13th December, 2015
1st ICCN, Hong Kong

Genomic studies in Diabetic Nephropathy

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2011 - 2015

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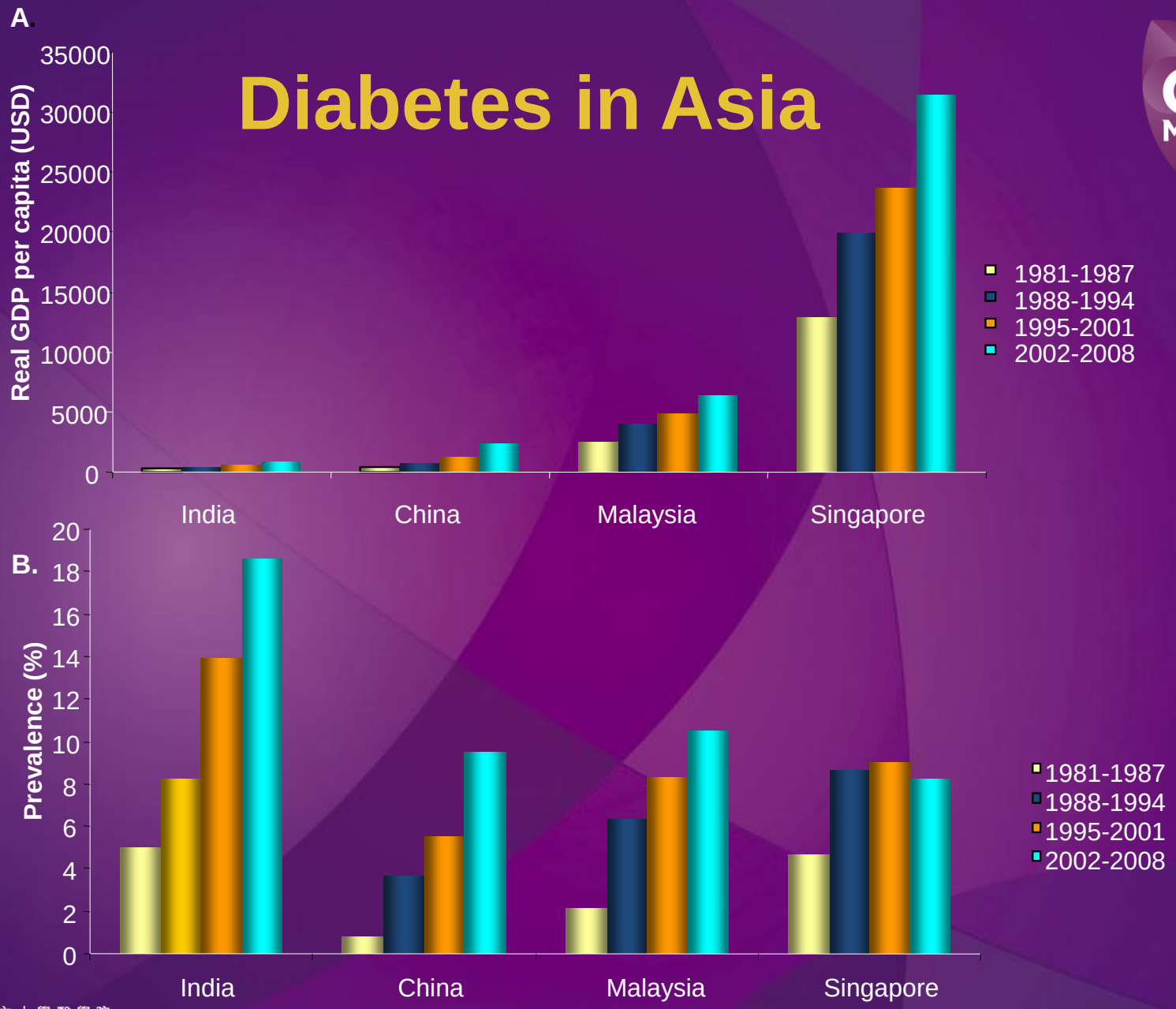
HONG KONG INSTITUTE OF DIABETES AND OBESITY
10th Anniversary

Prevent • Control • Cure

Diabetes in Asia



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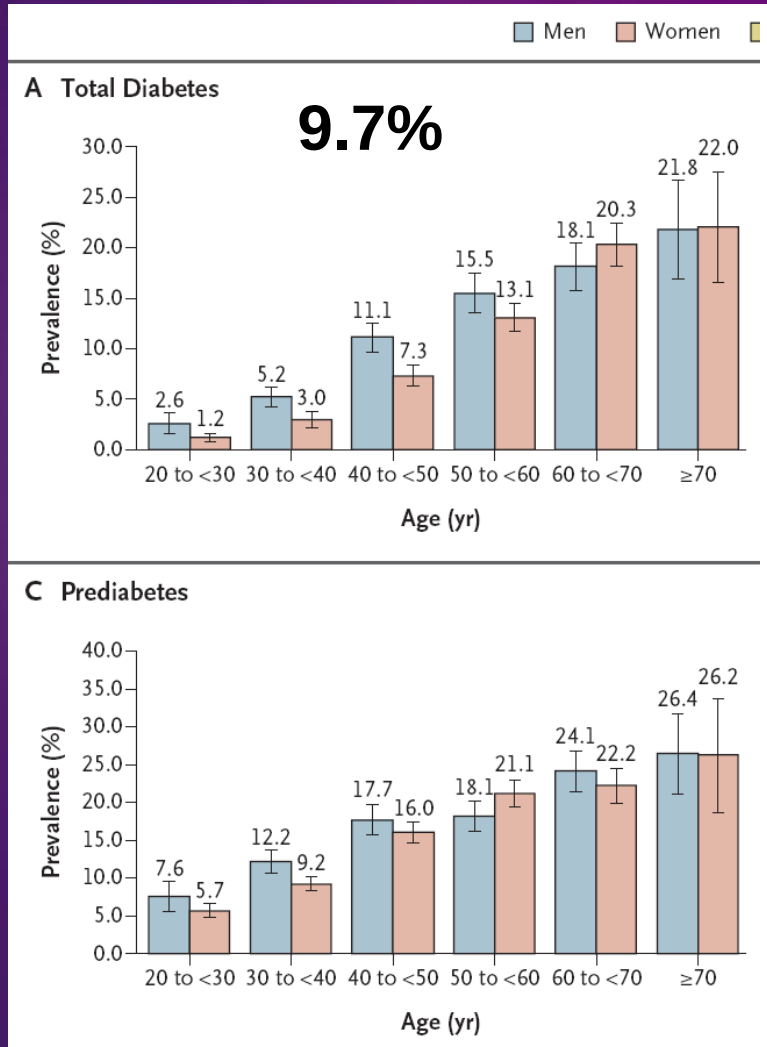
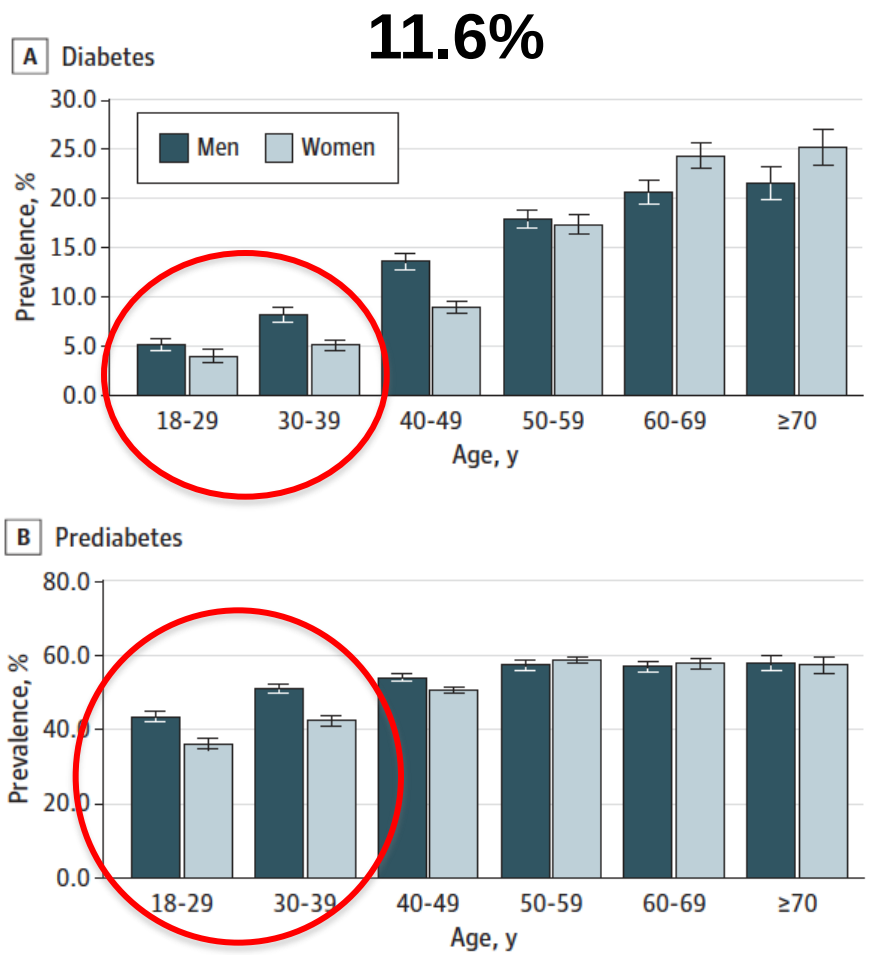
Faculty of Medicine
The Chinese University of Hong Kong

Ramachandran A, Ma RC and Snehalatha DC, Lancet 2010; 375: Jan 30

Prevalence of DM in China

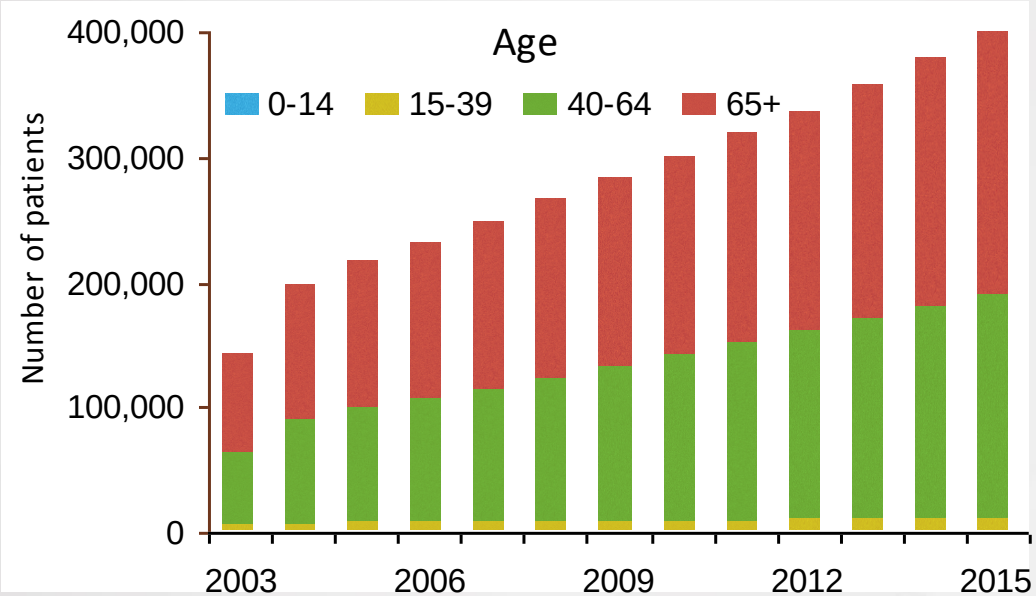




Yang W et al, NEJM 2010; March 25

Xu et al, J Am Med Asso 2013; Sept 4

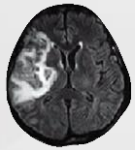
Cardiac and Renal Complications are the Major Healthcare Burden from Diabetes



