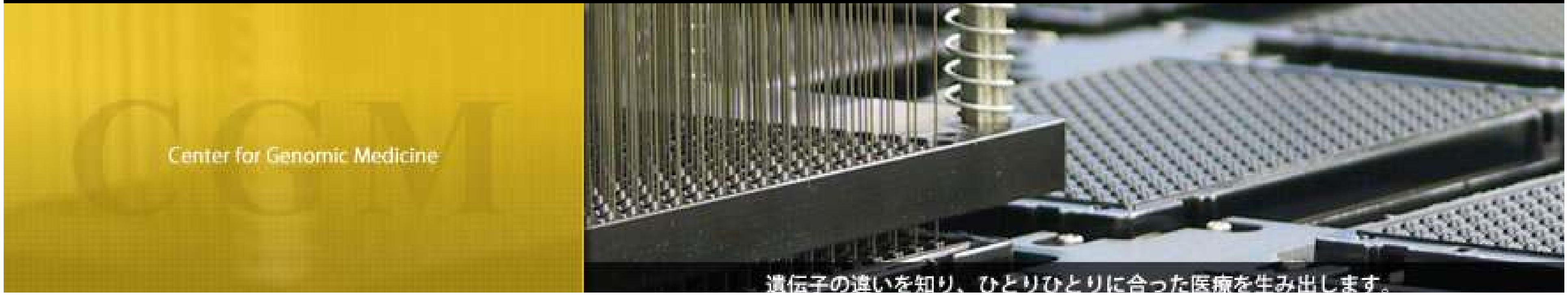
2012 International Conference on Diabetes and Metabolism (ICDM)

Symposium 11
Genetics in Diabetes

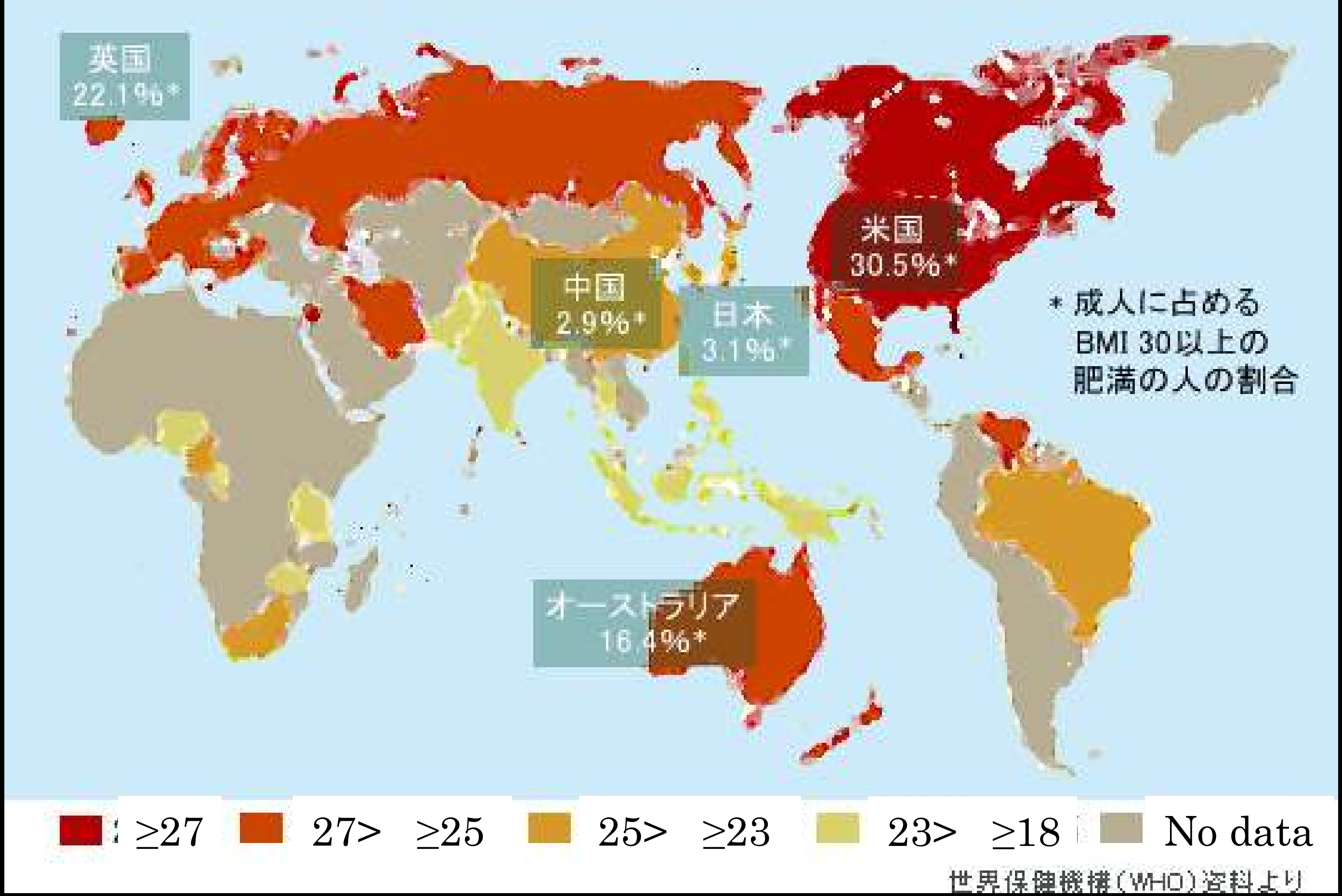
Genome-wide association study for type 2 diabetes in Japan

Laboratory for Endocrinology and Metabolism
RIKEN Center for Genomic Medicine

Shiro Maeda


CGM
Center for Genomic Medicine
遺伝子の違いを知り、ひとりひとりに合った医療を生み出します。

Mean BMI for subjects with age ≥ 15 at 2005


英国
22.1%*
中国
2.9%*
日本
3.1%*
米国
30.5%*
オーストラリア
16.4%*
≥27
27> ≥25
25> ≥23
23> ≥18
No data

