Genetic analysis for pathogenesis of type 2 diabetes and drug response in Thais.

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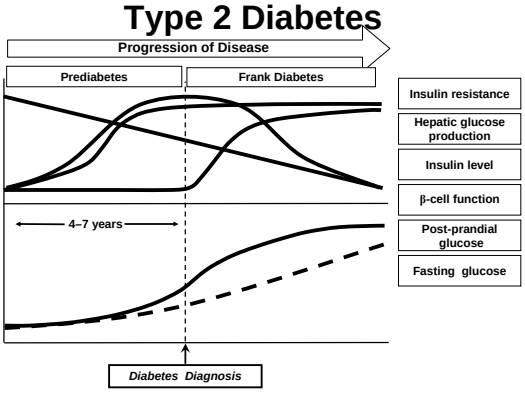
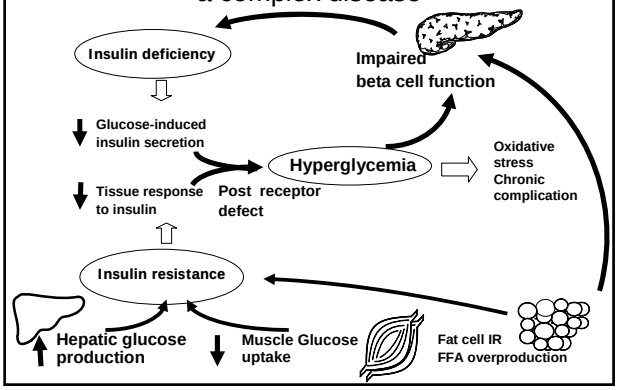
Development and Progression of Type 2 Diabetes

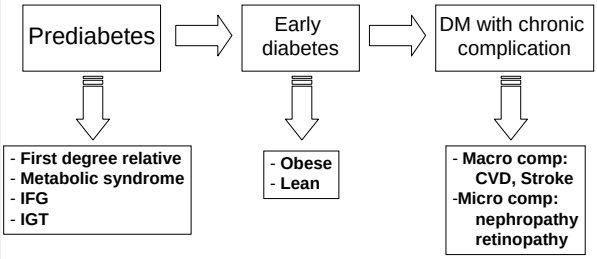


Pathophysiology of type 2 diabetes

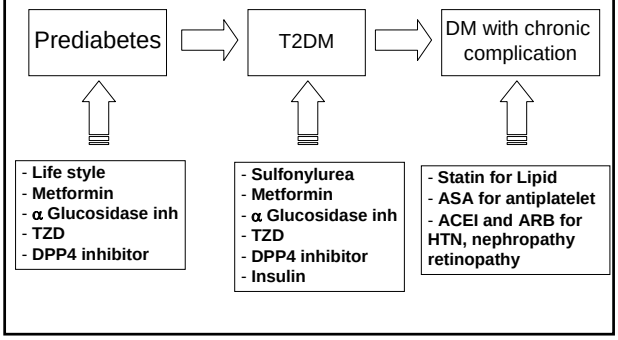
a complex disease



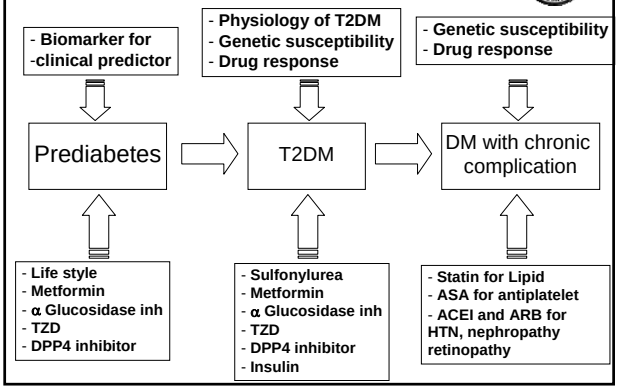
Subgroup Population According to Natural course of type 2 diabetes



Medication prescribed for T2D patients



Studies of Our Interest

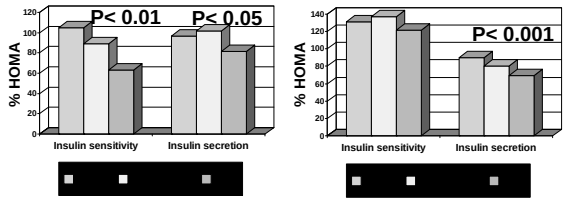


Current Studies of T2DM
Ramathibodi Hospital, Mahidol University

- Pathophysiology of T2DM in Thai.
- Genetic susceptibility of type 2 diabetes.
- Clinical predictor and pharmacogenetic study of drug response and ADR.