~20,000 new cases per year

32%

48%



27%

18%

6%

Hong Kong

Affects >10% of population
1.2million out-patient attendance/ year
280,000 hospital admissions/yr
Estimated costs: HKD 5 billion/yr
Majority due to diabetic complications

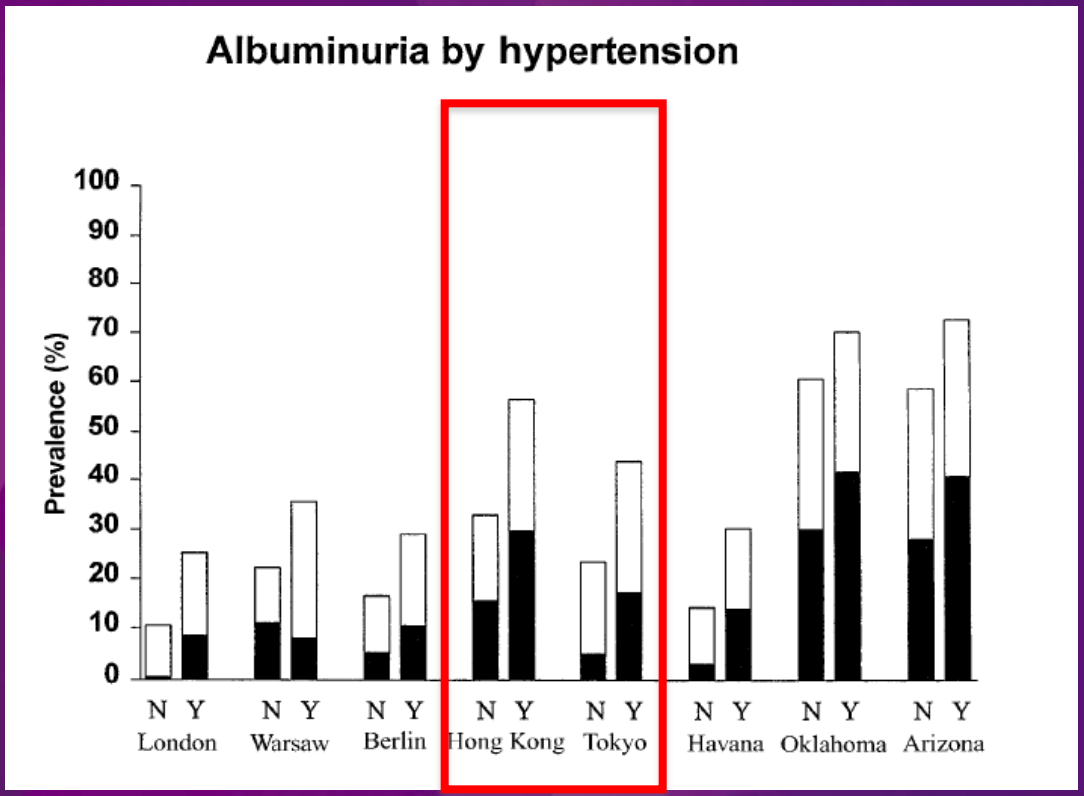
> 50% of DM patients die from cardiac and renal complications

Marked variation in the risk of diabetic nephropathy- WHO MSVDD

Microalbuminuria

Macroalbumininuriia

2033 patients from 8 centres

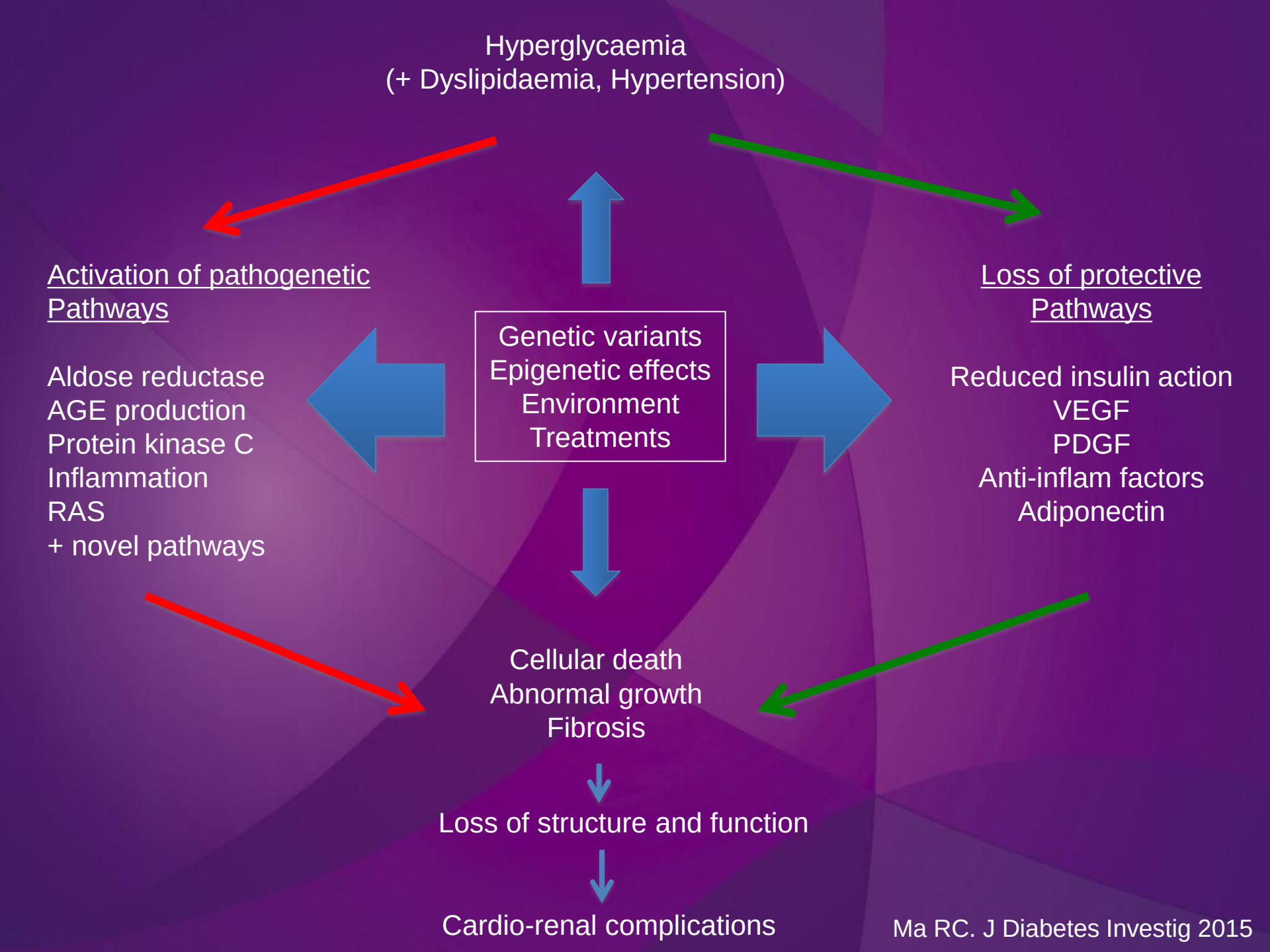


Main predictors:
Mean BP (all centres)
Retinopathy (not HK, Arizona, Warsaw)
Insulin treatment (HK, Tokyo, Berlin, Oklahoma)
CHD (HK, Arizona, London)

Bennett PH et al, Diabetologia 2001

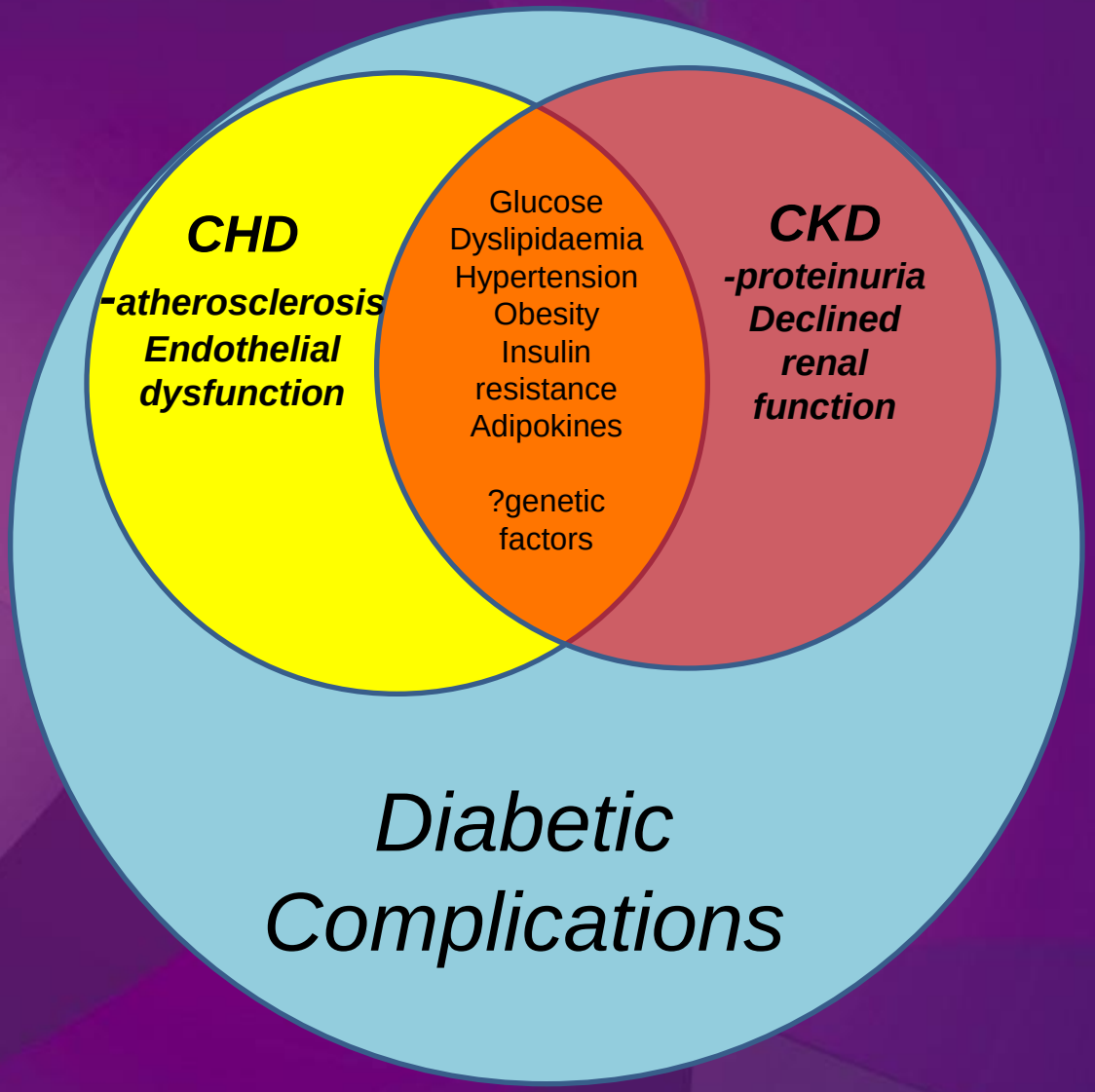
Overview

- Genetic markers for diabetic nephropathy
- Diabetic kidney complications
  - Candidate gene studies
  - Bioinformatics approach
  - GWAS
- Future directions



Ma RC. J Diabetes Investig 2015

Similarities And Differences



Ma RC. J Diabetes Investig 2015

The Hong Kong Diabetes Registry

Recruitment (1995-2005)

30-50 ambulatory DM patients/week

GPs
Community
Specialty clinics

→

Comprehensive assessment (every 12-18 months)

Risk factors

BMI, Waist circumference, BP
Glycemic control (PFG, HbA_{1c})
full lipid profile (TC, HDL-C, TG, LDL-C)
Albuminuria (e.g. spot urine albumin:creatinine ratio)