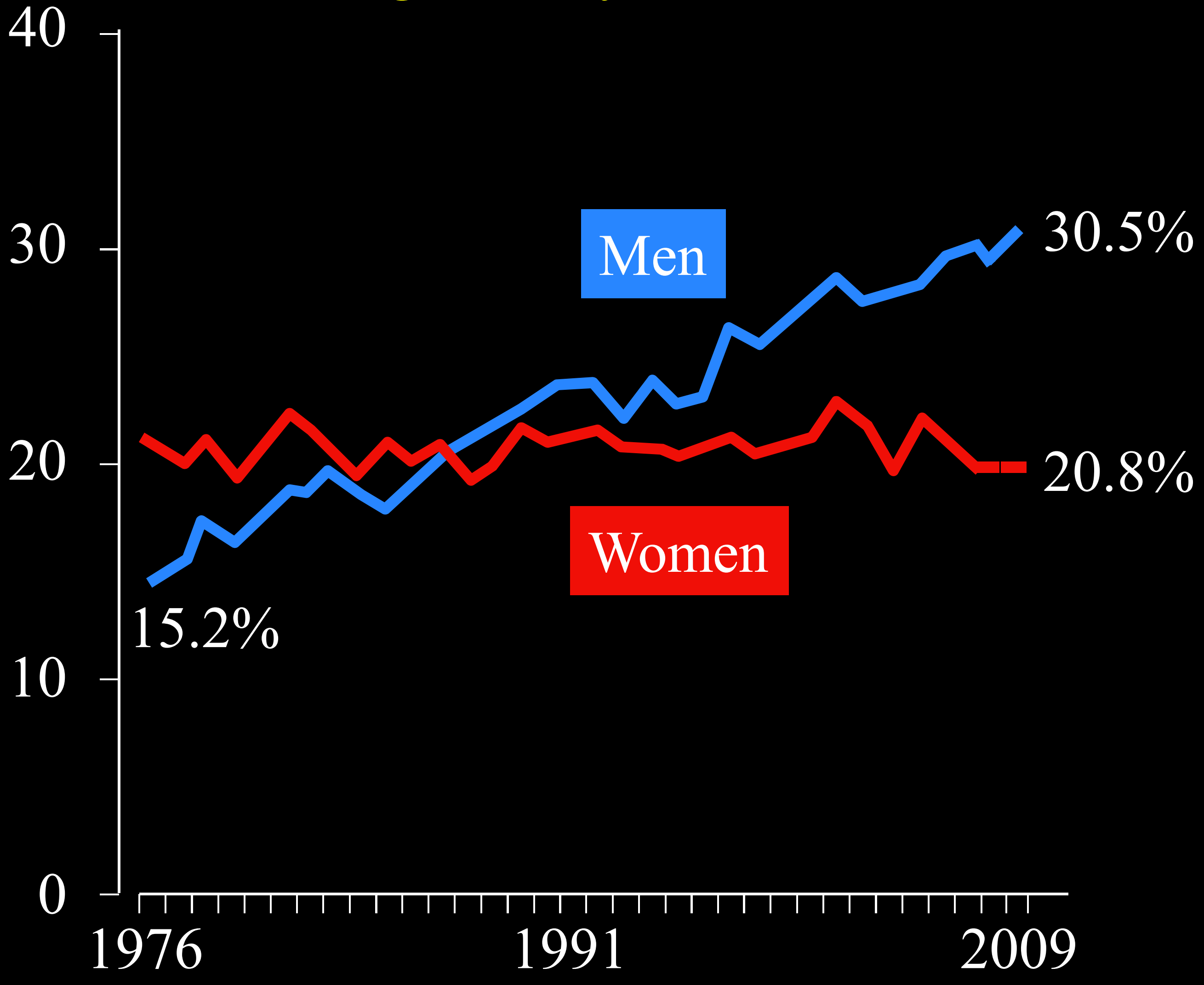
* 成人に占める
BMI 30以上の
肥満の人の割合

世界保健機構(WHO)資料より

Number of subjects with obesity (BMI ≥ 30): 700,000,000

Number of subjects with overweight (BMI ≥ 25): 2,300,000,000

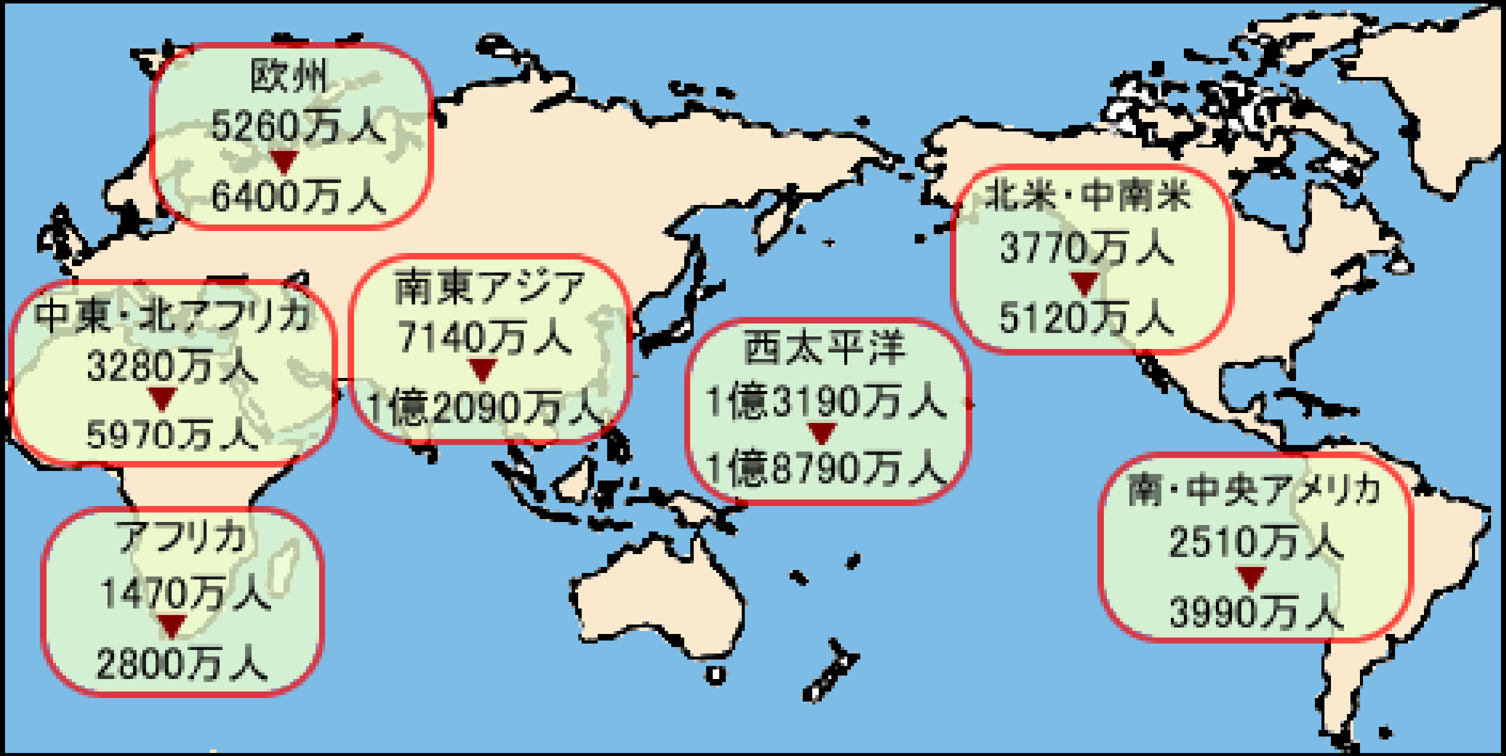
Percentage of subjects with BMI ≥ 25 in Japanese population
- Age > 20 years -



Ministry of Health, Labour and Welfare, 2009

Diabetes mellitus affects 366,200,000 individuals worldwide at 2011

2011年 3億6620万人 ▶ 2030年 5億5180万人


欧州
5260万人
6400万人
中東・北アフリカ
3280万人
5970万人
南東アジア
7140万人
1億2090万人
西太平洋
1億3190万人
1億8790万人
北米・中南米
3770万人
5120万人
アフリカ
1470万人
2800万人
南・中央アメリカ
2510万人
3990万人

International Diabetes Federation Diabetes Atlas 3rd Edition（2011）

551,800,000 peoples will be suffered from diabetes at 2030

Medical Cost for diabetes：302,500,000,000US＄

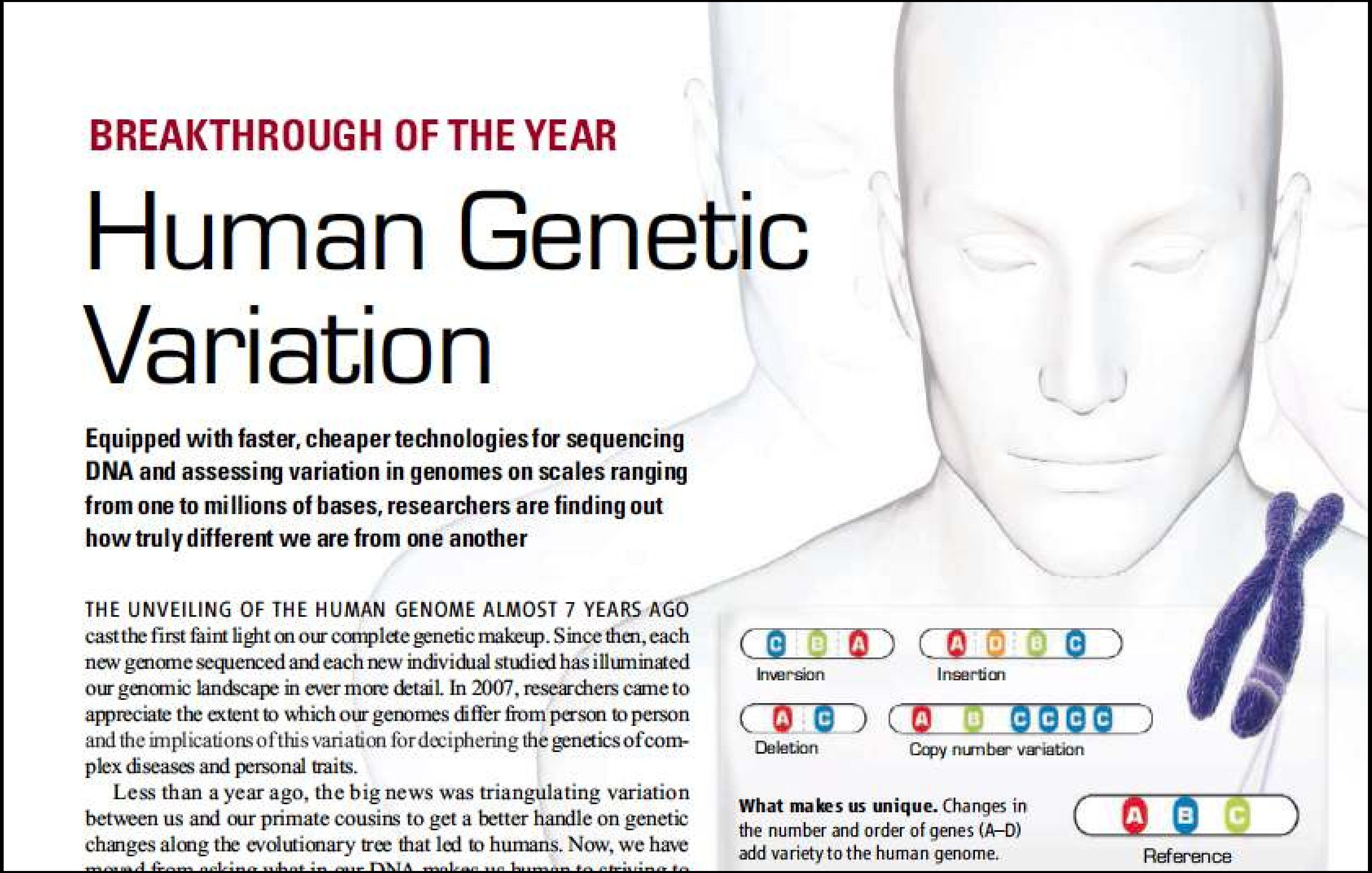
The Present State of Diabetes in Japan

Estimated Number of Diabetes : 10,700,000

Estimated Number of Pre-Diabetes : 14,900,000

One of three in age ≥ 40 might have diabetes

Medical Cost : 1,900,000,000,000 JYE



BREAKTHROUGH OF THE YEAR

Human Genetic Variation

Equipped with faster, cheaper technologies for sequencing DNA and assessing variation in genomes on scales ranging from one to millions of bases, researchers are finding out how truly different we are from one another

THE UNVEILING OF THE HUMAN GENOME ALMOST 7 YEARS AGO cast the first faint light on our complete genetic makeup. Since then, each new genome sequenced and each new individual studied has illuminated our genomic landscape in ever more detail. In 2007, researchers came to appreciate the extent to which our genomes differ from person to person and the implications of this variation for deciphering the genetics of complex diseases and personal traits.

