

Supplemental Data

Identification of *KCNJ15* as a Susceptibility Gene

in Asian Patients with Type 2 Diabetes Mellitus

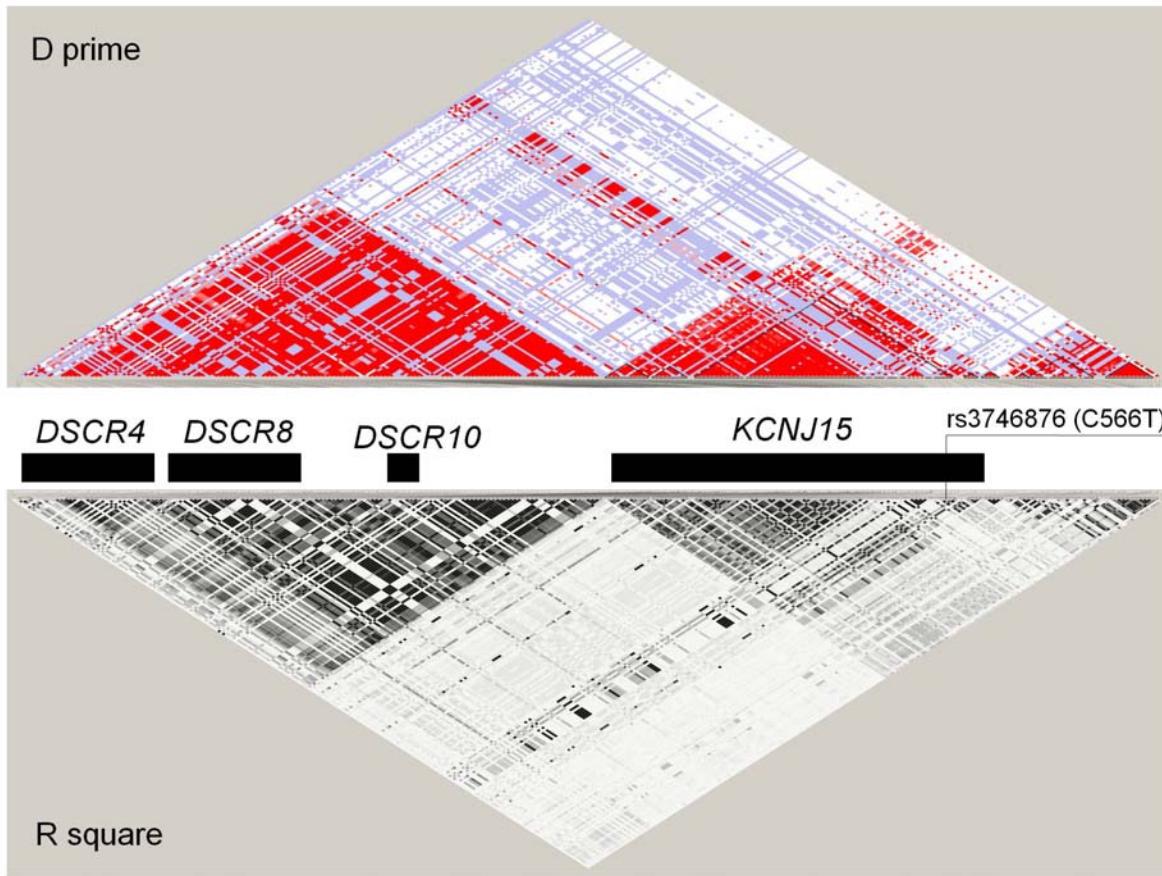
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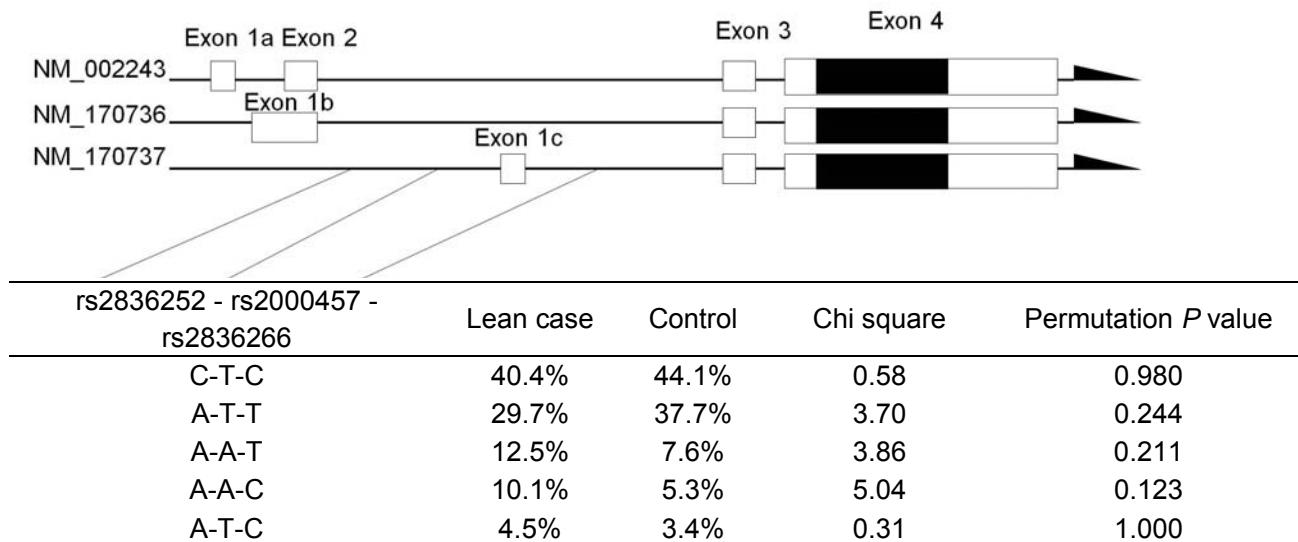
The Danish case-control study was supported by the Lundbeck Foundation Centre of Applied Medical Genomics for Personalized Disease Prediction, Prevention and Care (LUCAMP), and the Danish Diabetes Association. The Inter99 study was supported by the Danish Research Council, The Danish Centre for Health Technology Assessment, Novo Nordisk Inc., Research Foundation of Copenhagen County, Ministry of Internal Affairs and Health, The Danish Heart Foundation, The Danish Pharmaceutical Association, The Augustinus Foundation, and The Ib Henriksen Foundation. The Inter99 intervention was initiated by: Torben Jørgensen (PI), Knut Borch-Johnsen (co-PI), Hans Ibsen and Troels F. Thomsen. The steering committee comprises the former two and