Complications

Eye (visual acuity and fundoscopy)
Feet (skin changes, sensation and pulses)
Cardiovascular (ECG, stress test as appropriate)
Renal function test

→

Outcome Documentation

Drug use

Outcomes:
ESRF
Stroke
Death
CHD
CHF
Cancer

>10,000 patients
Follow-up >8 yr
Biobanked samples

Yang XL et al, Diabetologia 2006; 49: 2299-308
Yang XL et al, Diabetes Care 2007; 30: 65-70
Yang XL et al, Arch Intern Med 2008

The Burden of Diabetic Complications- The Hong Kong Diabetes Registry (1995-2005)

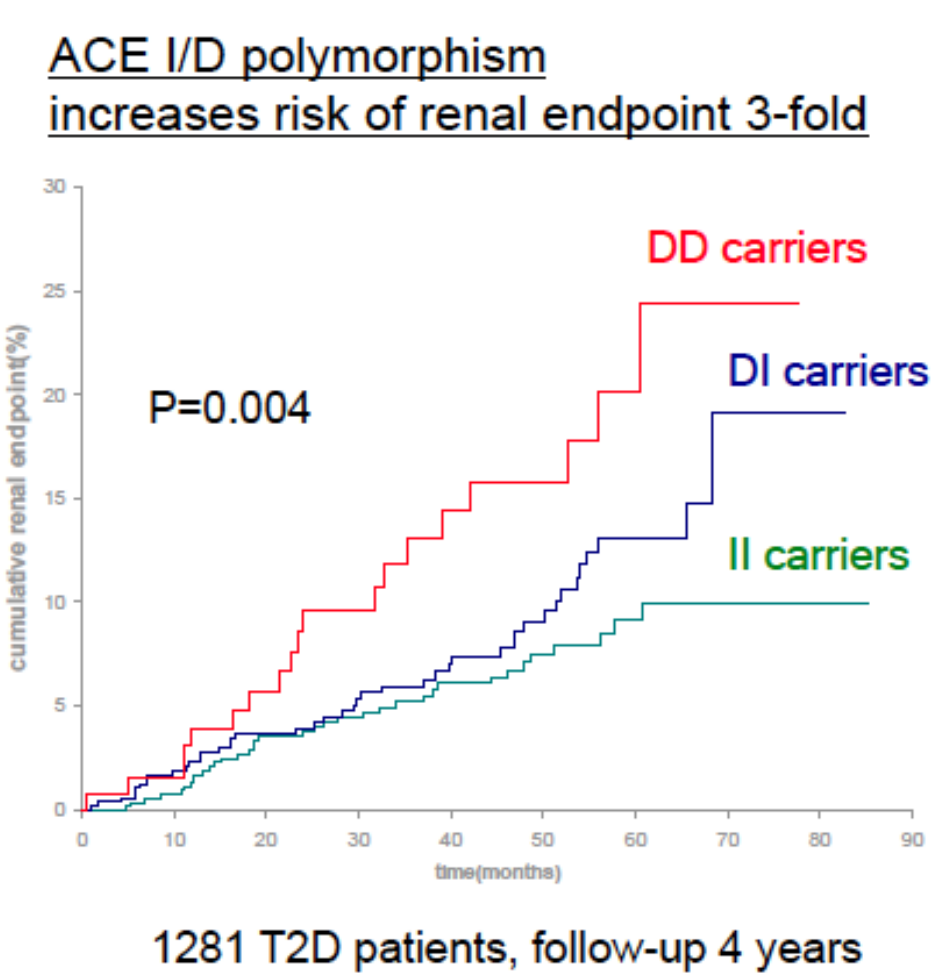
> 7,000 DM patients, mean duration of follow-up: 6 years

Death	10.1% (768)
Coronary heart disease	6.7% (507)
Cardiac failure	4.5% (340)
Stroke	5.6% (422)
End stage renal disease	10.5% (799)
Cancer	5.4% (413)
Composite events	32.9% (2492)

Endpoints captured in 2005, 2009, 2014

Yang et al, Arch Intern Med 2008; Am J Cardiol 2008, Diabetes Care 2007, Diabetologia 2007

ACE I/D polymorphism and DM complications



Variable	HR (95%CI)	P
RAS inh + II genotype	0.52 (0.3, 0.92)	0.02
RAS inh + DI genotype	0.43 (0.25, 0.72)	0.001
RAS inh + DD genotype	0.95 (0.42, 2.12)	0.91

Wang Y et al, Diabetes Care 2004

So WY, Ma RC et al, Kidney Int 2006; 69: 1438-44

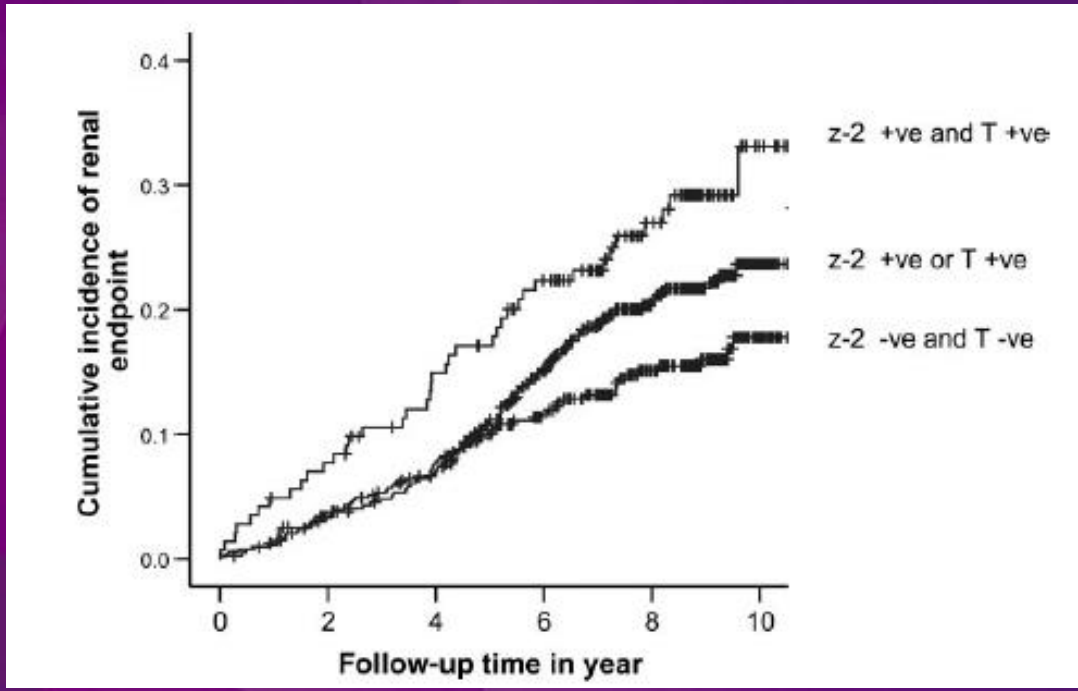
Genetic variants in the Aldose Reductase Pathway predicts DM complications

1,327 subjects with T2DM
Excluded those with
Baseline CKD, CHD

1,074 included in analysis

ALR2 z-2 allele
↑ risk of renal HR 1.53
(1.14-2.05)

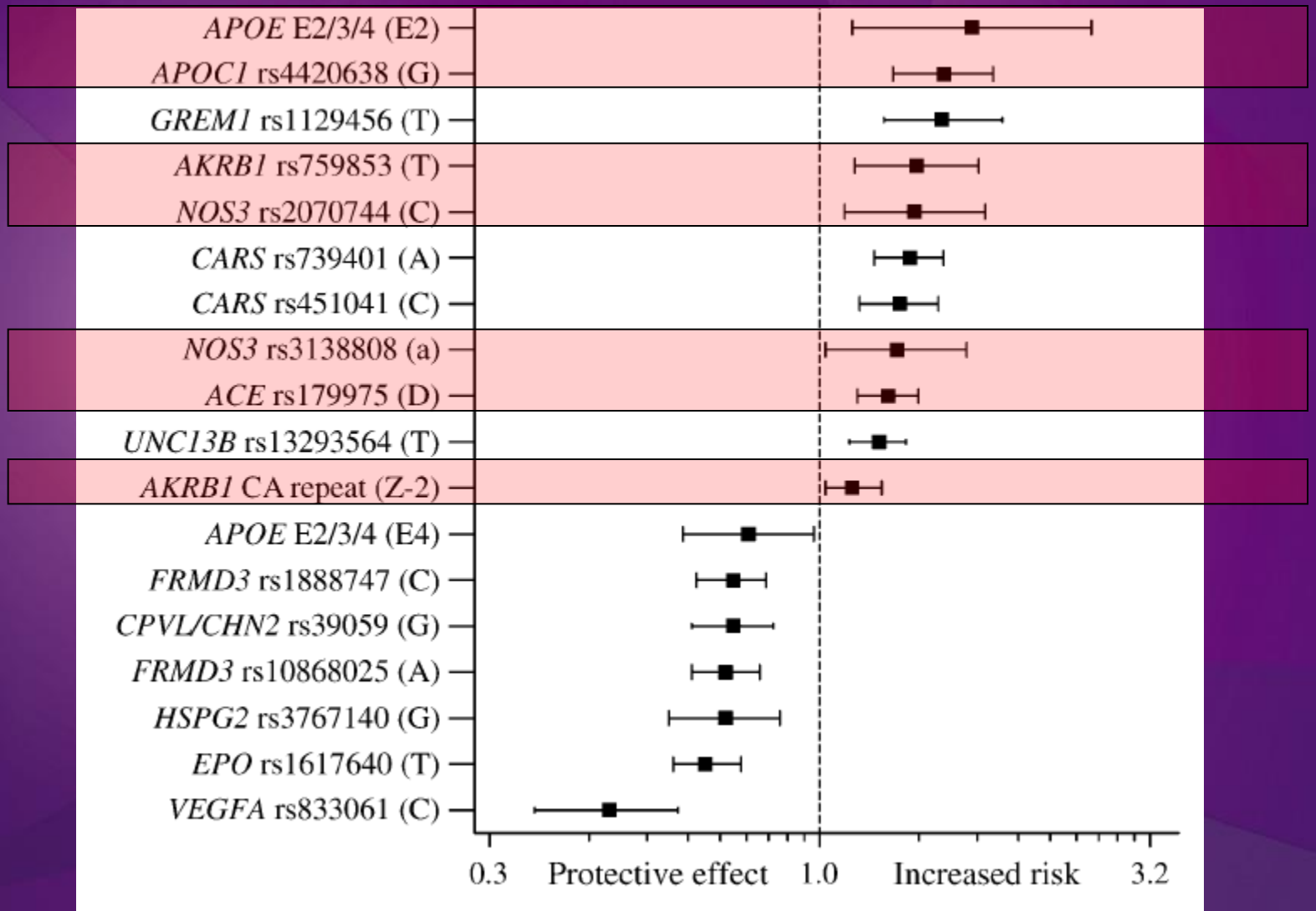
Or combined cardio-renal
Endpoints HR 1.49 (1.14-1.95)