Pathophysiology of type 2 diabetes in Thais

- Rationale:
  - Previous studies from Asia especially Japan showed that insulin secretion defect is the main defect of T2DM in Asians.
  - How about Thai?



Obese
BMI ≥ 25 kg/m^2

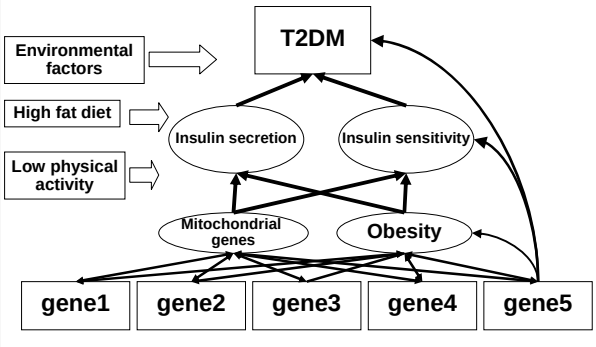
Lean
BMI < 25 kg/m^2

Insulin secretion defect is the main pathogenesis of nonobese T2DM meanwhile both insulin sensitivity and insulin secretion defects play important role for obese T2DM

Genetic susceptibility of T2DM

- Subjects: ~300 T2DM vs ~ 400 normal control by OGTT criteria
- Methods: genotyping at Research center, Ramathibodi Hosp.
  - Candidate genes association study
    select genes of interest
  - Whole genome association analysis
    perform whole genome genetic markers scan

Hypothesis



Previous reported genes relating to T2DM

- By candidate gene approaches

Gene	Common name	Polymorphisms	Population with evidence for association	Reference supporting association	Population no evidence of association	Reference not supporting association
NOS3	eNOS	(–)786T>C, D298, IVS18 + 27C	Japanese, Caucasian–Italian	[225, 226]		
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit p85α	Met326Ile	Pima	[227]	Japanese, Caucasian–Danish	[228, 217]
PON2	Paraoxonase 2	Ala148Gly	Oji–Cree	[229]	Scandinavian, Saguenay–Lac-Saint-Jean region of Quebec	[143]
PPARG	Peroxisome proliferator activated receptor, γ	Pro12Ala	Scandinavian, Saguenay-Lac-Saint-Jean region of Quebec, Japanese-American; meta-analysis	[145, 230, 231]	Caucasian–UK; French; Italian	[151, 232–235]
PPARGC1	PGC1; peroxisome proliferator activated receptor, γ, co-activator 1	Gly482Ser and other SNPs	Caucasian-Danish, Japanese	[236, 237]	Caucasian-French, Pima	[238, 239]
PPP1R3A	Protein phosphatase 1, regulatory (inhibitor) subunit 3A	Arg905Lys, 3′-UTR indel and haltrap	Pima, Caucasian-Danish, UK	[68, 151, 240, 241]	Oji–Cree; Caucasian–Swedish, Japanese	[242, 243]
RRAD	Ras-related associated with diabetes	Tetranucleotide repeat	Caucasian-American	[244]		[245]
SLC2A2	GLUT2 glucose transporter 2	The 1101le and other polymorphism	Caucasian-UK	[151]	Pima, West Indian, Caucasian–Danish, Caucasian–Italian	[246–250]
TNF	Tumour necrosis factor α	–238 A/G	North European	[251]	Scandinavian, Saguenay Lac-Saint-Jean region of Quebec	[145]
UCP2	Uncoupling protein 2	–866 G/A and A55V	Caucasian-French	[233]	Caucasian–Danish, Japanese	[234, 235]
UCP3	Uncoupling protein 3	–55 C/T	Caucasian-French, Chinese	[252]		

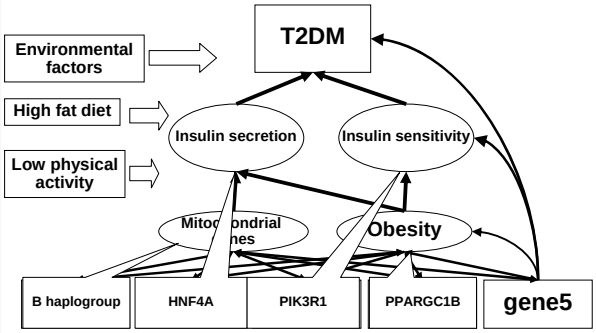
Only associations with T2D are reported; no associations with insulin resistance or other diabetes-related quantitative traits are included in this table.