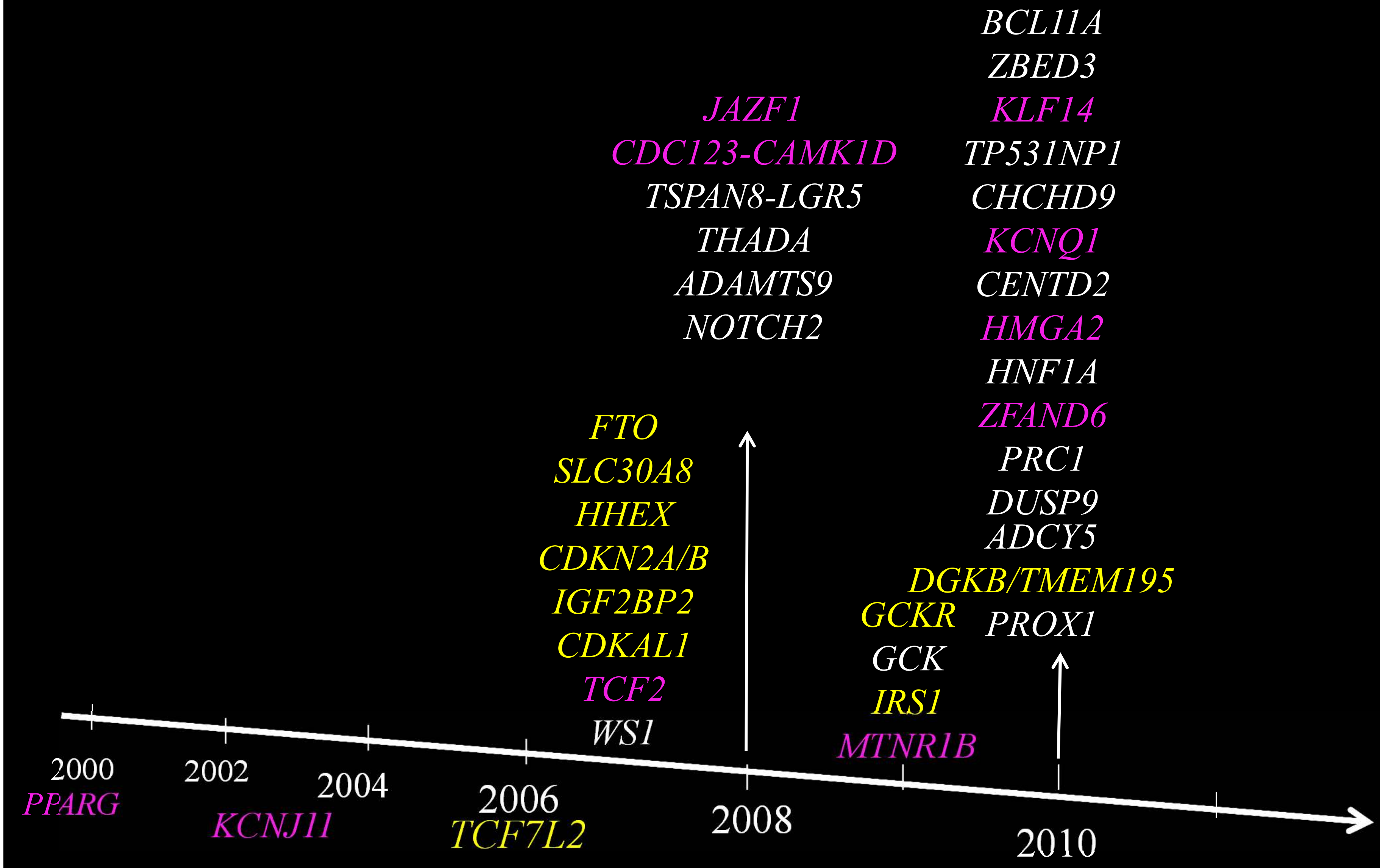
Less than a year ago, the big news was triangulating variation between us and our primate cousins to get a better handle on genetic changes along the evolutionary tree that led to humans. Now, we have

What makes us unique. Changes in the number and order of genes (A–D) add variety to the human genome.

GENOME WIDE ASSOCIATION STUDY

(Science 318; 1842, 2007)

Discovery of genetic loci for type 2 diabetes



2000
PPARG

2002
KCNJ11

2004

2006
TCF7L2

FTO
SLC30A8
HHEX
CDKN2A/B
IGF2BP2
CDKAL1
TCF2
WS1

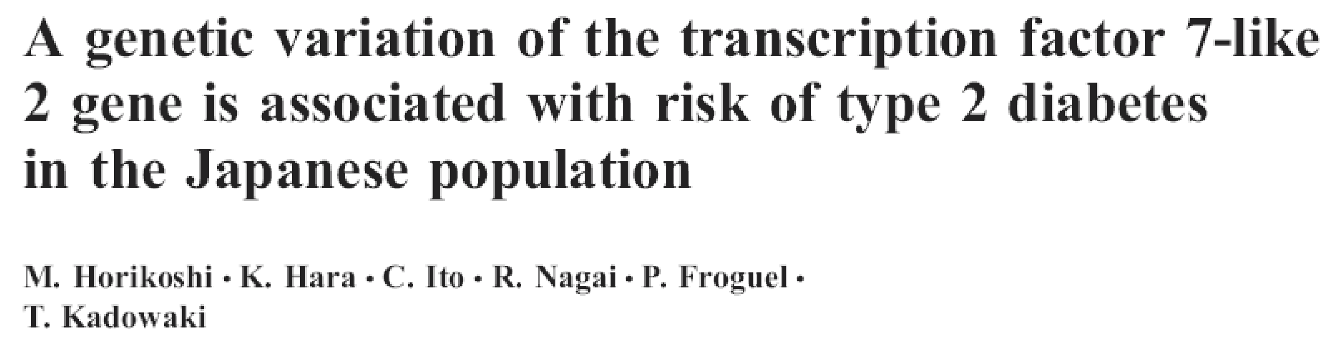
2008

JAZF1
CDC123-CAMK1D
TSPAN8-LGR5
THADA
ADAMTS9
NOTCH2

GCKR
GCK
IRS1
MTNR1B

2010

BCL11A
ZBED3
KLF14
TP531NP1
CHCHD9
KCNQ1
CENTD2
HMGA2
HNF1A
ZFAND6
PRC1
DUSP9
ADCY5
DGKB/TMEM195
PROX1



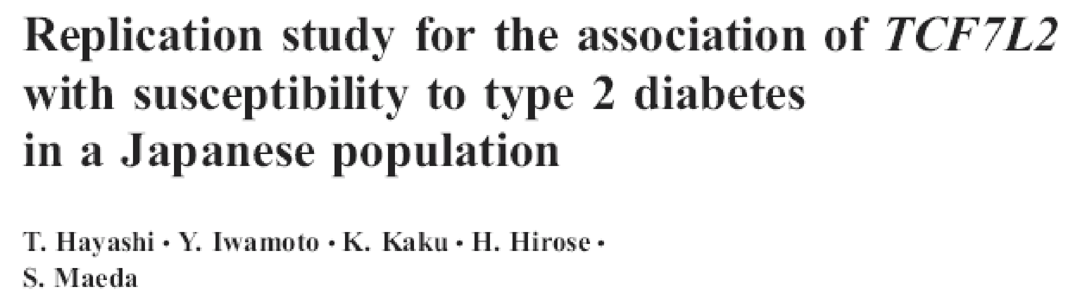
A genetic variation of the transcription factor 7-like 2 gene is associated with risk of type 2 diabetes in the Japanese population

M. Horikoshi · K. Hara · C. Ito · R. Nagai · P. Froguel ·
T. Kadowaki

Association of *TCF7L2* polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects

Kishio Nanjo · Takashi Kadowaki · Masato Kasuga

Variations in *TCF7L2* are associated with type 2 diabetes also in the Japanese



Replication study for the association of *TCF7L2* with susceptibility to type 2 diabetes in a Japanese population

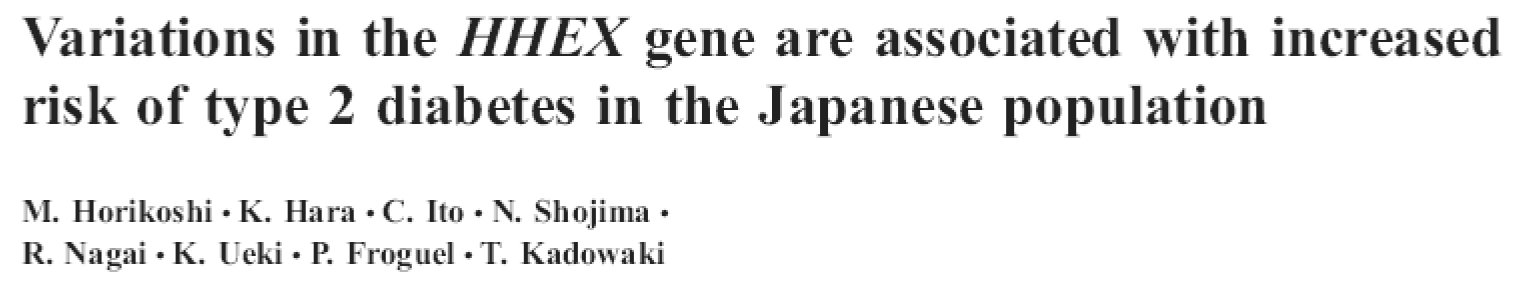
T. Hayashi · Y. Iwamoto · K. Kaku · H. Hirose ·
S. Maeda

Association of polymorphisms in TCF7L2 with type 2 diabetes in a Japanese population

- Type 2 diabetes (T2D) 1,630 vs. Control (CN)1,064 -

	Risk allele frequencies (%)				*P*	
	Japanese		European			
	T2D	CN	T2D	CN	Japanese	European
DG10S478	3.5	2.3	36	28	0.019	$2x10^{-9}$($5x10^{-18}$)
rs12255372	3.5	2.2	36	28	0.002	$5x10^{-8}$($3x10^{-16}$)
rs7903146	5.4	4.2	39	30	0.048	$2x10^{-9}$($2x10^{-17}$)

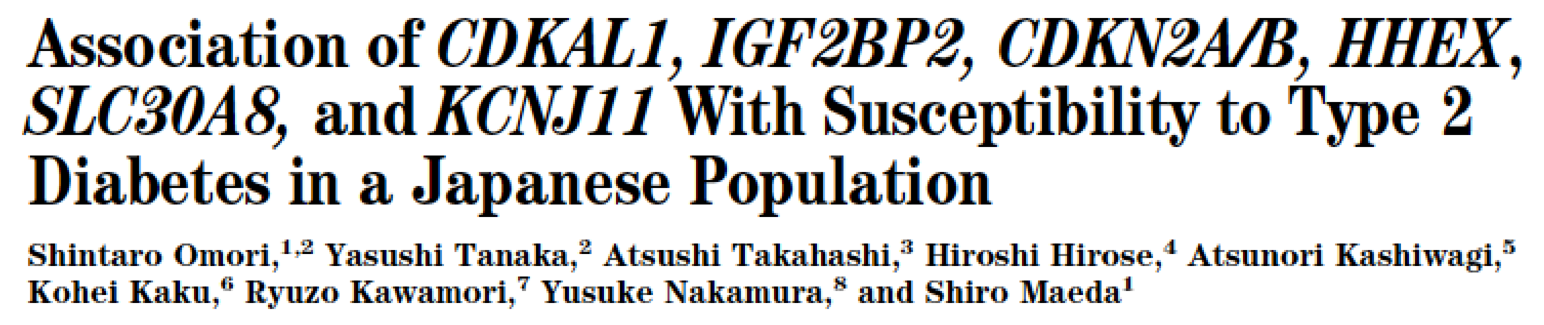
(Hayashi T et al. *Diabetologia*, 2007)



Variations in the *HHEX* gene are associated with increased risk of type 2 diabetes in the Japanese population

M. Horikoshi · K. Hara · C. Ito · N. Shojima ·
R. Nagai · K. Ueki · P. Froguel · T. Kadowaki

Many of European GWAS-derived loci are associated with type 2 diabetes also in the Japanese, but the others are not.