So WY et al Diabetes Care 31: 2148-2153, 2008

Few known genetic variants for diabetic kidney disease

AL Mooyaart et al
Diabetologia 2011



Association study of *PRKCB1* and diabetic nephropathy

Prospective study of 1172 patients

T2 DM free of renal disease

Mean duration of follow-up 7.9 years

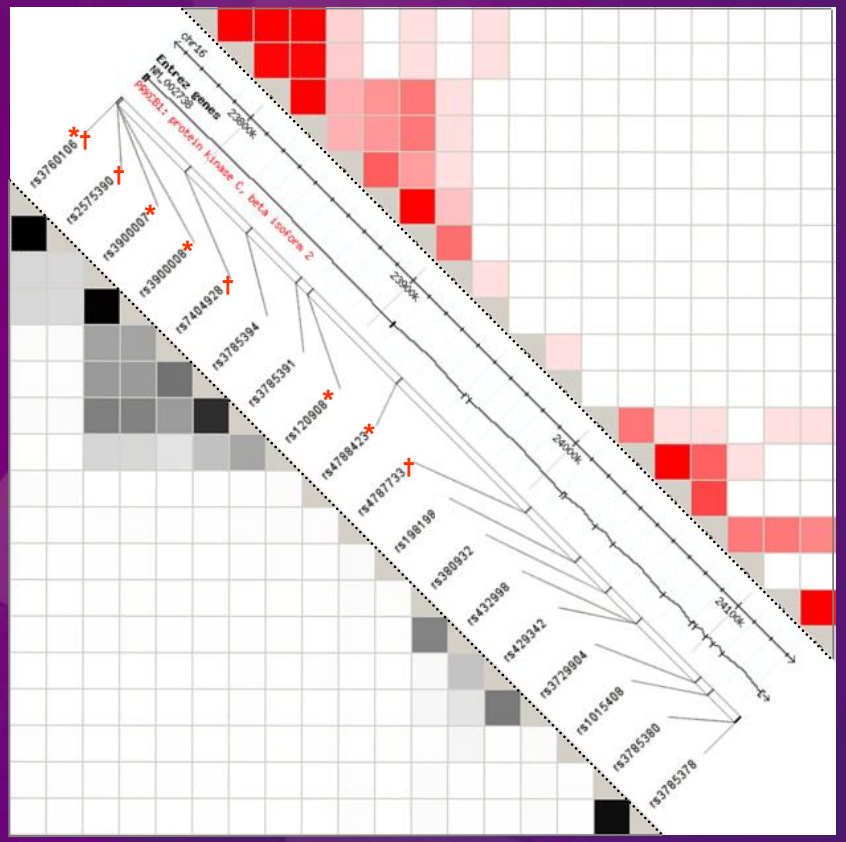
Endpoints:

- 90 out of 1172 developed ESRD

18 Tag SNPs

Cox-proportional hazard regression model

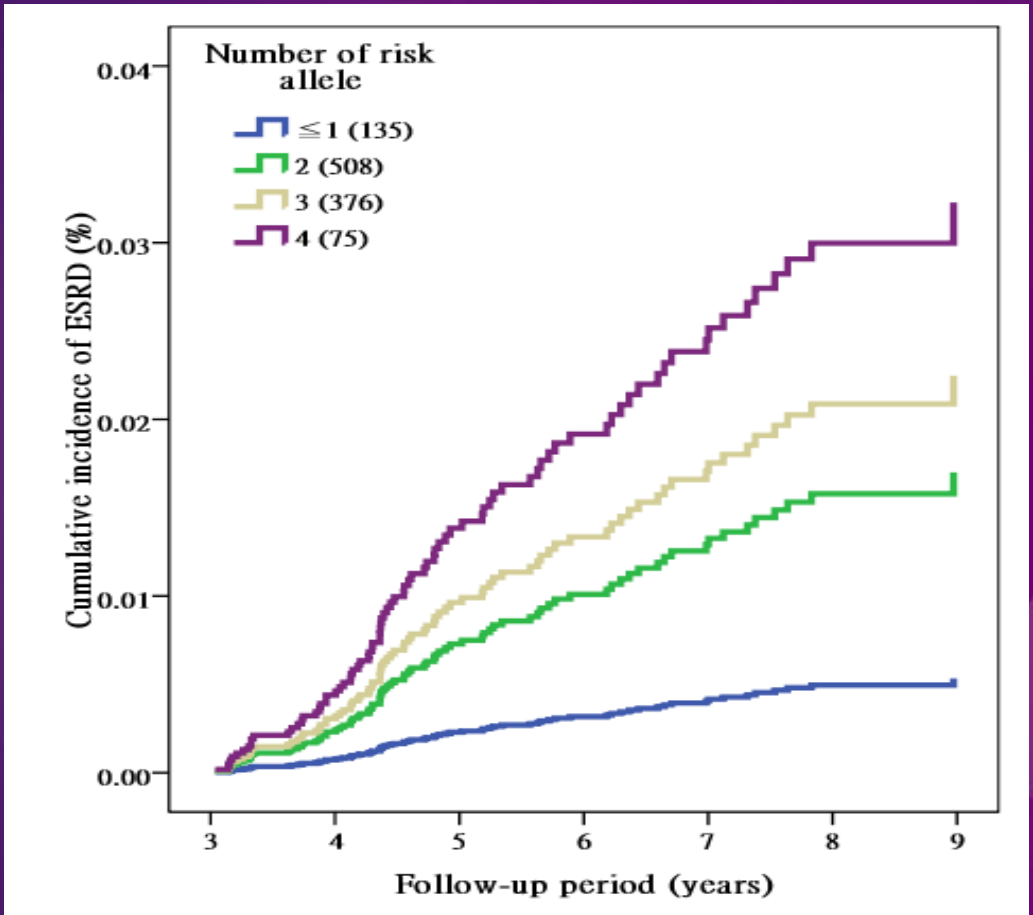
Adjusted for conventional Risk factors



Structure of *PRKCB1* gene

Variants in PRKCB1 predict new-onset ESRD

90 out of 1172 developed ESRD during 7.9 yr follow-up



The HR for ESRD increased with increasing number of risk alleles (P=0.0007) in the joint effect analysis.

The adjusted risk for ESRD was 6.04 (2.00-18.31) for patients with four or more alleles compared to patients with one or less risk allele

Adjusted for conventional risk factors (age, gender, duration of DM, SBP, DBP, HbA1c, Cholesterol, Ln AER, eGFR, retinopathy, use of ACEI.ARB)

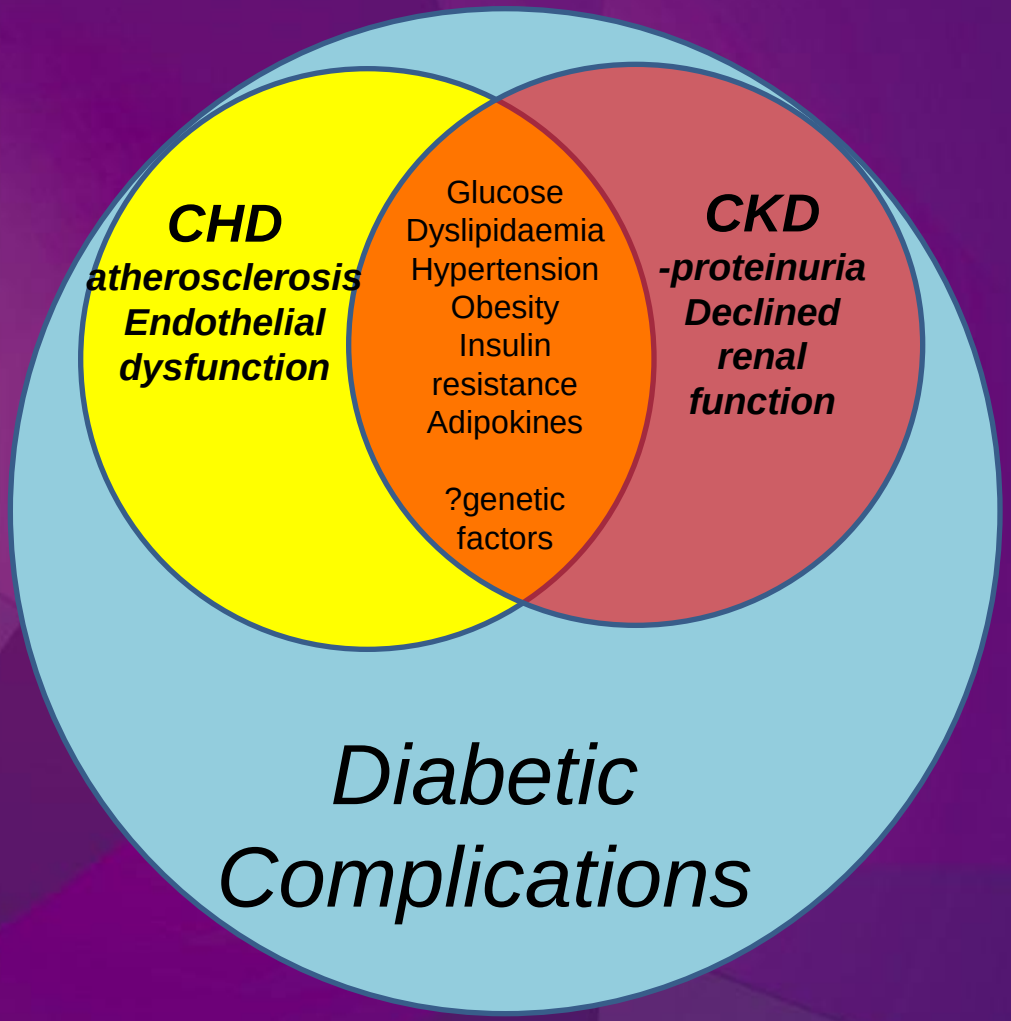
Ma RC et al, *JAMA* 2010, Aug 25

How to identify predictors from a dataset containing clinical and genetic variables?

Traditional analyses

Neglects potential interaction or multiplicative effect between variables

Are subjects with increased risk of DM complications enriched for SNPs related to diabetes or related traits?



Identifying predictors of diabetic kidney disease

2,755 subjects from registry with complete data and genotype data

673 (24.4%) developed CKD during median FU 7.7years

36 SNPs relating to T2DM, obesity, glucose traits

25 clinical variables including age, sex, eGFR, as well as drug use information

1) a stepwise model selection procedure based on the Akaike Information Criterion (AIC) was repeatedly conducted (using 200 subsample aggregation each covering 2/3 of the cases and controls);

2) the category-free net reclassification improvement (NRI) was employed to evaluate the contribution of top SNPs towards the prediction model;

3) the associations of identified genetic variants with outcomes were further validated in independent samples from collaborators