Gene	Common name	Polymorphisms	Population with evidence for association	Reference supporting association	Population no evidence of association	Reference not supporting association
ABCC8	SUR1	C/T intron 22), Ser1370Ala and other polymorphisms	Caucasian-Danish; Caucasian-Finnish; Caucasian-UK; Caucasian-Northern European; Caucasian-Scandinavian	[145, 151, 166–171]	Caucasian-Danish; Japanese	[156, 172]
ADRB3	β3-adrenergic receptor	Trp64Arg	Japanese	[173]	Scandinavian; Caucasian-Finnish; Japanese	[145, 174–176]
ADRB2	β2-adrenergic receptor	Gln27Glu	Japanese	[177]	Scandinavian	[145]
APM1	Adiponectin	SNP45 and SNP276	Japanese; Caucasian-French	[178, 179]		
CAPN10	Calpain 10	SNP43, SNP44), haplotype effects and other polymorphisms	Mexican-American; African-Americans; Japanese; meta-analysis	[96, 104, 106, 180, 236]	Samoans, Japanese, Pima, Oji-Cree, Caucasian-UK; meta-analysis	[98–102, 103, 107, 181]
ENPP1	PC-1	Lys121Gln and haplotype effects			Oji-Cree; Caucasian-Danish; Swedish; Finnish; Caucasian-UK	[99, 151, 182, 183]
FABP2	Liver fatty acid binding protein	Ala54Thr	Pima Indians; Guadeloupe	[184, 185]	Scandinavian; African-American	[145, 186]
GC	DBP; vitamin D binding protein	Asp416Glu, Thr420Lys	Japanese	[187, 188]	Caucasian-American, French	[189–191]
GCGR	Glucagon receptor	Gly40Ser	Caucasian-French and Sardinian families	[192, 193]	Japanese; Finland; Sardinian; Russian	[194–197]
GYS1	Glycogen synthase	Met416Val	Finnish, French and Pima	[198–202]	Japanese, Finland; Scandinavian	[145, 203, 204]
HNF4A	Hepatocyte nuclear factor 4α	Thr130Ile, Val255Met; promoter SNPs, haplotype	Japanese; Caucasian-UK; Finish; Ashkenazim	[151–154]	Caucasian-Danish, Caucasian-Swedish, Caucasian-Finnish	[125, 205–207]
IAPP	Insulin amyloid protein	Ser20Gly	Japanese	[208]	Scandinavian	[145]
IGF1	Insulin growth factor 1	CA microsatellite—1 kb 5′ from IGF1 start	Caucasian-Dutch	[209]	Caucasian-UK	[210]
INS	Insulin	−23A/T (HphI); VNTR	Caucasian-UK	[151, 157]	Scandinavian	[145]
INSR	Insulin receptor	Val985Met, and other polymorphisms	Caucasian-Netherlands, Caucasian-UK	[151, 211, 212]	Caucasian-Welsh, Danish	[213–215]
IRS1	Insulin receptor substrate 1	Gly Arg972; Ala 513Pro	Caucasian-Netherlands; meta-analysis	[155, 212, 216, 217]	Scandinavian	[145]
IRS2	Insulin receptor substrate 2	Gly1057Asp	Overweight Caucasian-Italian; Pima Indians	[218, 219]	Finnish; Chinese; Italian	[220, 221]
KCNJ11	KIR6.2	Glu23Lys	Caucasian-French, UK, Danish	[146–151, 222]	Caucasian, Scandinavian	[145, 223, 224]

Genes which have been studied in our institute and results

	Relate to DM	Relate to clinical feature	Relate to intermediate phenotypes	
			insulin secretion	insulin sensitivity
HNF4A	0.67	NS	0.007	NS
PHF15	0.005	NS	NS	0.019
Mitrochondrial haplogroup B	0.017	NS	NA	NA
PDK1	NS	NS	NS	NS
PIK3R1	NS	NS	NS	P = 0.008 with adiponectin
PPARGC1B	NS	BMI p<0.01 Waist, p=0.03	NS	NS