Association of *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8*, and *KCNJ11* With Susceptibility to Type 2 Diabetes in a Japanese Population

Shintaro Omori,[1,2] Yasushi Tanaka,[2] Atsushi Takahashi,[3] Hiroshi Hirose,[4] Atsunori Kashiwagi,[5]
Kohei Kaku,[6] Ryuzo Kawamori,[7] Yusuke Nakamura,[8] and Shiro Maeda[1]

Comparison of risk allele frequencies between Japanese and European populations

Gene	Chromosome	SNP	Japanese(%)	European(%)
TCF7L2	10q25.3	rs7903146	4.2	30.0
IGF2BP2	3q27.2	rs4402960	29.3	31.3
CDKN2A/B	9p21	rs10811661	56.1	84.1
HHEX	10q23	rs1111875	28.4	57.7
SLC30A8	8q24.11	rs13266634	60.0	63.4
CDKAL1	6p22.3	rs7756992	47.4	23.2
FTO	16q12.2	rs9939609	19.4	38.5
KCNJ11	11p15.1	rs5219	35.5	46.4
PPARG	3p25	rs1801282	97.3	82.4
JAZF1	7p15	rs864745	78.5	50.1
CDC123-CAMK1D	10p13	rs12779790	14.9	18.3
TSPAN8-LGR5	12q21	rs7961581	21.3	26.9
THADA	2p21	rs7578597	100	90.2
ADAMTS9	3p14	rs4607103	59.9	76.1
NOTCH2	1p13-p11	rs10923931	1.7	10.6

(Hayashi et al. *Diabetologia*, 2007, Omori et al. *Diabetes*, 2008)

Genome-Wide Association Studies in Japan

1. Millennium Genome Project
2. BioBank Japan Project

個性にあった未来の医療
オーダーメイド医療の実現をめざして

文部科学省リーディングプロジェクト

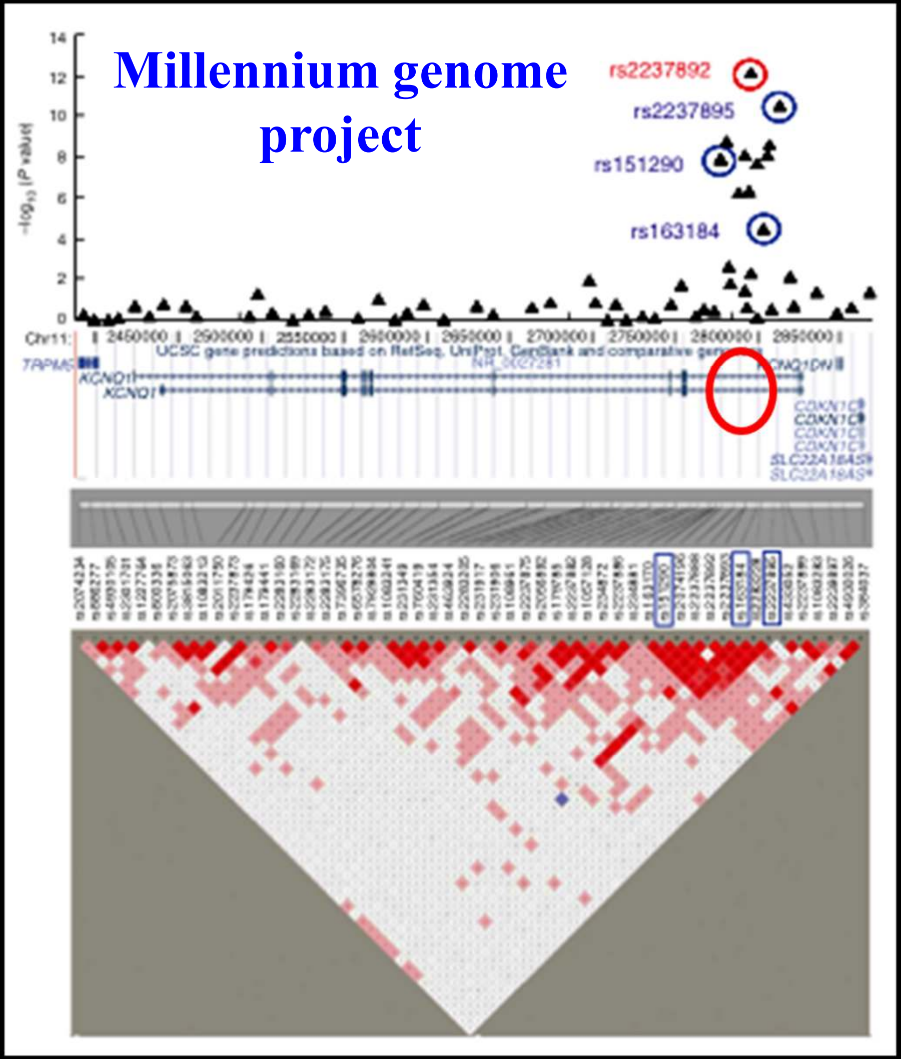
個人の遺伝情報に応じた医療の実現プロジェクト
（オーダーメイド医療実現化プロジェクト）

ホームページアドレス
http://biobankjp.org/

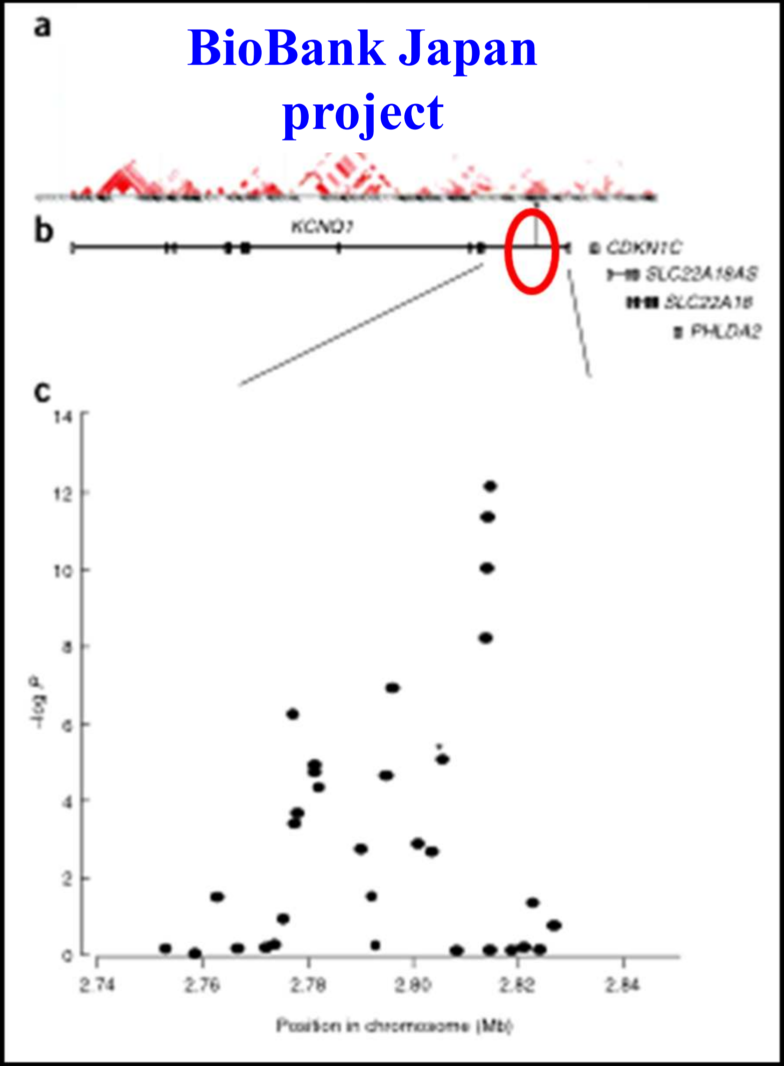
さらにひろがる
みんなの想い

個人の遺伝情報に応じた医療の実現プロジェクト
オーダーメイド医療実現化プロジェクト

Two Japanese GWAS identified *KCNQ1* at 11q15 as strong susceptibility locus for type 2 diabetes



Millennium genome project



BioBank Japan project

Association of SNPs in the KCNQ1 with type 2 diabetes across different ethnicities

	risk allele freq			
rs2237897	T2DM	control	OR (95% CI)	*P*
Japanese(3522:1320)	0.68	0.61	1.41(1.29-1.55)	6.8×10^{-13}
Singaporean(1433:1735)	0.69	0.65	1.22(1.09-1.35)	2.4×10^{-4}
Dane(3891:4888)	0.97	0.96	1.36(1.16-1.60)	1.2×10^{-4}

(Unoki H et al. *Nat Genet*, 2008)