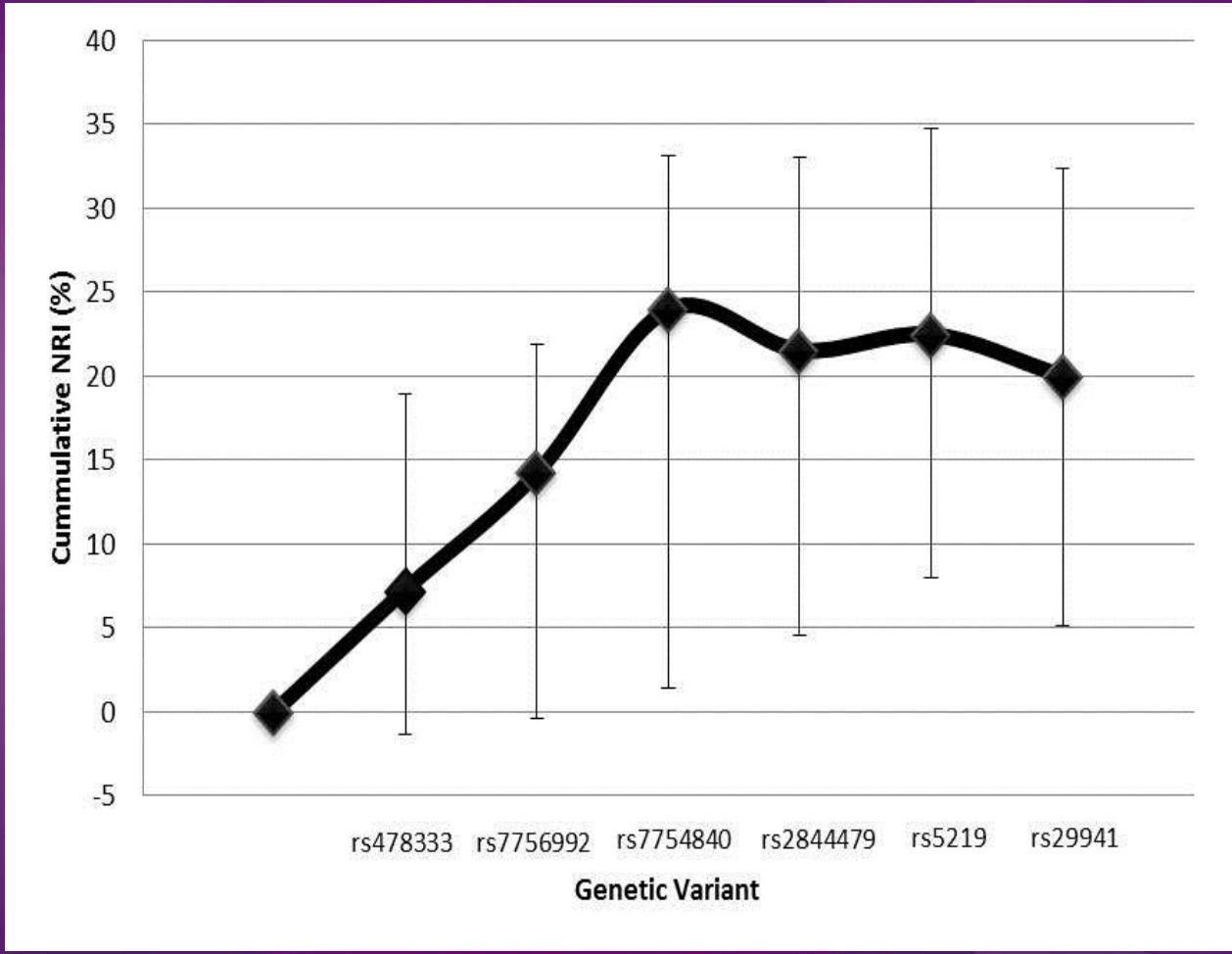
Selected Variable	Inclusion Frequency (%)		
	genetic model	clinical model	clinico-genomic model
ACR	/		
Age	/		
eGFR	/		
HbA1c	/		
Insulin	/		
Sensory neuropathy	/		
ACEIs or ARBs	/		
CHD history	/		
rs478333 (*G6PC2*)	63.5		
Retinopathy	/		
rs7756992 (*CDKAL1*)	5		
Triglyceride	/		
rs7754840 (*CDKAL1*)	2		
rs2844479 (*LST1/AIF1*)	74		
rs5219 (*KCNJ11*)	3		
rs29941 (*KCTD15*)	11.5		
LDL cholesterol	/		
rs4430796 (*HNF1B*)	59.5		
AIC value*	6496(7.8)		

Selected Variable	Inclusion Frequency (%)		
	genetic model	clinical model	clinico-genomic model
ACR	/	100	
Age	/	100	
eGFR	/	100	
HbA1c	/	100	
Insulin	/	100	
Sensory neuropathy	/	100	
ACEIs or ARBs	/	100	
CHD history	/	99	
rs478333 (*G6PC2*)	63.5	/	
Retinopathy	/	80.5	
rs7756992 (*CDKAL1*)	5	/	
Triglyceride	/	68	
rs7754840 (*CDKAL1*)	2	/	
rs2844479 (*LST1/AIF1*)	74	/	
rs5219 (*KCNJ11*)	3	/	
rs29941 (*KCTD15*)	11.5	/	
LDL cholesterol	/	53	
rs4430796 (*HNF1B*)	59.5	/	
AIC value*	6496(7.8)	5736(28.9)	

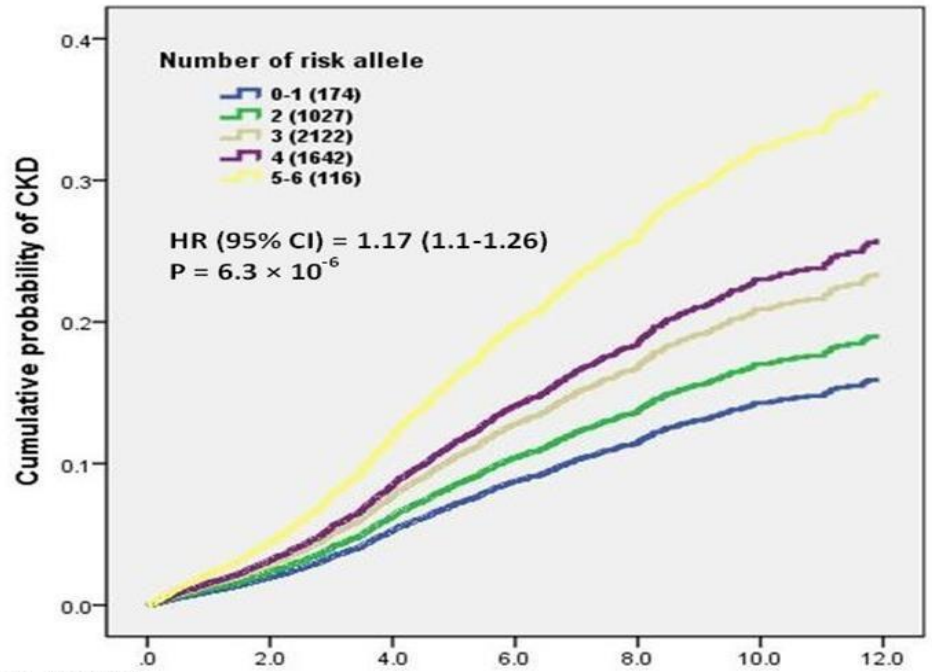
Selected Variable	Inclusion Frequency (%)		
	genetic model	clinical model	clinico-genomic model
ACR	/	100	100
Age	/	100	100
eGFR	/	100	100
HbA1c	/	100	100
Insulin	/	100	100
Sensory neuropathy	/	100	100
ACEIs or ARBs	/	100	99.5
CHD history	/	99	99.5
rs478333 (*G6PC2*)	63.5	/	95.5
Retinopathy	/	80.5	80.5
rs7756992 (*CDKAL1*)	5	/	67
Triglyceride	/	68	65.5
rs7754840 (*CDKAL1*)	2	/	64
rs2844479 (*LST1/AIF1*)	74	/	59.5
rs5219 (*KCNJ11*)	3	/	59
rs29941 (*KCTD15*)	11.5	/	58
LDL cholesterol	/	53	57.5
rs4430796 (*HNF1B*)	59.5	/	4
AIC value*	6496(7.8)	5736(28.9)	5722(29.4)

Selected Variable	Inclusion Frequency (%)		
	genetic model	clinical model	clinico-genomic model
ACR	/	100	100
Age	/	100	100
eGFR	/	100	100
HbA1c	/	100	100
Insulin	/	100	100
Sensory neuropathy	/	100	100
ACEIs or ARBs	/	100	99.5
CHD history	/	99	99.5
rs478333 (*G6PC2*)	63.5	/	95.5
Retinopathy	/	80.5	80.5
rs7756992 (*CDKAL1*)	5	/	67
Triglyceride	/	68	65.5
rs7754840 (*CDKAL1*)	2	/	64
rs2844479 (*LST1/AIF1*)	74	/	59.5
rs5219 (*KCNJ11*)	3	/	59
rs29941 (*KCTD15*)	11.5	/	58
LDL cholesterol	/	53	57.5
rs4430796 (*HNF1B*)	59.5	/	4
AIC value*	6496(7.8)	5736(28.9)	5722(29.4)

Evaluating the contribution of selected genetic variants to the prediction model

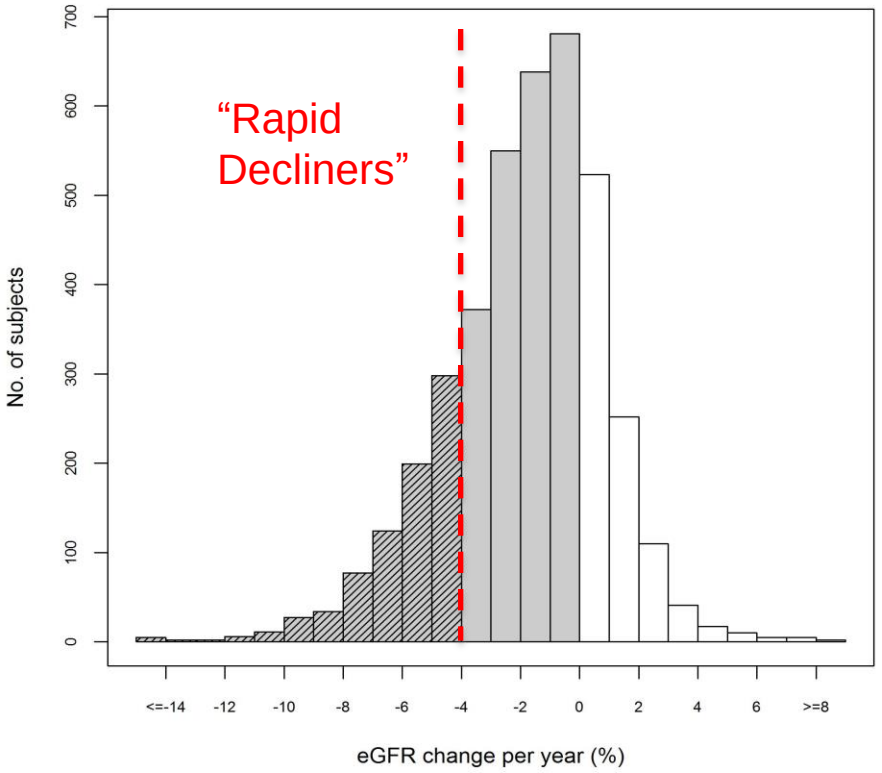


Variants related to glucose metabolism predicts development of CKD in T2DM



No. of at-risk by allele count							
0-1	174	167	156	123	102	76	37
2	1027	968	922	772	569	401	170
3	2122	1996	1878	1521	1154	824	373
4	1642	1526	1438	1186	901	606	248
5-6	116	110	103	83	67	49	22