Hypothesis



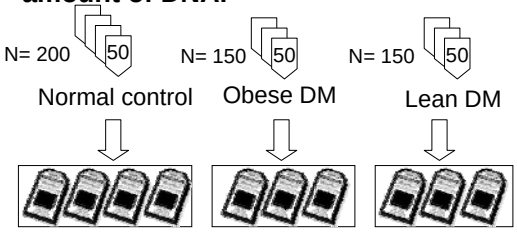
Previous reported genes relating to T2DM

By whole genome scan approach (n=32554)

	FUSION		DGI		WTCCC/UKT2D		All Samples	
Gene	OR	p-value	OR	p-value	OR	p-value	OR	p-value
TCF7L2	1.34	1.3×10^{-8}	1.38	2.3×10^{-21}	1.37	6.7×10^{-13}	1.37	1.0×10^{-40}
IGF2BP2	1.18	2.1×10^{-4}	1.17	1.7×10^{-7}	1.11	1.6×10^{-4}	1.14	8.9×10^{-16}
CDKN2A/B	1.20	.0022	1.20	5.4×10^{-6}	1.19	4.9×10^{-7}	1.20	7.8×10^{-15}
FTO	1.11	0.016	1.03	0.25	1.23	7.3×10^{-14}	1.17	1.3×10^{-12}
CDKAL1	1.12	0.0095	1.08	0.0024	1.16	1.3×10^{-5}	1.12	4.1×10^{-11}
KCNJ11	1.11	0.013	1.15	1.0×10^{-7}	1.15	0.0013	1.14	6.7×10^{-11}
HHEX	1.10	0.026	1.14	1.7×10^{-4}	1.13	4.6×10^{-4}	1.13	5.7×10^{-10}
SLC30A8	1.18	7.0×10^{-5}	1.07	0.047	1.12	7.0×10^{-4}	1.12	5.3×10^{-8}
Chr 11	1.48	5.7×10^{-5}	1.16	0.12	1.13	0.068	1.23	4.5×10^{-7}
PPARG	1.20	0.0014	1.09	0.019	1.23	0.0013	1.14	1.7×10^{-4}

Our strategies of pooled DNA microarray SNP genotyping study

- Genotyping one marker at a time, of large numbers of samples by **pooling equal amount of DNA.**



Affymetrix GeneChip 10K microarray

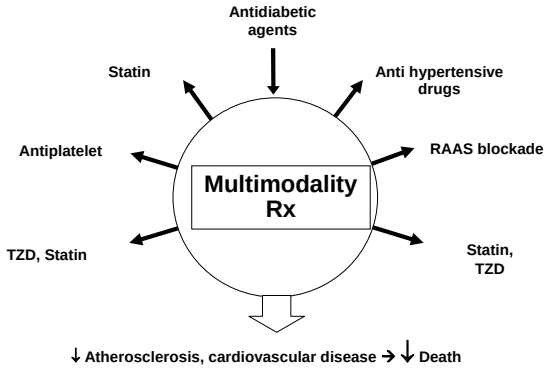
ID	k	Average DM_con	Avg freq DM	Avg freq con	P value
SNP_A-1518521	0.414065	0.152345401	0.46712561	0.27755485	8.7349E-05
SNP_A-1518574	0.277495	0.19148986	0.68331683	0.49625613	0.000100036
SNP_A-1514639	0.4045275	0.151524299	0.8195553	0.65877149	0.000135619
SNP_A-1512095	0.440626667	0.169945081	0.38122773	0.20662063	0.000137803
SNP_A-1511658	0.49554375	-0.155470749	0.22836888	0.38901135	0.000298795
SNP_A-1512664	0.625958333	-0.154919136	0.55510641	0.72177152	0.000449091
SNP_A-1516996	0.2089	-0.114938087	0.07184856	0.17714862	0.0005948
SNP_A-1512479	0.182772727	-0.192523733	0.35687672	0.52084677	0.000644421
SNP_A-1511559	0.561077778	-0.149326421	0.41497343	0.57854005	0.000759537
SNP_A-1515181	0.43186875	-0.084847437	0.03153207	0.10812095	0.000915287

