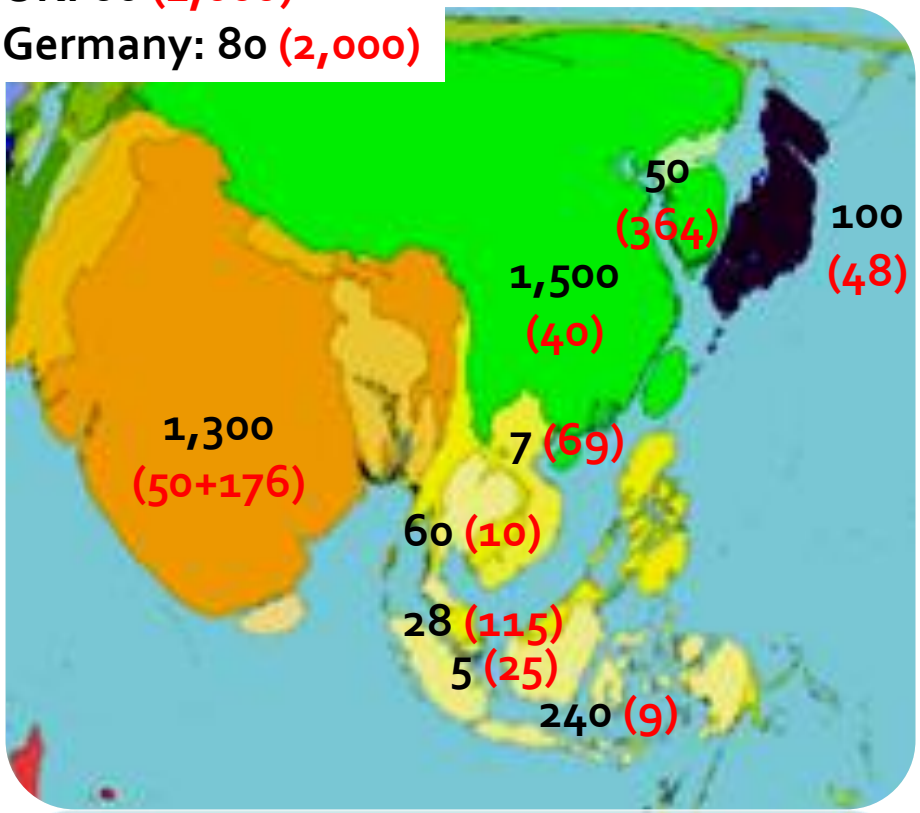


Genes	Breast cancer		Ovarian cancer	
	Risk estimates	Prevalence [1]	Risk estimates	Prevalence [1]
BRCA1	44-75%	1-3%	43-76%	4.9-9.6%
BRCA2	41-70%	1-3%	44-80%	0.9-5.7%
TP53	> 90%	< 1%	?	?
PALB2	26-46%	< 1%	?	< 1%

[1] Prevalence in unselected population-based studies

Gaps in access to genetic testing in Asia

UK: 60 (2,000)
Germany: 80 (2,000)



Population (millions)
Carrier families (number)

[aka 2010]

Challenge

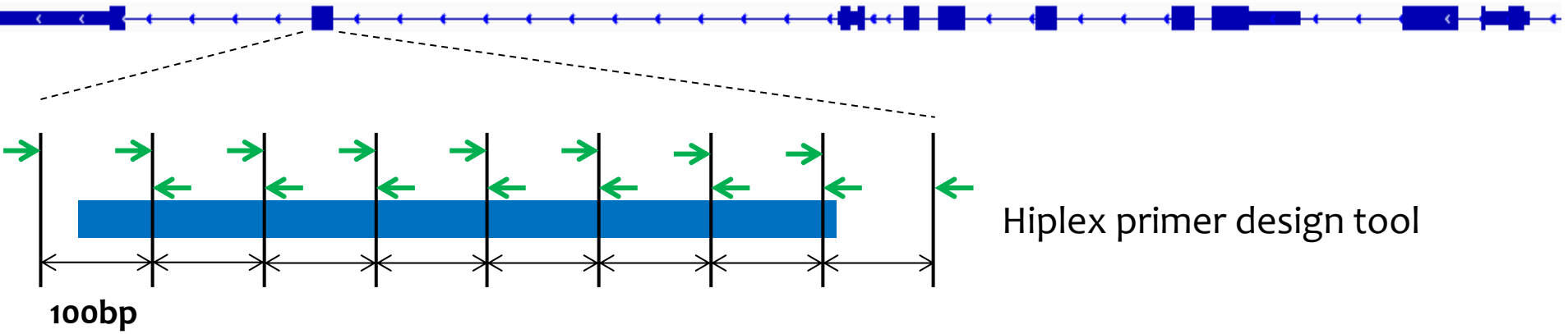
- Severe under-testing in Asia
- <1% of newly diagnosed breast cancer patients receive genetic testing
- Main reason: Cost