Decline in renal function per year



rs478333 of *G6PC2*
rs7754840 of *CDKAL1*
rs7756992 of *CDKAL1*

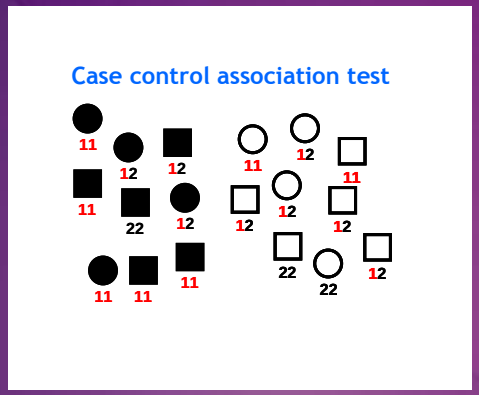
Jiang G et al, Kidney Int, *in press*

DM complications	Phenotype	Study type	Ethnic group	Polymorphism	Candidate gene/nearest gene	Chrm location	References
Type 2 diabetes mellitus	Nephropathy	Candidate gene	Multi-ethnic	rs179975	ACE	17q23	Mooyart *et al.*, Diabetologia (2011)
		Candidate gene		rs4646994 rs 4344	ACE	17q23	Ng *et al.* (2005), Wang *et al.* (2012)
		Candidate gene	Asian	rs4646994	ACE	17q23	Zhong *et al.*, JRAAS (2015)
			Asian (incident DN)	rs4646994	ACE	17q23	Zhong *et al.*, JRAAS (2015)
				rs759853	Aldose reductase	7q35	So *et al.*, Diabetes Care (2008)
				Microsatellite	Aldose reductase	7q35	So *et al.*, Diabetes Care (2008)
					APOE	19q13	
		Candidate gene	Multi-ethnic	rs1801282	PPARG	3p25	Herrmann *et al.*, Diabetes (2002); Liu *et al.*, Diabetes Care (2010)
		Candidate gene	Japanese	rs2237897	KCNQ1	11p15	Ohshige *et al.*, Diabetes Care (2010)
					APOE	19q13	Li *et al.*, Mol Biol Rep (2011)
			Multi-ethnic	D18S880	CNDP1	18q22	Janssen *et al.*, Diabetes (2005); Mooyart *et al.*, Diabetologia (2011)
		Candidate gene	European	rs1799883	FABP2	4q28	Canani *et al.*, Diabetes (2005)
		Candidate gene	European	rs451041	CARS	11p15	Pezzolesi *et al.*, Kidney Int (2011)
		Candidate gene		rs1411766		13q	Pezzolesi *et al.*, Kidney Int (2011)
		Candidate gene	European	rs1531343	HMGA2	12q15	Alkayyali *et al.*, Diabetologia (2013)
		Replication	Japanese	rs1411766	Near IRS2	13q	Maeda *et al.*, Diabetes (2010)
		GWAS	Japanese	Arg913Gln	SLC12A3	16q13	Tanaka *et al.*, Diabetes (2003)
		GWAS	Japanese	rs741301	ELMO1	7p14	Shimazaki *et al.*, Diabetes (2005)
		GWAS	Japanese	rs2268388	ACACB	12q24.1	Maeda *et al.*, PLoS Genet (2010)
	ESRD	Candidate gene	Multi-ethnic	rs4646994	ACE	17q23	Yu *et al.*, Nephrology (2012)
		Candidate gene	Asian	rs4646994	ACE	17q23	Yu *et al.*, Nephrology (2012)
		Candidate gene	Chinese	rs3760106	PRKCB1	16p11	Ma *et al.*, JAMA (2010)
		Candidate gene	African American	rs7754586	ENPP1	6q24-27	Keene *et al.*, Diabetes (2008)
		Candidate gene	African American	rs4478844	OR2AK2	1q44	Cooke Bailey *et al.*, Hum Genet (2014)
		Candidate gene	Euriopean	rs3747154	LIMK2	22q12	Cooke Bailey *et al.*, Hum Genet (2014)
		Replication	European	rs11769039	ELMO1	7p14	
		Replication	African American	rs1345365	ELMO1	7p14	Leak *et al.*, Ann Hum Genet (2009)
	ESRD in type 2 diabetes mellitus	GWAS	Pima Indians	rs2720709	PVT1	8q24	Hanson *et al.*, Diabetes (2007)
		Resequencing		rs2648875	PVT1	8q24	Hanson *et al.*, Diabetes (2007)

CHD, coronary heart disease; CV, cardiovascular; DM, diabetes mellitus; ESRD, end-stage renal disease; GWAS, genome-wide association studies.

Ma RC. J Diabetes Investig 2015

Identification of novel genetic predictors of DMN in Chinese by GWAS

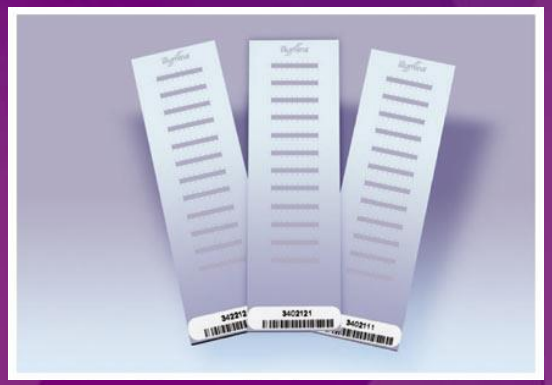


Select 400 cases without DN

Matched for duration of DM, age using prospective design

200 cases developed DN
200 cases no DN on FU
from database

DNA extracted →



Genome-wide genotyping using high density chips (Illumina 610 Quad)

→ **Data Analysis**

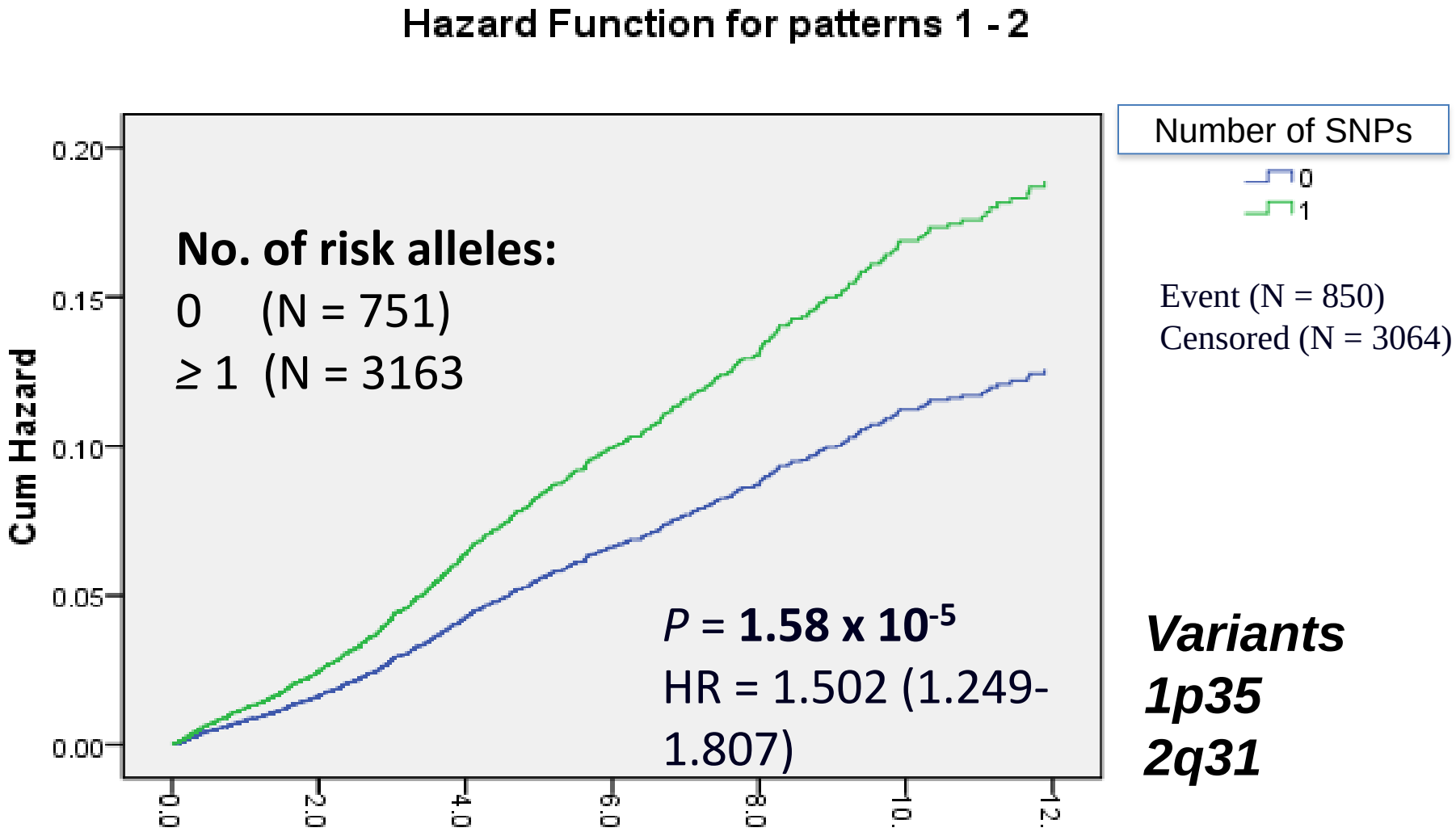
Quality control
Marker quality control
Population substructure

↓

Preliminary data
for top association signals
Plan for future
Replication studies

Supported by the Innovation and Technology Fund

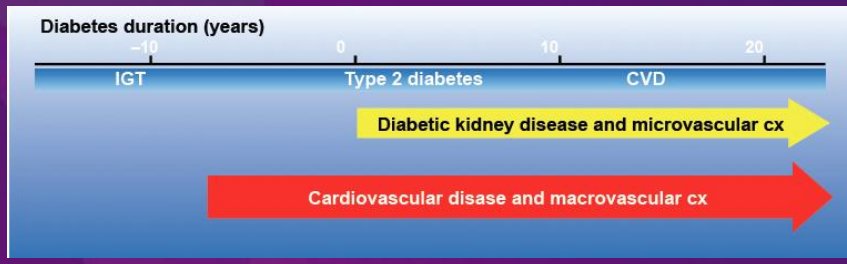
Joint analysis of top 2 replicated SNPs



Variants
1p35
2q31

Abstract presentation EASD 2014

Theme-based Research Scheme on DM cardio-renal complications



Hong Kong Diabetes Registry

Since 1995
>10,000 DM subjects
Prospective follow-up
Regular assessment
Detailed clinical info
Medication use
Baseline DNA and blood

Genome (GWAS)

Methylome (EWAS)

MiRNA (micRNA array)

Transcriptome (RNA-seq)

Transomic Integrative analysis

Functional studies and animal studies

Pathways

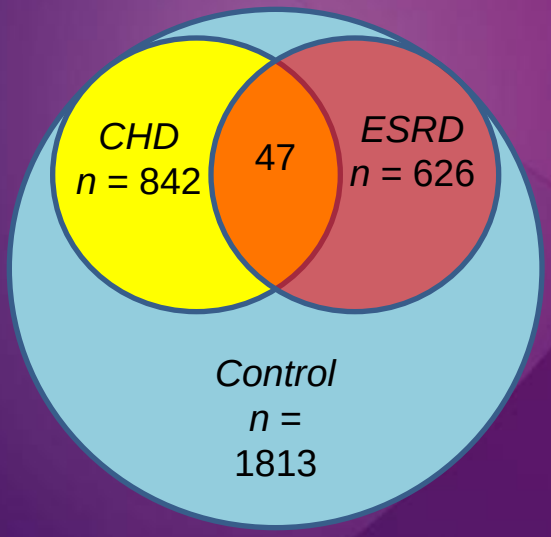
Replication samples from Hong Kong Diabetes Biobank (10 centres across Hong Kong)

Replication in external cohorts from collaborators

Prof HY Lan
Prof CC Szeto

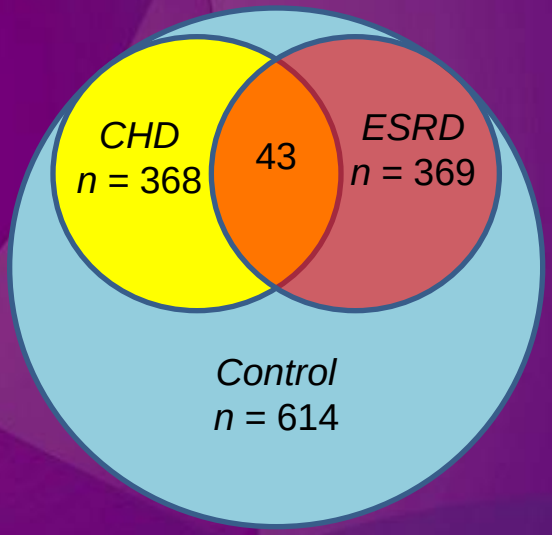
GWAS
Genetic markers

Whole-genome genotyping
Cover 2.5m+ genetic variants
1st phase 3008 sample
Total > 6,500 samples