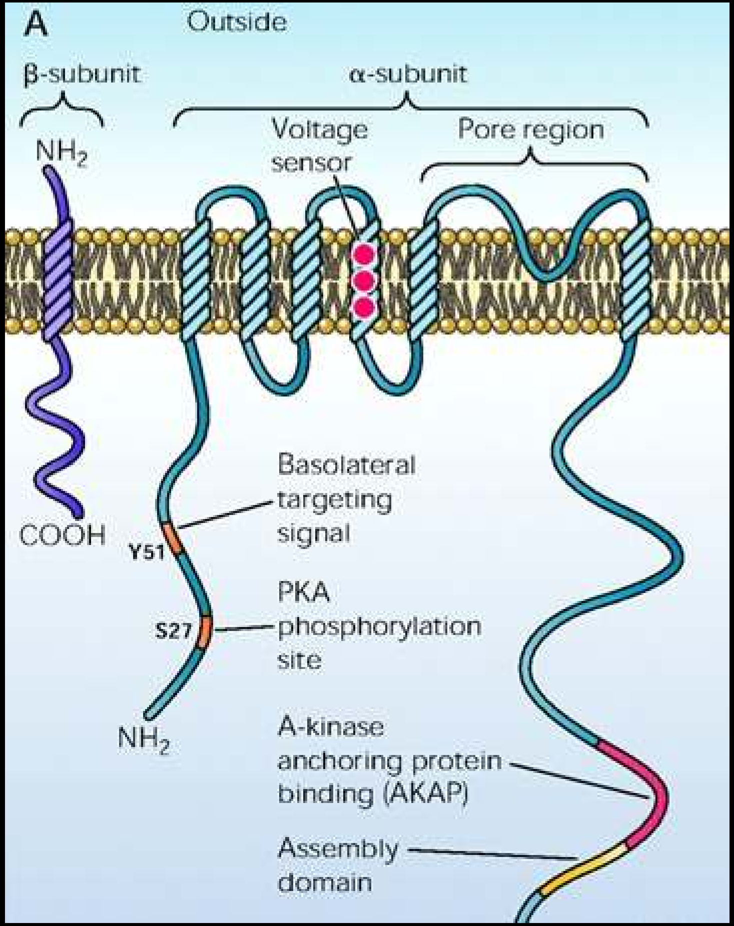
	risk allele freq			
rs2237892	T2DM	control	OR (95% CI)	*P*
Japanese 1(1414:1421)	0.69	0.60	1.49(1.34-1.66)	1.7×10^{-12}
Japanese 2(1481:1513)	0.66	0.59	1.39(1.25-1.54)	1.6×10^{-9}
Japanese 3(1420:1431)	0.68	0.60	1.41(1.26-1.57)	1.1×10^{-9}
Chinese (1356:1540)	0.72	0.65	1.38(1.24-1.55)	4.2×10^{-9}
Korean (752:628)	0.69	0.61	1.41(1.21-1.65)	1.0×10^{-5}
Swedish(2759:3426)	0.95	0.93	1.29(1.11-1.50)	7.2×10^{-4}

(Yasuda K et al. *Nat Genet*, 2008)

KCNQ1 (KVLQT1)

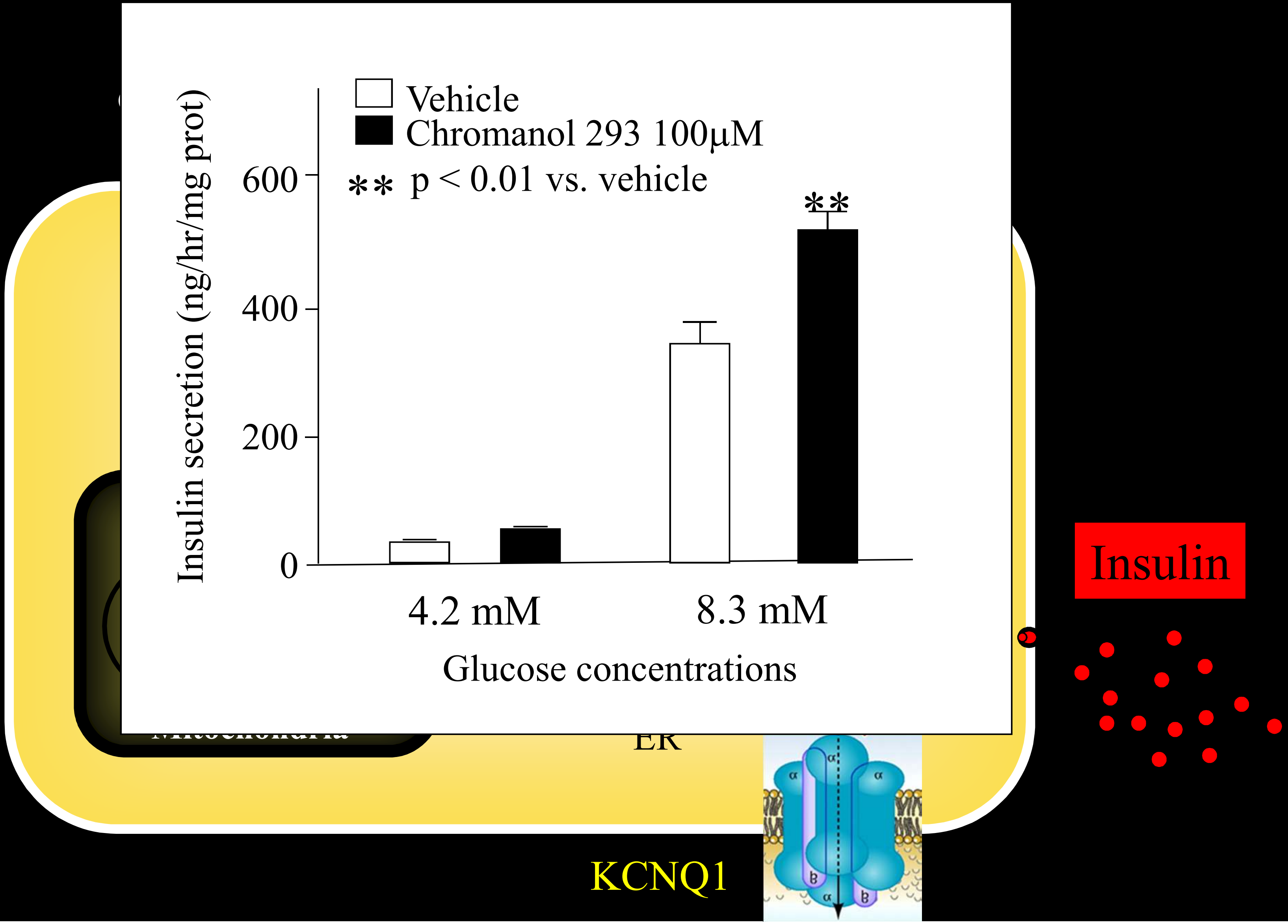
(pottasium votage-gated channel, KQT-like subfamily, member 1)

11p15.5

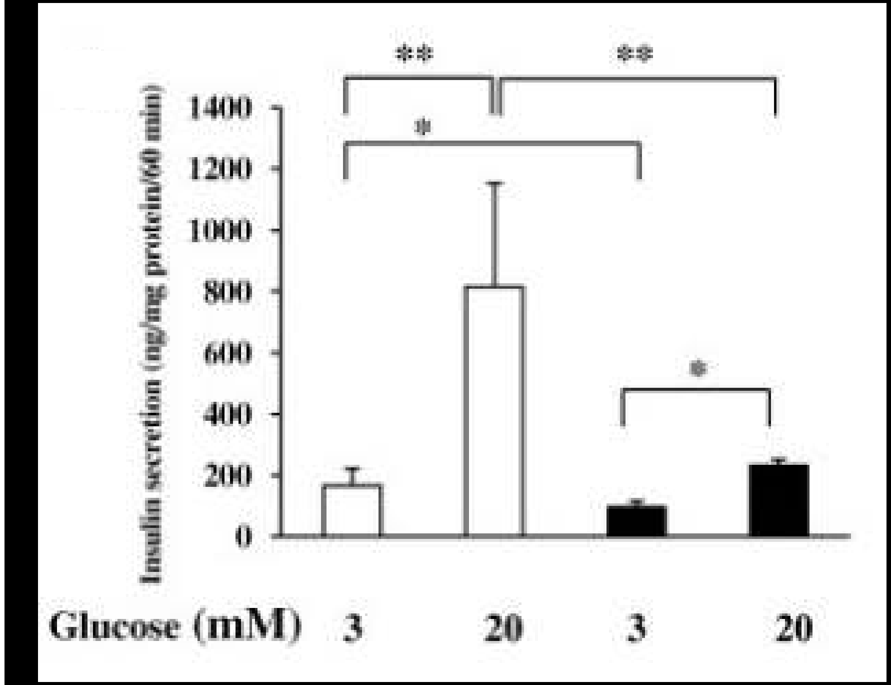

A
Outside
β-subunit
α-subunit
Voltage sensor
Pore region
NH2
COOH
Basolateral targeting signal
Y51
PKA phosphorylation site
S27
NH2
A-kinase anchoring protein binding (AKAP)
Assembly domain

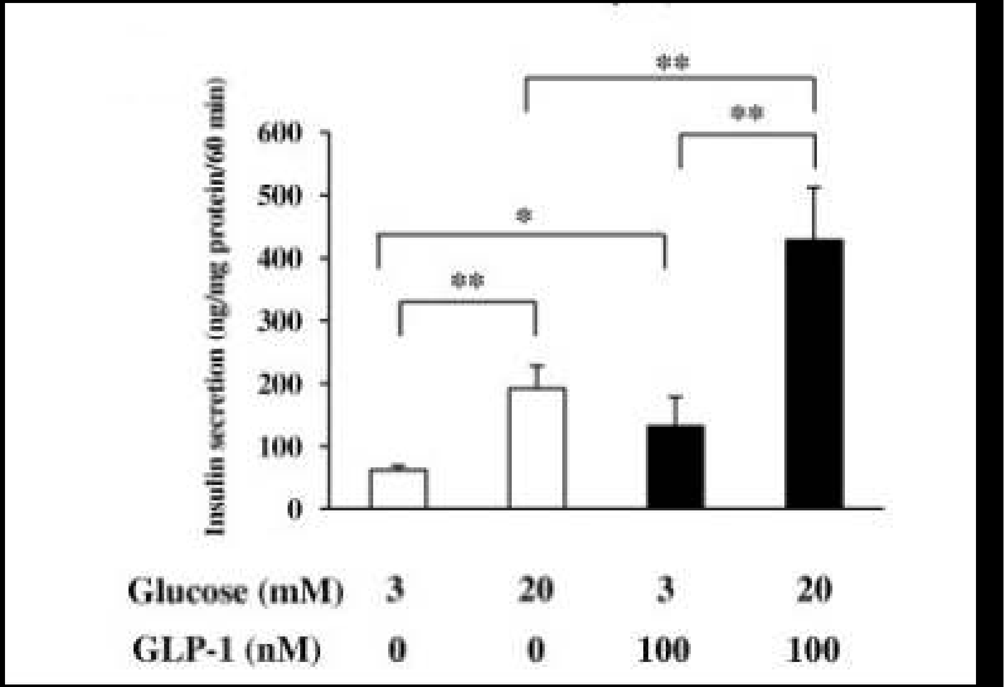
(Jespersen T et al. Physiology, 2005)