Nakamura, Public Health Genomics, 2016

Our approach

To develop a high-throughput and cost-efficient genetic testing method to increase affordability and accessibility

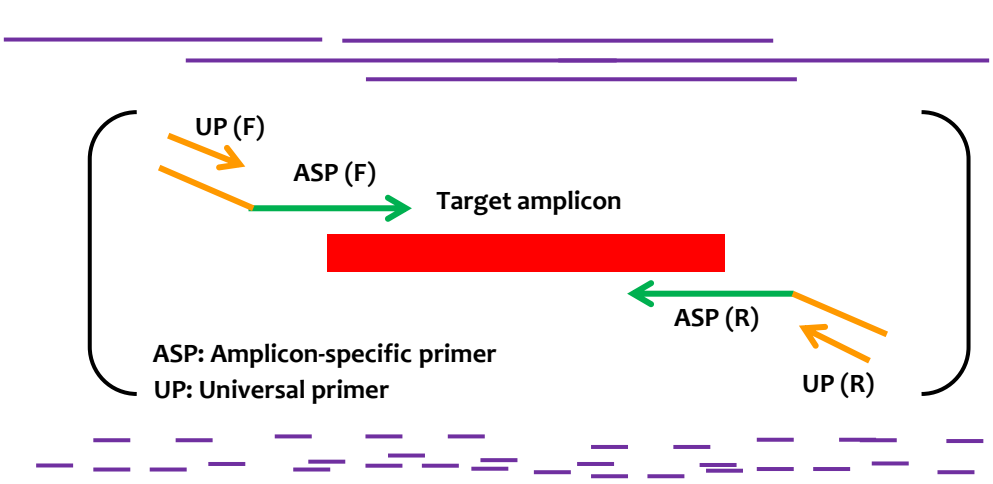
Hi-Plex work flow

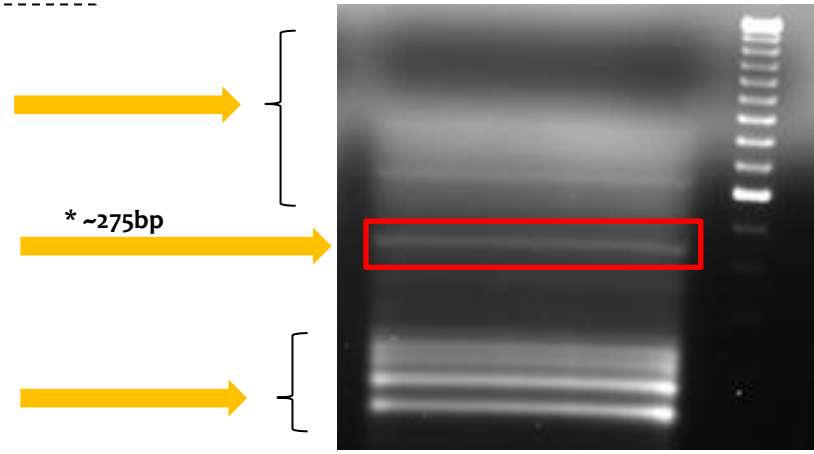


Hiplex primer design tool

259 amplicons to cover *BRCA1*, *BRCA2*, *TP53* , *PALB2*

Step-wise annealing temperature of 55,60,65,70 °C





Nguyen-Dumont, Biotechniques, 2015

Bioinformatics workflow

- Sequencing platform: MiSeq
- Reads alignment: Bowtie2
- Variant calling: ROVER
  - Variant present on both read-pairs
  - Variant present in at least 2 read-pairs
  - Variant present at least 15% of all read-pairs
- Variant annotation: ANNOVAR
- Variant prioritisation
  - Exclude variants with MAF >1%
    - dbSNP138
    - 1000genome
    - ESP6500
    - Japanese cohort
  - Exclude variants on intronic sites
- Candidate variants