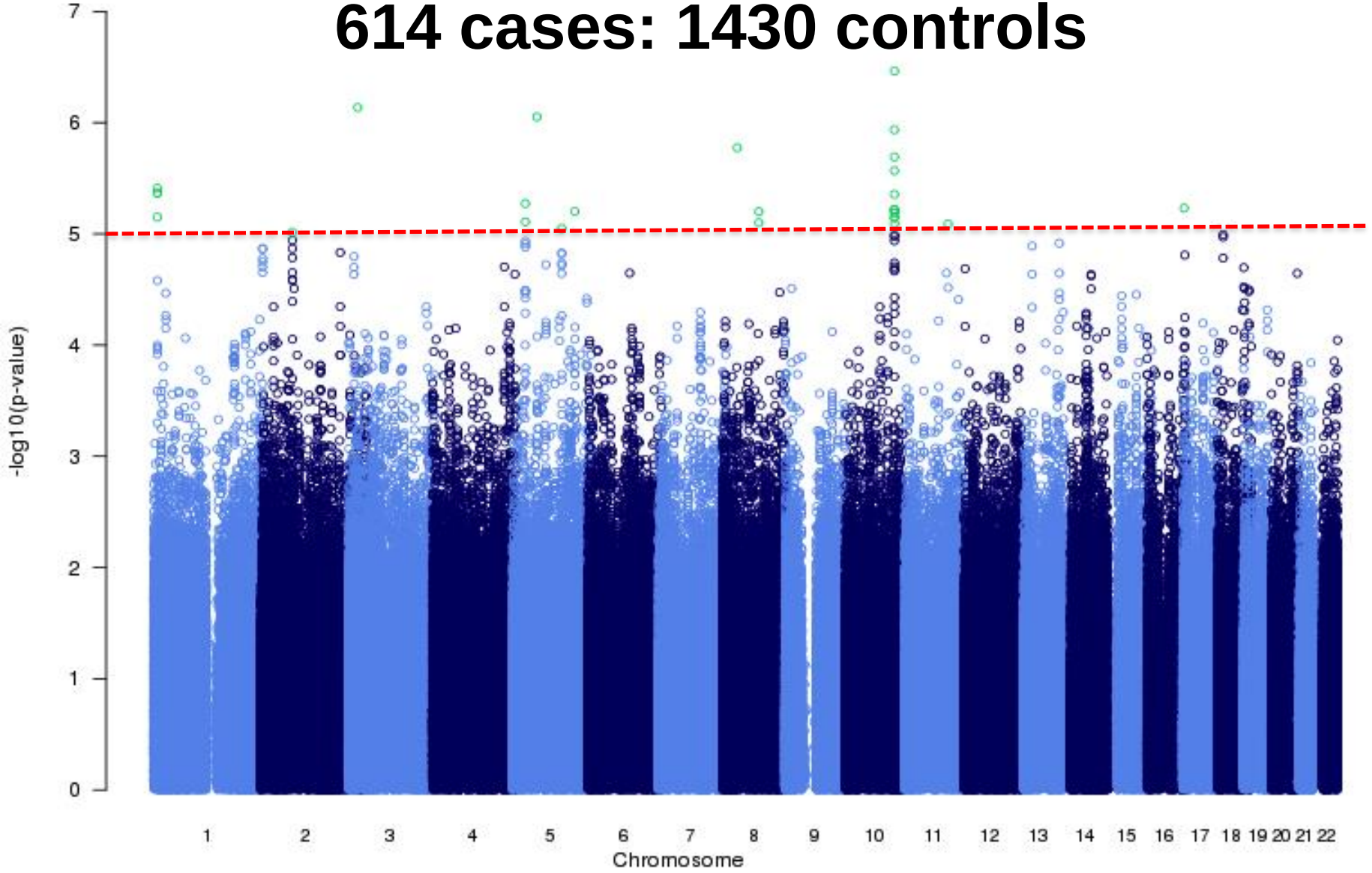


EWAS
Epigenetic markers

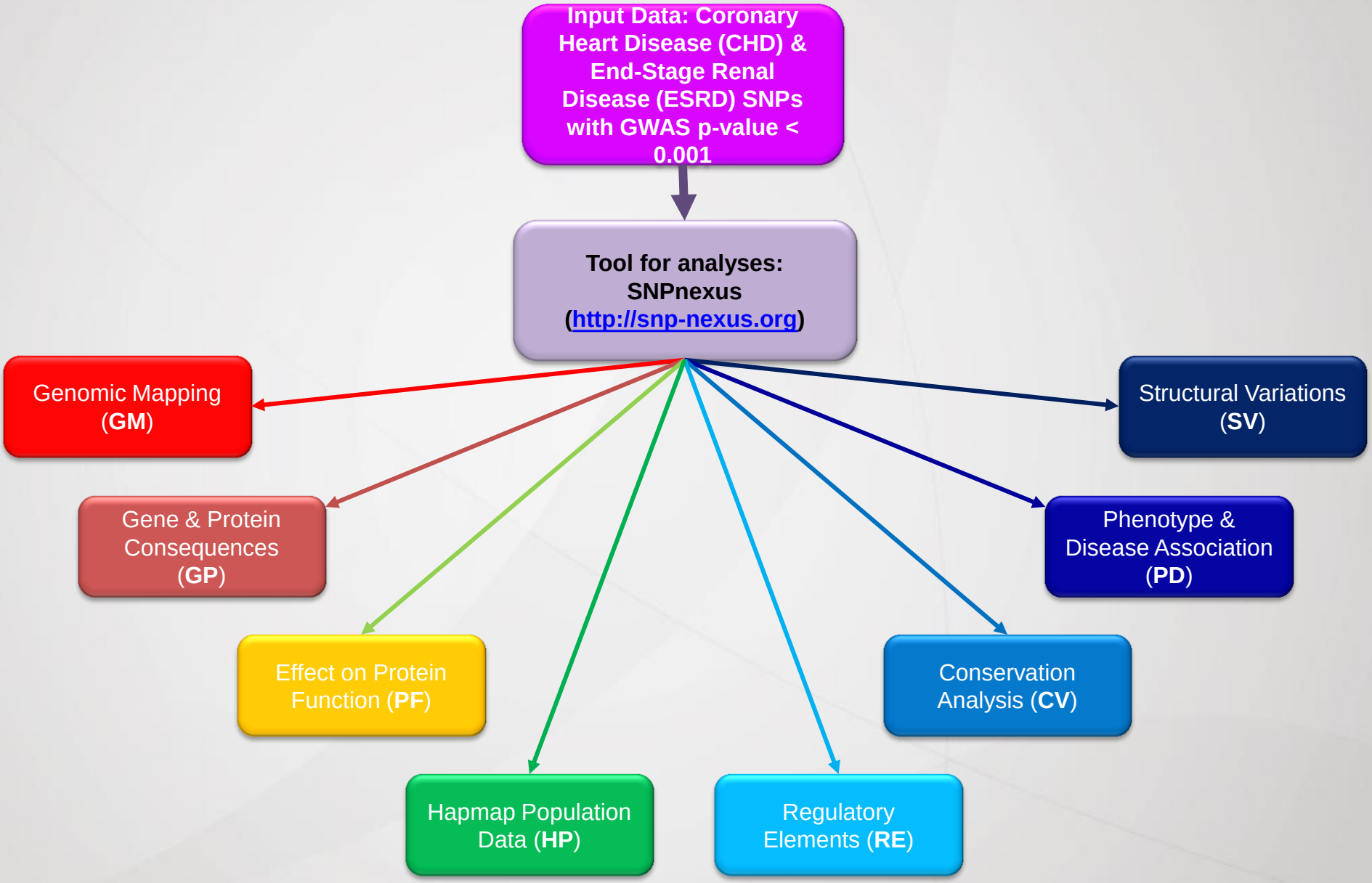
Epigenome-wide association
485K methylation sites
Incident DKD or CHD



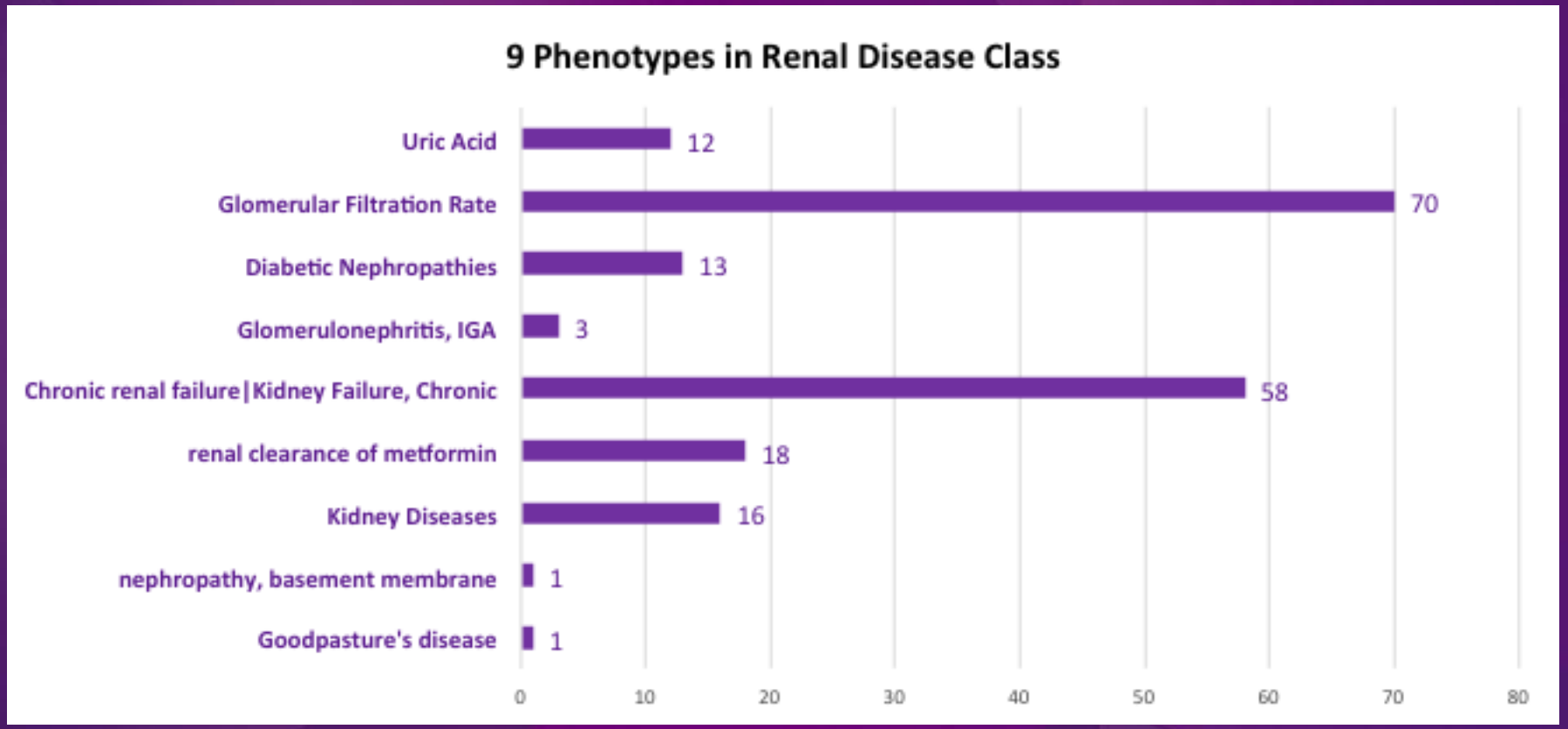
GWAS for ESRD in Type 2 Diabetes
614 cases: 1430 controls