Role of potassium channels in insulin secretion from pancreatic β cells



Over expression of *KCNQ1* inhibits glucose-induced insulin secretion in MIN6 cells





(Yamagata K et al. *Biochem Biophys Res Commun*, 2011)

Association of SNPs in the *KCNQ1* with type 2 diabetes across different ethnicities

rs2237897	risk allele freq T2DM	risk allele freq control	OR (95% CI)	*P*
Japanese(3522:1320)	0.68	0.61	1.41(1.29-1.55)	6.8×10^{-13}
Dane(3891:4888)	0.97	0.96	1.36(1.16-1.60)	1.2×10^{-4}

194 T2D vs. 1,558 controls for 207,097 SNPs

(Unoki H et al. *Nat Genet*, 2008)

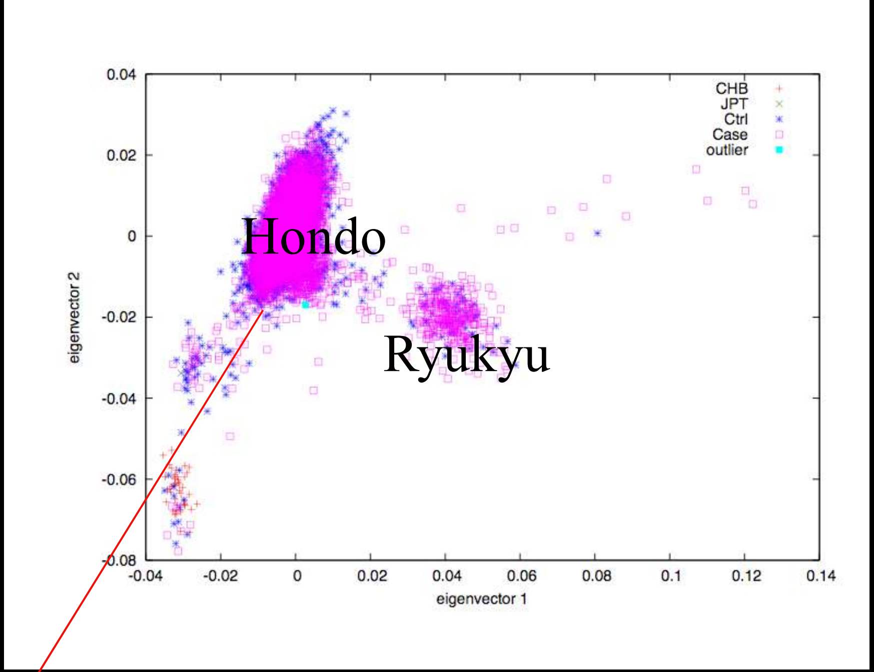
rs2237892	risk allele freq T2DM	risk allele freq control	OR (95% CI)	*P*
Japanese 1(1414:1421)	0.69	0.60	1.49(1.34-1.66)	1.7×10^{-12}
Ja				$^{-9}$
Japanese 3(1420:1431)	0.68	0.60	1.41(1.26-1.57)	1.1×10^{-9}
Chinese (1356:1540)	0.72	0.65	1.38(1.24-1.55)	4.2×10^{-9}
Korean (752:628)	0.69	0.61	1.41(1.21-1.65)	1.0×10^{-5}
Swedish(2759:3426)	0.95	0.93	1.29(1.11-1.50)	7.2×10^{-4}

187 T2D vs. 752 controls for 82,343 SNPs

(Yasuda K et al. *Nat Genet*, 2008)

Genome-wide association study in the Japanese

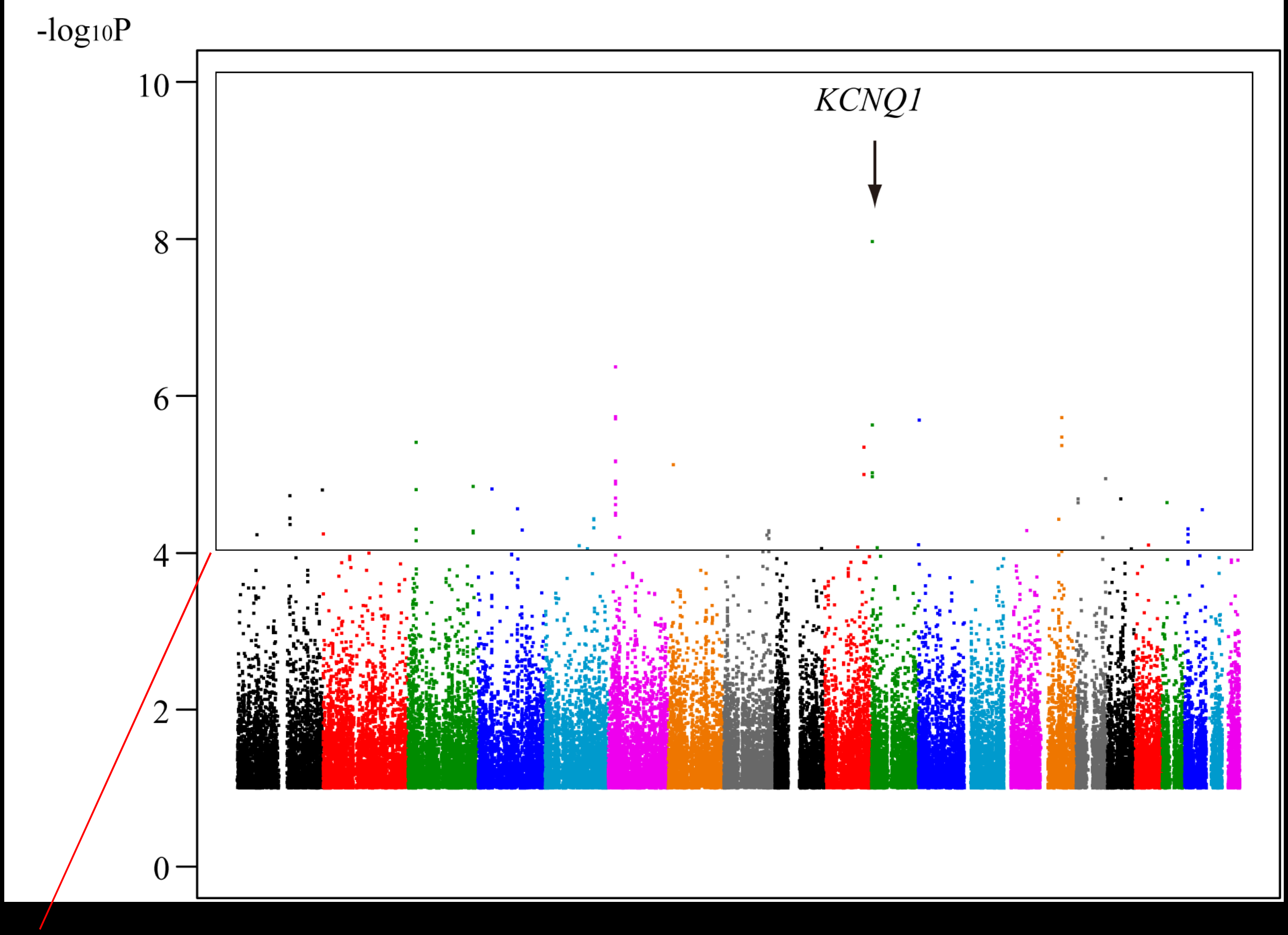
1st stage : **4,878 type 2 diabetes vs. 3,345 controls**



4,470 type 2 diabetes vs. 3,071 controls belong to the Hondo cluster

For 459,359SNPs

Result of 1st stage analysis



Top 100SNPs were forwarded to stage 2 analysis

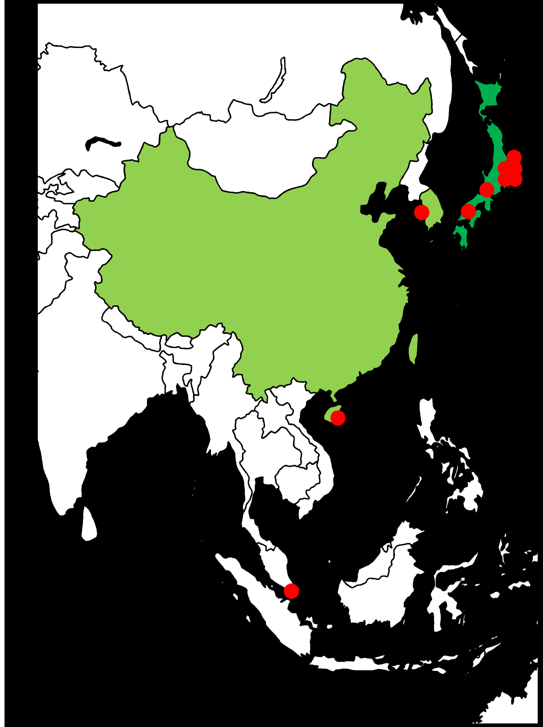
2nd stage： 2,886 type 2 diabetes vs. 3,087 controls