Langmead, Nat Methods, 2012
Pope, Source Code Biol Med, 2014
Wang, Nucleic Acid Res, 2010
Nagasaki, Nat Commun, 2015

Panel evaluation: Sensitivity assessment

Genes	Region covered (bp)	Variants tested	Variant type						Variants detected
			Deletion			Insertion		SNV	
			1-3bp	4-6bp	7-9bp	1-3bp	4-6bp		
BRCA1	7,100	83	19	-	-	4	-	60	83
BRCA2	11,900	121	13	8	1	6	-	93	120
TP53	2,000	7	1	-	-	-	1	5	7
PALB2	4,900	5	2	2	-	1	-	-	5
Total	**25,900**	**216**	**35**	**10**	**1**	**11**	**1**	**158**	**215**
		176 patients							

- 215 of 216 variants successfully detected
- Sensitivity of 99.5%

Panel evaluation: Specificity assessment

- 3,209 variants detected by our panel
- Filtered away polymorphisms (n=2,717), intronic variants (n=265), variants on homopolymer region (n=1)
- 215 variants previously reported + 11 rare coding variants previously not reported

No.	Gene	Genomic change	Nucleotide change	Amino acid change	Reconfirmed to be true?
1	*BRCA1*	chr17:g.41245675G>A	c.1873C>T	p.L625L	Yes
2	*BRCA1*	chr17:g.41245465C>T	c.2083G>A	p.D695N	Yes
3	*BRCA1*	chr17:g.41251820A>T	c.519T>A	p.P173P	Yes
4	*BRCA2*	chr13:g.32912750G>T	c.4258G>T	p.D1420Y	Yes
5	*BRCA2*	chr13:g.32913919C>T	c.5427C>T	p.C1809C	Yes
6	*BRCA2*	chr13:g.32968854C>T	c.9285C>T	p.D3095D	Yes
7	*BRCA2*	chr13:g.32953550G>A	c.8851G>A	p.A2951T	Yes
8	*BRCA2*	chr13:g.32914277A>G	c.5785A>G	p.I1929V	Yes
9	*BRCA2*	chr13:g.32972626A>T	c.9976A>T	p.K3326*	Yes
10	*BRCA2*	chr13:g.32913919C>T	c.5427C>T	p.C1809C	Yes
11	*PALB2*	chr16:g.23647121G>A	c.746C>T	p.P249L	Yes

Panel application: Breast & ovarian cancer patients

Genes	Breast cancer (N = 438) [1]			Ovarian cancer (N = 286) [2]		
	Deleterious	VUS [3]	Non-carriers	Deleterious	VUS [3]	Non-carriers
BRCA1	12 (2.7%)	4 (0.9%)	422 (96.3%)	19 (6.6%)	4 (1.4%)	263 (92.0%)
BRCA2	16 (3.7%)	16 (3.7%)	406 (92.7%)	12 (4.2%)	3 (1.0%)	271 (94.8%)
TP53	2 (0.5%)	1 (0.2%)	435 (99.3%)	-	-	286 (100%)
PALB2	3 (0.7%)	3 (0.7%)	432 (98.6%)	1 (0.3%)	2 (0.7%)	283 (99.0%)
Total	**33 (7.5%)**	**24 (5.5%)**	**381 (87.0%)**	**32 (11.2%)**	**9 (3.1%)**	**245 (85.7%)**

[1] High risk, hospital-based cohort
[2] Unselected, hospital-based cohort
[3] Non-C0 missense and inframe indels