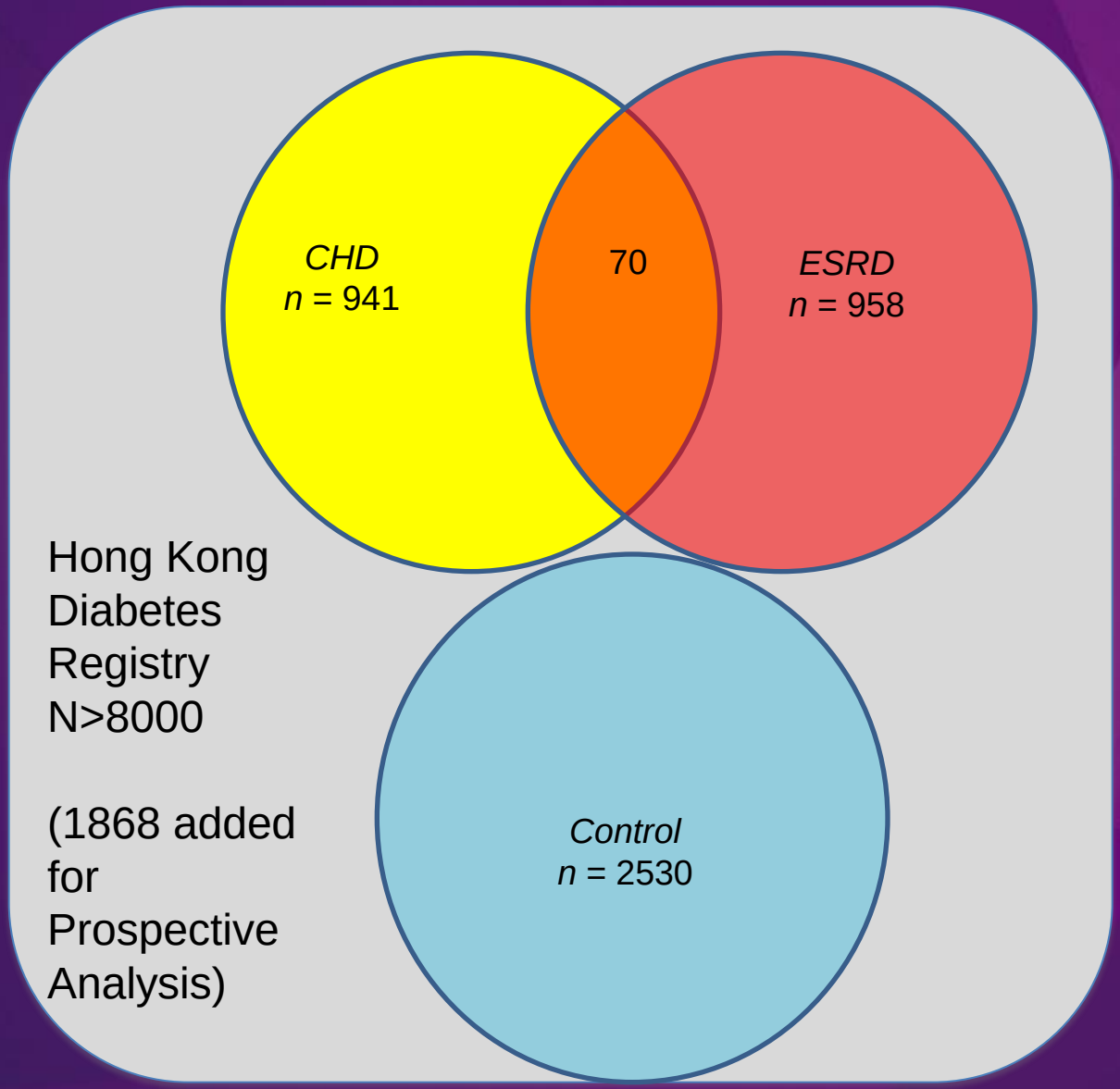
Workflow


Input Data: Coronary Heart Disease (CHD) & End-Stage Renal Disease (ESRD) SNPs with GWAS p-value < 0.001
Tool for analyses: SNPnexus (http://snp-nexus.org)
Genomic Mapping (GM)
Structural Variations (SV)
Gene & Protein Consequences (GP)
Phenotype & Disease Association (PD)
Effect on Protein Function (PF)
Conservation Analysis (CV)
Hapmap Population Data (HP)
Regulatory Elements (RE)

Totally 154 of the top ESRD SNPs were classified to 9 phenotypes in RENAL disease class



GWAS (phase 1+2=7003)



Hong Kong Diabetes Registry N>8000

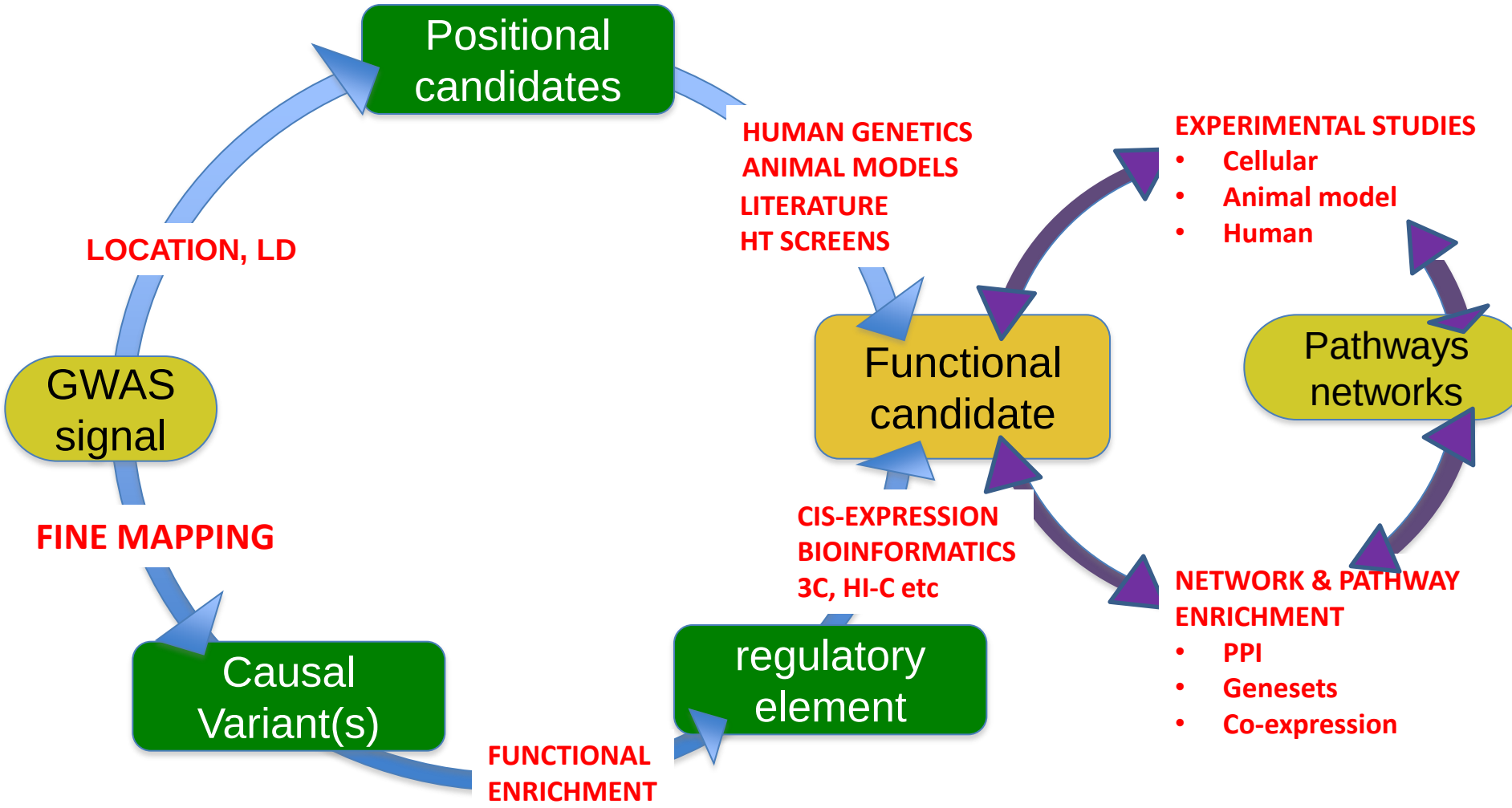
(1868 added for Prospective Analysis)

ESRD: *n* = 1028

1. ESRD endpoint (till 2013); no CVD and ESRD history
2. ESRD history; no CVD history
3. Complete phenotype data

Control: *n* = 2530

1. no CVD and CKD history
2. no CVD and CKD endpoint (till 2013)
3. have lab test results
4. Follow-up + duration of DM ≥10 years
5. Complete phenotype data



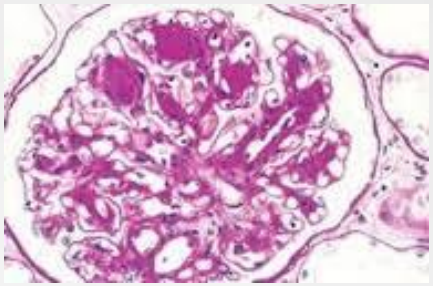
NEPHROMINE

GTEx Portal

Adapted from McCarthy MI

Identifying gene changes in DM nephropathy

Recruitment
Consented



Laser-capture
Microdissection

glomeruli tubules

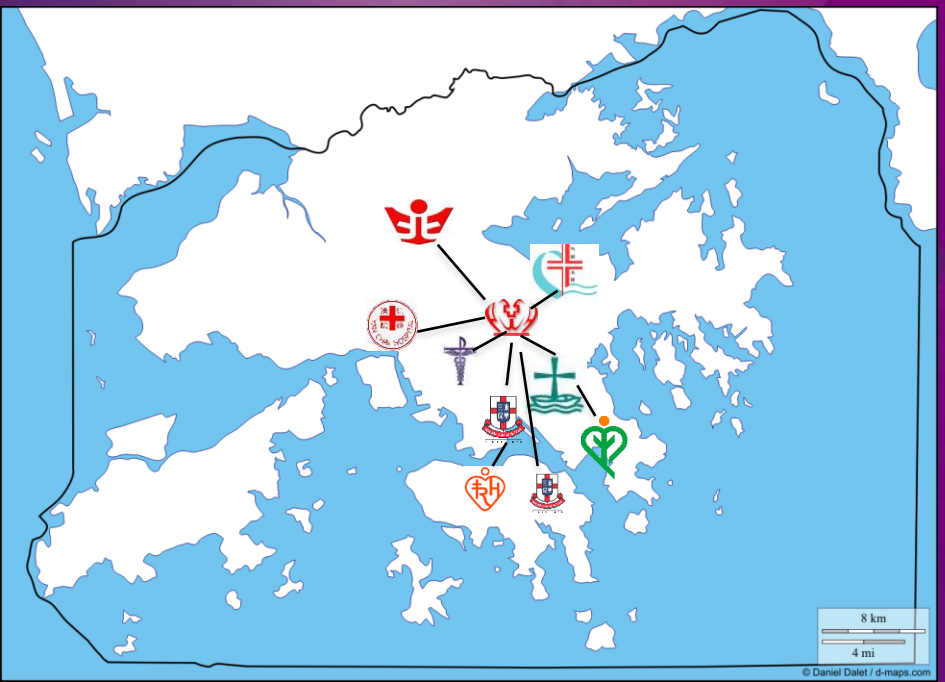
RNA : RNA-seq

Call back
DM complication sx
Blood taking

Whole blood DNA
Methylation array
Genotyping
miRNA

HONG KONG DIABETES
BIOBANK
香港糖尿病生物庫

- 10 hospitals across different regions in Hong Kong
- Aim to recruit over >10,000 Chinese patients with T2DM
- Baseline clinical information at complications screen
- Follow-up for development of diabetic complications, tx failure
- Recruited over 6,000 patients



	With CKD	W/out CKD
N	1109 (26.7%)	3033
M/F	677/ 432	1742/ 1291
Age	67.2	58.6

Summary

- Genetic studies have identified few genetic factors associated with the risk of diabetic kidney disease
- Novel data-mining approaches may uncover association not previously identified using conventional approaches
- Genetic predictors of DM complications may aid identification of high-risk subjects
- Translation to better management of diabetes and associated risk factors and provide novel drug targets

TRANSCEND CONSORTIUM
TRANS-OMICS ANALYSIS OF COMPLICATIONS AND ENDPOINTS IN DIABETES

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Innovation and Technology Commission
The Government of the Hong Kong Special Administrative Region

Research Fund Secretariat
Food and Health Bureau
The Government of the Hong Kong Special Administrative Region

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NIDDK
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

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Thank you
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