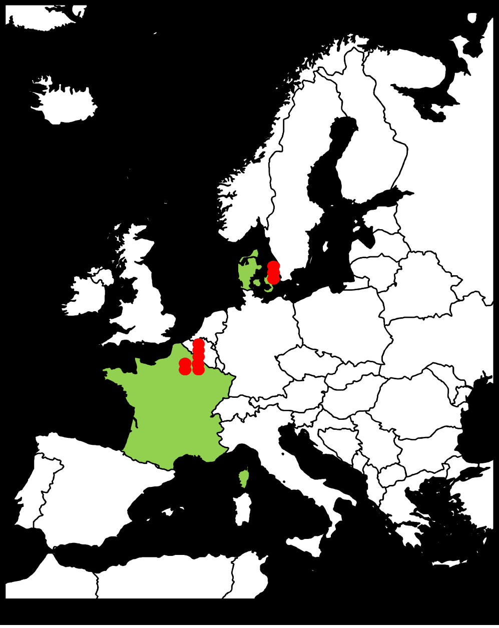
Combined results of 1st and 2nd stage analyses

SNP	Ch	nearest gene	*P* *	OR (95 %CI)
rs2237892	11	*KCNQ1*	6.66×10^{-18}	1.25 (1.19 – 1.31)
rs2206734	6	*CDKAL1*	1.86×10^{-13}	1.20 (1.14 – 1.26)
rs2383208	9	*CDKN2B*	1.45×10^{-11}	1.19 (1.13 – 1.24)
rs7901695	10	*TCF7L2*	4.53×10^{-9}	1.41 (1.26 – 1.58)
rs6780569	3	*UBE2E2*	6.76×10^{-9}	1.21 (1.14 – 1.30)
rs1470579	3	*IGF2BP2*	5.20×10^{-8}	1.15 (1.09 – 1.21)
rs7172432	15	*C2CD4A/B*	7.48×10^{-8}	1.14 (1.09 – 1.20)

*Combined P values for 1st and 2nd stage calculated by Mantel-Haenszel test

(Yamauchi et al. *Nat Genet,* 2010)





Stage 3 analysis

3,622 type 2 diabetes vs. 2,356 controls

Replication study in other East Asian populations
or in European populations

East Asian

(Singapore, Hong-Kong, South Korea）

4,184 type 2 diabetes vs. 4,154 controls

European

(France, Denmark）

6,980 type 2 diabetes vs. 8,615 controls

	T2D	Control	P	OR (95% CI)
***UBE2E2*: rs7612463**				
Japanese	0.854	0.832	2.27×10^{-9}	1.19 (1.12 – 1.26)
Other East Asian	0.825	0.811	0.0306	1.09 (1.01 – 1.18)
East Asian	0.846	0.825	9.16×10^{-10}	1.15 (1.10 – 1.21)
European	0.590	0.566	6.36×10^{-5}	1.10 (1.05 – 1.15)
Total	0.606	0.582	8.78×10^{-14}	1.11 (1.08 – 1.14)

SNP	n	p値	OR (95% C))
rs7612463	9580 T2D vs. 53,810 CN	9.8×10^{-4}	1.10 (1.04 – 1.16)
rs1496653	34.840 T2D vs. 114,981 CN	3.6×10^{-9}	1.09 (1.06 – 1.12)

(Yamauchi et al. *Nat Genet,* 2010)

UBE2E2 (ubiquitin-conjugating enzyme E2E2)

- Location: chromosome 3 (3p24.2)
- Expression: ubiquitous, including pancreas, liver, adipose
- Ubiquitin-proteasome system plays a pivotal role in maintaining normal insulin biosynthesis, secretion and signalling especially under conditions that increase ER stress in pancreatic β cells

C2CD4A/B (C2 calcium-dependent domain containing 4*A/B*)

Alternative symbols: *NLF 1/2, FAM148A/B*

- Location: chromosome 15 (15q22.2)
- Expression: pancreas, insulin secreting cell lines
- Expressions of C2CD4A/B have been shown to be increased by treatment with pro-inflammatory cytokines

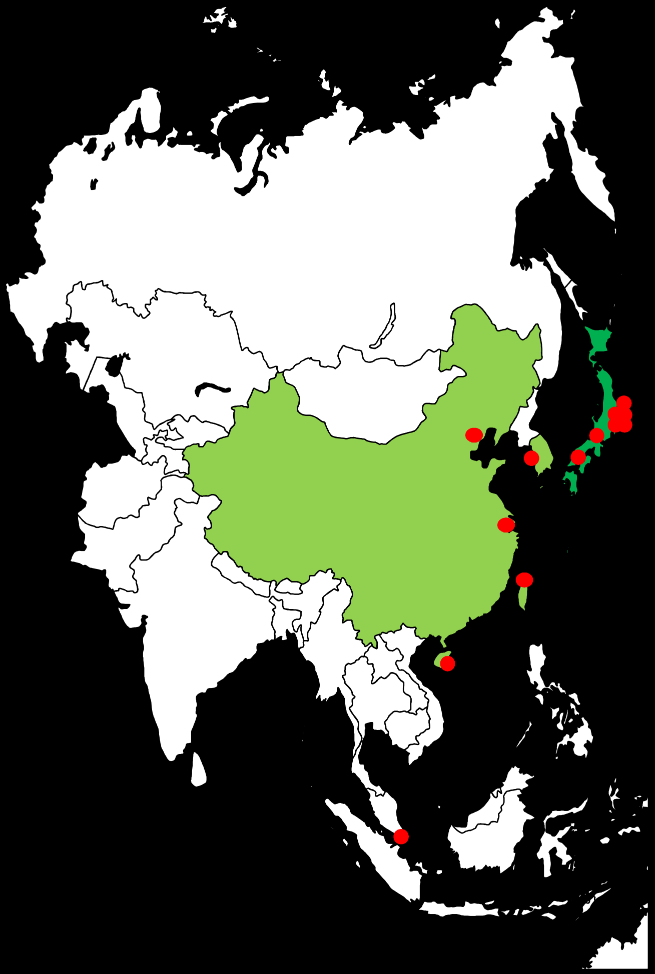
Imputation based GWAS identified a new susceptibility loci for type 2 diabetes at *ANK1* locus
~2,229,890 imputed SNPs~

Association of rs515071 within *ANK1* with type 2 diabetes

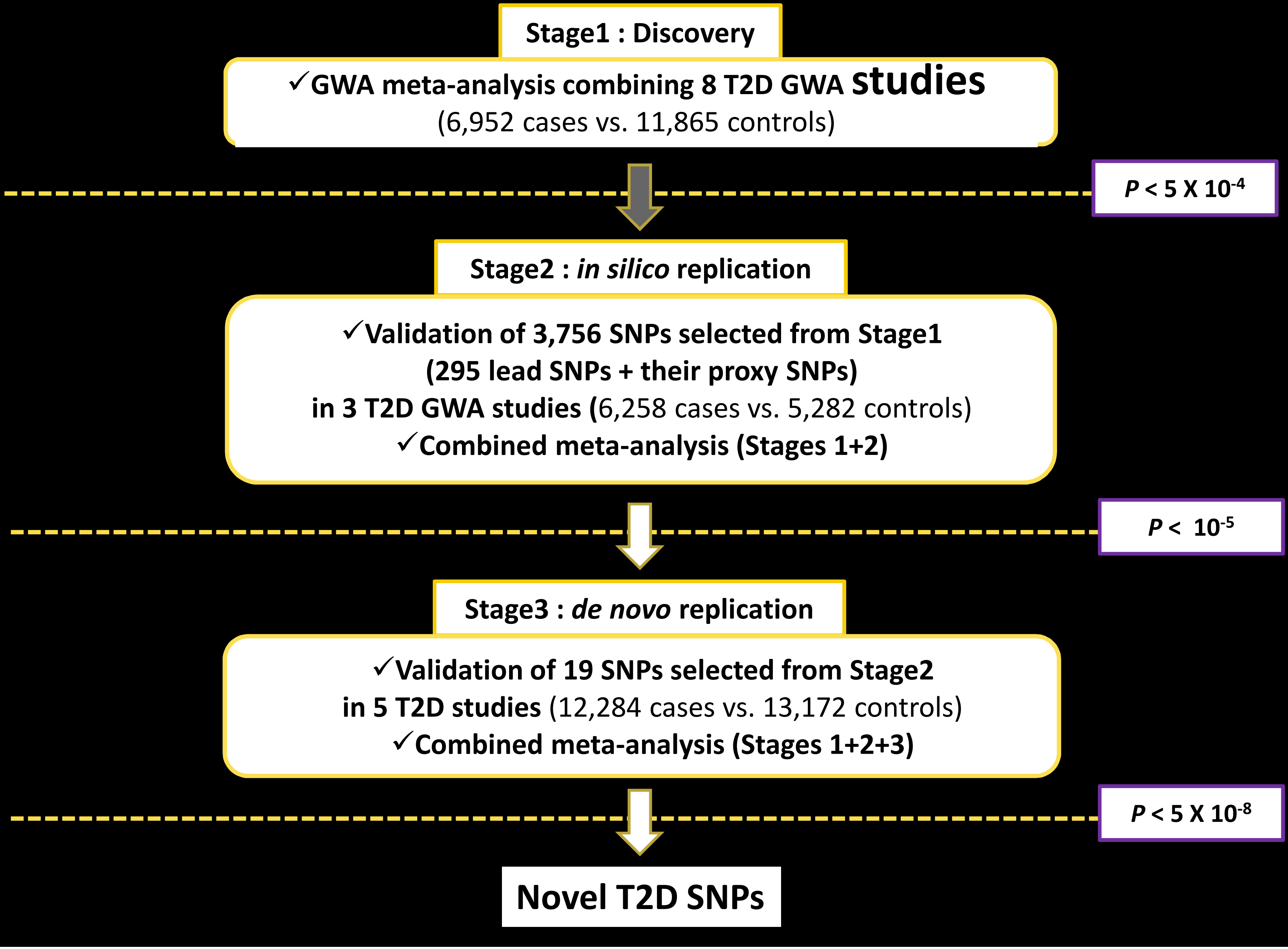
population	n	*P*	OR (95% CI)
Japanese	16,902	1.37×10^{-8}	1.18 (1.12 – 1.25)
Eurpean	22,570	8.54×10^{-4}	1.09 (1.03 – 1.14)