We demonstrated the most difference in allele frequency* is SNP_A_1518521

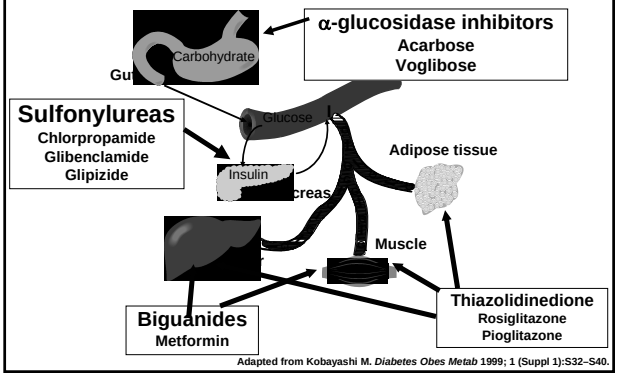
	Estimated allele freq	
Normal	0.53	
Obese DM	0.29	
Lean DM	0.33	
Δ Allele frequency between control and Obese DM	0.24	
Chi square value (RASav)	12.97	P <0.001
Δ Allele frequency between control and lean DM	0.20	
Chi square value (RASav)	8.16	P <0.01

Pharmacogenetic study

Drug prescribed in T2DM



Primary sites of action of oral anti-diabetic agents



Adapted from Kobayashi M. *Diabetes Obes Metab* 1999; 1 (Suppl 1):S32–S40.

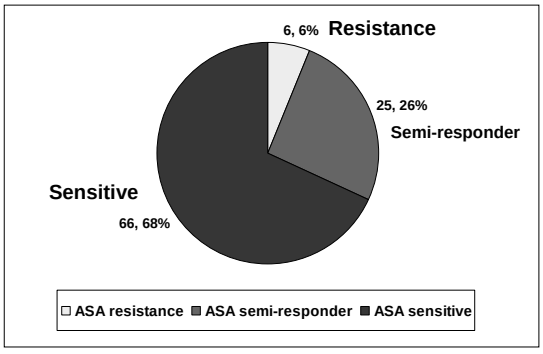
Pharmacogenetic study of drug response and ADR.

1. Effect of Insulin Resistance on Platelet Aggregation in Type 2 Diabetic Patients with Stable Cardiovascular Disease Treated with Aspirin.

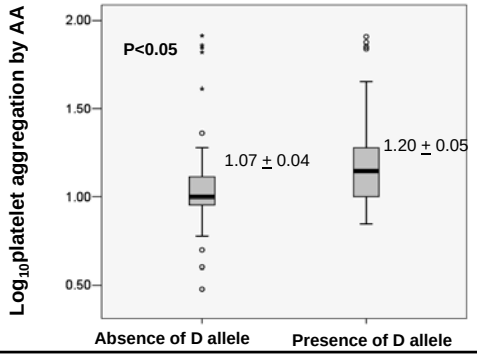
Rationale …

There have been emerging evidences that aspirin nonresponder subjects have higher chance of cardiovascular events.

The frequency of ASA response according to cutoff criteria of both AA and ADP mediated platelet aggregation analysis (Gum's definition)



Subjects with presence of D allele in VNTR of *GPIBA* had a poor response to aspirin.



Multivariate regression analysis included HbA1C, thromboxane B2 levels and presence of D allele in VNTR polymorphisms of *GP1BA*

Parameters	Standardized coefficient (β)	95% CI of β	Sig.*
Thromboxane B2	0.08	0.04-0.12	P=0.04
Presence of D allele in VNTR polymorphism of *GPIBA*	0.11	0.001-0.23	

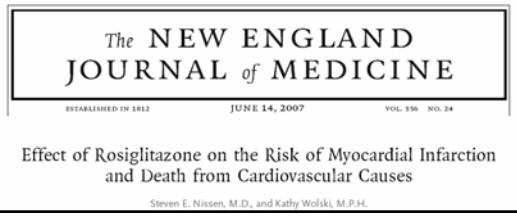
Only presence of D allele and thromboxane 2 levels were significant independent predictor of platelet aggregation function induced by AA.

*** R^2 = 0.19**

Pharmacogenetic study of drug response and ADR.

2. Study of the frequency and associated factors of thiazolidinedione induced fluid retention in type 2 diabetes patients.

Rationale …


The NEW ENGLAND JOURNAL of MEDICINE

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Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes

Steven E. Nissen, M.D., and Kathy Wolski, M.P.H.