Conclusion

- We developed a high-throughput and cost-efficient genetic testing panel for four clinically relevant breast and ovarian cancer genes
  - Sensitivity of **>99%**
  - Specificity of **>99%**

- Application of panel on high risk breast patients
  - **7.5%** of patients are carriers of these genes
  - *BRCA1/2* carriers were more likely to have younger age at diagnosis, have family history of breast cancers, and have triple-negative breast cancers
  - **2** *TP53* carriers identified have no known family history of Li-Fraumeni Syndrome cancers but were early-onset (<35yo)
  - **3** *PALB2* carriers identified have family history of breast cancer

- Application of panel on unselected ovarian cancer patients (Hasmad, Gynecol Oncol, 2016)
  - **10.8% of** patients are carriers of *BRCA1* and *BRCA2*
  - Mutation carriers were more likely to be Indian, have serous ovarian cancer, and have more relatives with breast or ovarian cancer
  - **42%** of mutation carriers did not have any family history of breast or ovarian cancer
  - Offering genetic counselling and genetic testing only to women with family history would mean that **35%** of *BRCA1* mutation carriers and **57%** of *BRCA2* mutation carriers would not be offered genetic testing
  - Emphasis on genetic screening on all unselected ovarian cancer patients

Acknowledgment

Core Team

Lai Kah Nyin
Joanna Lim
Teo Soo Hwang

Familial Team

Lee Sheau Yee
Tiara Hassan
Daphne Lee
Caziena Krishnan
Yoon Sook Yee

Breast Cancer Team

Nadia Rajaram
Kwan Pui Yoke
Jaslyn Soo Sian Siu
Shivaani Mariapun
Siti Norhidayu Hasan
Patsy Ng Pei Sze

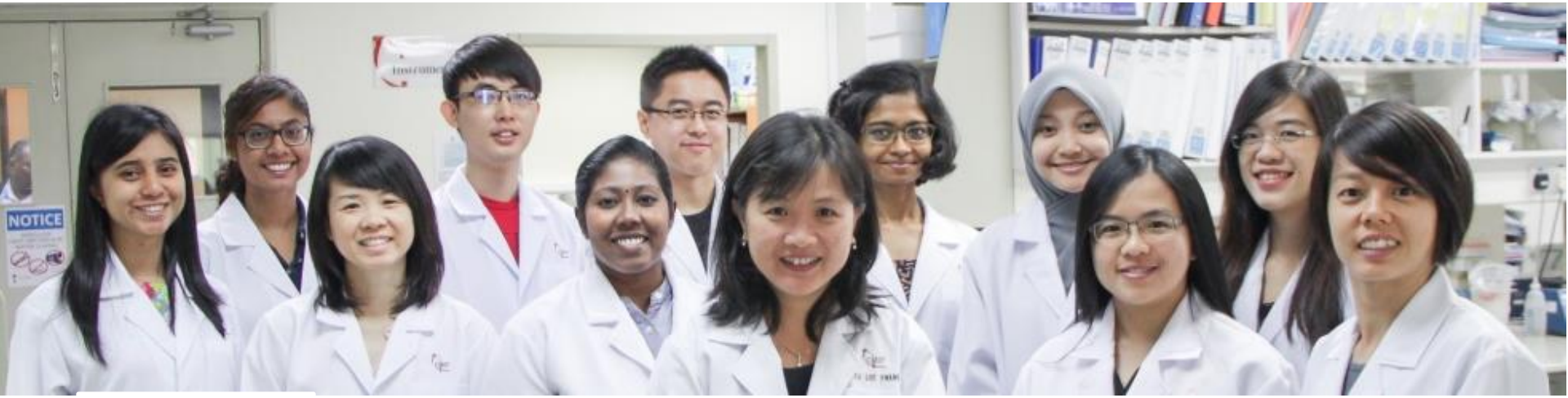
Clinicians

Nur Aishah Mohd Taib
Yip Cheng Har
Woo Yin Ling

Collaborators

Daniel J Park
Tu Nguyen-Dumont
Fleur Hammet

and many, MANY more individuals...



Thank you 고맙습니다