(Imamura M et al. Hum Mol Genet, 2012)

Asian Genetic Epidemiology Network (AGEN)



East Asian T2D GWA meta-analysis



Stage1 : Discovery

✓**GWA meta-analysis combining 8 T2D GWA studies**
(6,952 cases vs. 11,865 controls)

P < 5 X 10^{-4}

Stage2 : *in silico* replication

✓**Validation of 3,756 SNPs selected from Stage1**
(295 lead SNPs + their proxy SNPs)
in 3 T2D GWA studies (6,258 cases vs. 5,282 controls)
✓**Combined meta-analysis (Stages 1+2)**

P < 10^{-5}

Stage3 : *de novo* replication

✓**Validation of 19 SNPs selected from Stage2**
in 5 T2D studies (12,284 cases vs. 13,172 controls)
✓**Combined meta-analysis (Stages 1+2+3)**

P < 5 X 10^{-8}

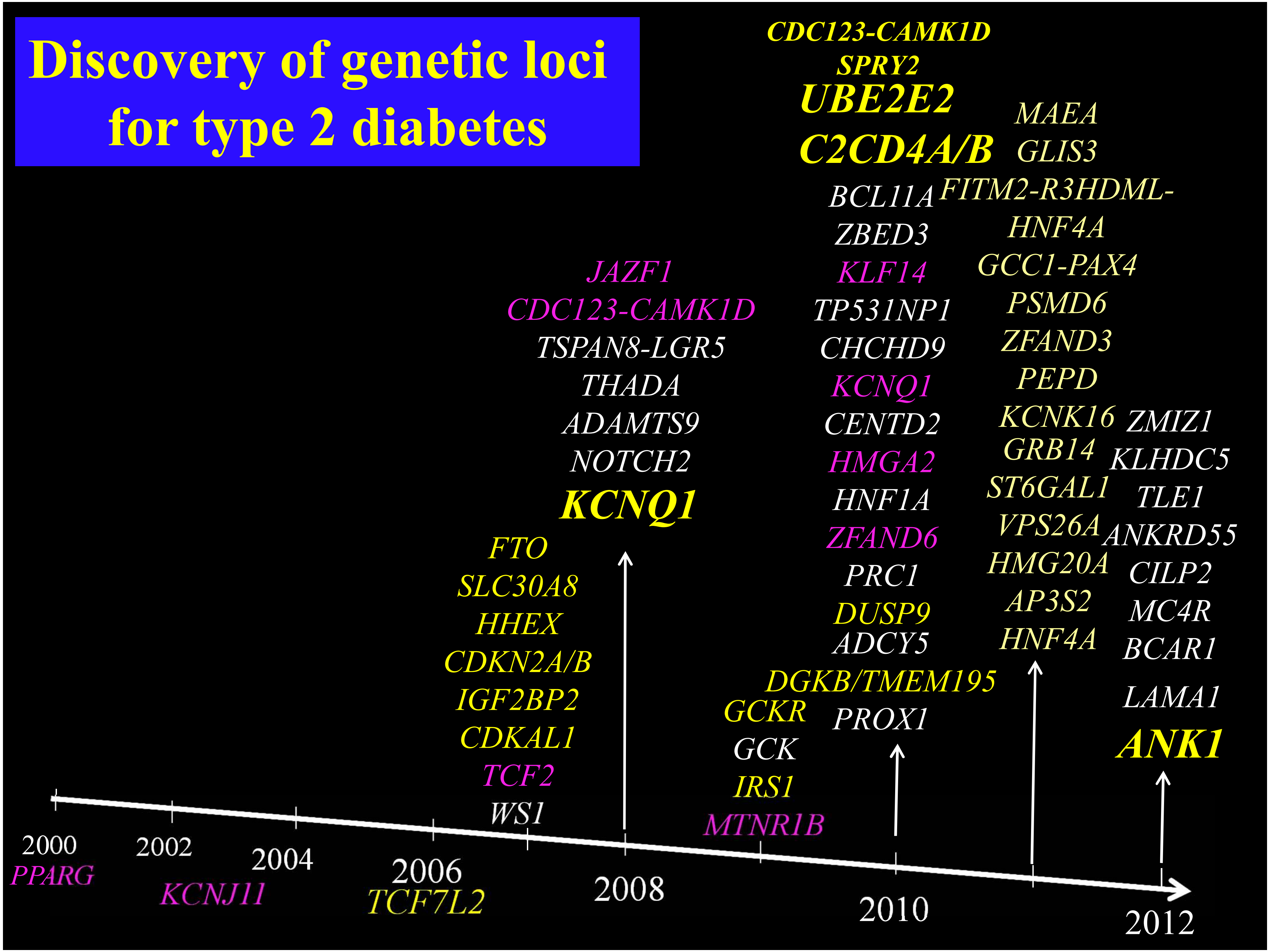
Novel T2D SNPs

East Again GWAS meta-analysis identified 8 novel loci for type 2 diabetes

SNP	chr	Nearest gene(s)	P	P in DIAGRAM*
rs6815464	4	*MAEA*	1.57×10^{-20}	0.00656 (-)
rs7041847	9	*GLIS3*	1.99×10^{-14}	0.0643 (0.00072)
rs6017317	20	*FITM2-R3HDML-HNF4A*	1.12×10^{-11}	0.0147 (-)
rs6467136	7	*GCC1-PAX4*	4.96×10^{-11}	0.489 (0.55)
rs831571	3	*PSMD6*	8.41×10^{-11}	0.445 (0.18)
rs9470794	6	*ZFAND3*	2.06×10^{-10}	0.4 (0.81)
rs3786897	19	*PEPD*	1.30×10^{-8}	0.361 (0.037)
rs1535500	6	*KCNK16*	2.30×10^{-8}	0.921 (-)

* Combined data for stage 1 (DIAGRAMv3) and stage 2 in recent NG paper by Morris AP *et al*. are shown in parenthesis

Discovery of genetic loci for type 2 diabetes



2000
PPARG

2002
KCNJ11

2004

2006
TCF7L2

FTO
SLC30A8
HHEX
CDKN2A/B
IGF2BP2
CDKAL1
TCF2
WS1

JAZF1
CDC123-CAMK1D
TSPAN8-LGR5
THADA
ADAMTS9
NOTCH2
KCNQ1

2008

GCKR
GCK
IRS1
MTNR1B

CDC123-CAMK1D
SPRY2
UBE2E2
C2CD4A/B
BCL11A
ZBED3
KLF14
TP531NP1
CHCHD9
KCNQ1
CENTD2
HMGA2
HNF1A
ZFAND6
PRC1
DUSP9
ADCY5
DGKB/TMEM195
PROX1

2010

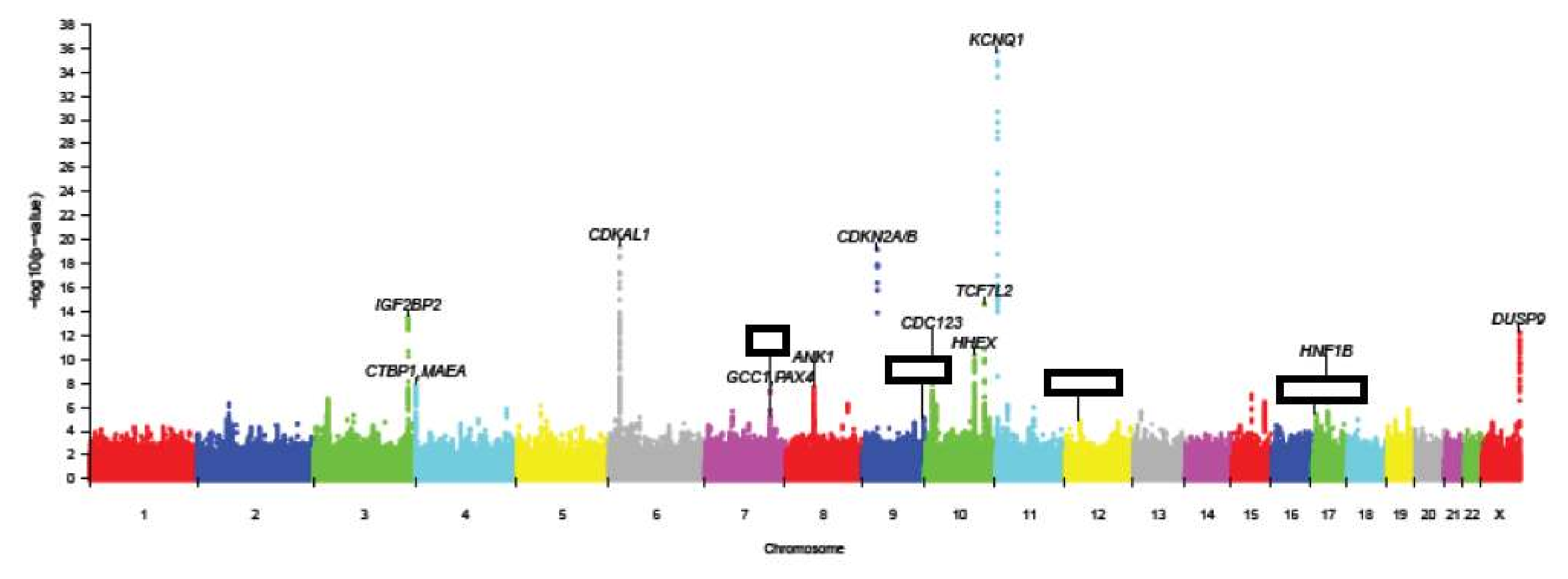
MAEA
GLIS3
FITM2-R3HDML-HNF4A
GCC1-PAX4
PSMD6
ZFAND3
PEPD
KCNK16
GRB14
ST6GAL1
VPS26A
HMG20A
AP3S2
HNF4A

ZMIZ1
KLHDC5
TLE1
ANKRD55
CILP2
MC4R
BCAR1
LAMA1
ANK1

2012

Manhattan plot for the discovery analysis of directly genotyped and imputed SNPs in 5,976 T2D cases and 20,829 controls.

6,209,637 SNPs
(459,359 genotyped＋ 5,750,278 imputed)



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