Frequencies of thiazolidinediones on weight change, fluid retention

	Fluid retention				
	NiL (<1.8 kg)	Mild (1.8 – 3kg)	Moderate (3.1-5kg)	Severe (>5 kg)	Peripheral Edema
Pioglitazone (N =55)	48 (87.30%)	6* (10.90%)	1 (1.80%)	-	6 (10.90%)
Rosiglitazone (N=77)	70 (90.90%)	4 (5.20%)	2 (2.60%)	1* (1.30%)	4 (5.20%)
Total (N= 132)	118 (89.3%)	10 (7.58%)	3 (2.27%)	1 (0.76%)	10 (7.58%)

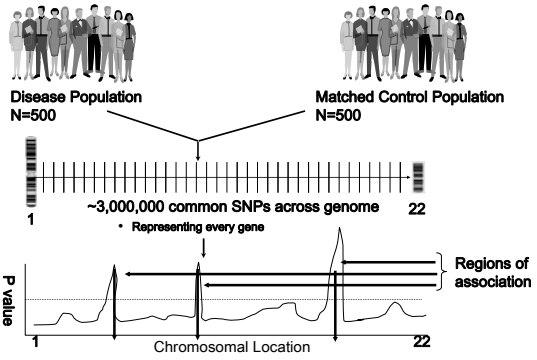
(body weight rising > 0.05 kg/day)
*3 of 21 experienced both edema and weight gain.

Multivariate analysis showed previous CVD is the only independently risk factor associated with fluid retention after TZD prescription.

Factor	Coefficient	SE	OR (95%CI)	P-value
CAD	2.039	0.81	7.686	0.012 *

Ongoing Studies ….

Whole Genome Associations

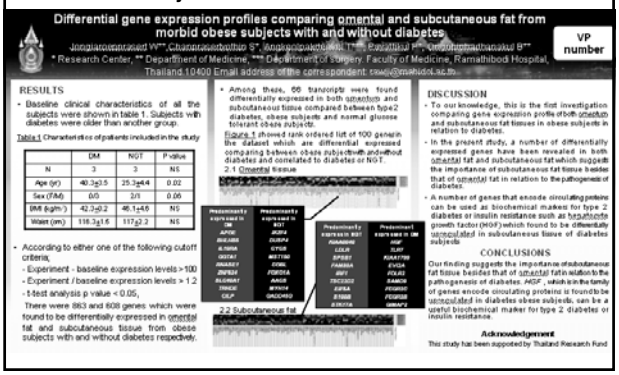


Informatics to ID gene(s) mapped to associated SNP

Whole Genome Associations

- Pharmacogenomic study of Aspirin responsiveness in Thais.
- Genome wide association study using 500K microarrays on pooled DNA approaches.

Differential gene expression profiles comparing subcutaneous and visceral fat from morbid obese subjects with and without diabetes


Differential gene expression profiles comparing omental and subcutaneous fat from morbid obese subjects with and without diabetes

VP number

RESULTS

DISCUSSION

CONCLUSIONS

Acknowledgement

This study has been supported by Thailand Research Fund

Biomarkers to Predict T2DM

- Finding of serum biomarkers to predict T2DM
  - Select candidate protein from expression study
  - Test hypothesis in a prospective cohort of 300 healthy subjects underwent 75 gm OGTT and FU for 4 years.

Acknowledgement

• Our residents, endocrine fellows, Master degree and PhD students
• Our patients from Ramathibodi Hospital

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Collaboration

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- RIKEN SNP Research Center, Japan
  - Prof Yusuke Nakamura
- Department of pharmacology Chulalongkorn University
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- King's Mongkut University of Technology Thonburi
  - ดร.อัศวิน มีชัย
  - ดร. Jonathan Chan

What do we have and need …

Our facility

ABI 16-capillary automated sequencing 3130

Taqman real time PCR

Our facility

Affymetrix Microarray platform

Manpower

- 2 fulltime clinical research nurses
  - Conduct all clinical study not only DM
- 2 fulltime technicians
  - Master degree
- 2 fulltime biostatisticians cover all biomedical science study in Ramathibodi hospital
- None of bioinformatic technicians
- Computer scientists → cover all the hospital database stuff

What we need ……..

- Funding agency who appreciated in basic science knowledge
- More biostatisticians
- Bioinformatic computer scientist
- Powerful and efficient database system
- PhD scientists
- More collaboration from other institutes

Thank you for your attention