Charlotta Pisinger. The ADDITION study Denmark was supported by the National Health Services in the counties of Copenhagen, Aarhus, Ringkøbing, Ribe and South Jutland, together with the Danish Research Foundation for General Practice, Danish Centre for Evaluation and Health Technology Assessment, the diabetes fund of the National Board of Health, the Danish Medical Research Council, the Aarhus University Research Foundation and the Novo Nordisk Foundation. The study received unrestricted grants from Novo Nordisk, Novo Nordisk Scandinavia, Astra Denmark, Pfizer Denmark, GlaxoSmithKline Pharma Denmark, Servier Denmark and HemoCue Denmark.

Figure S1. LD Block Structure of the Target Region Extracted from HapMap Japanese Data



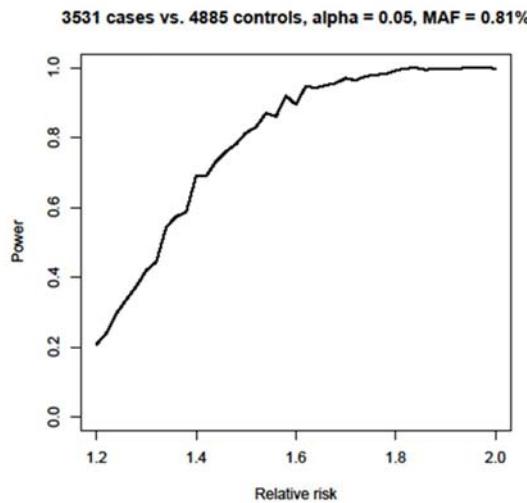
Haplotype structure and gene annotation. The plot includes pairwise r^2 values and D prime from the HapMap database for the JPT population.

Figure S2. Estimated Haplotype Frequencies in Intron 2 in Case and Control Samples



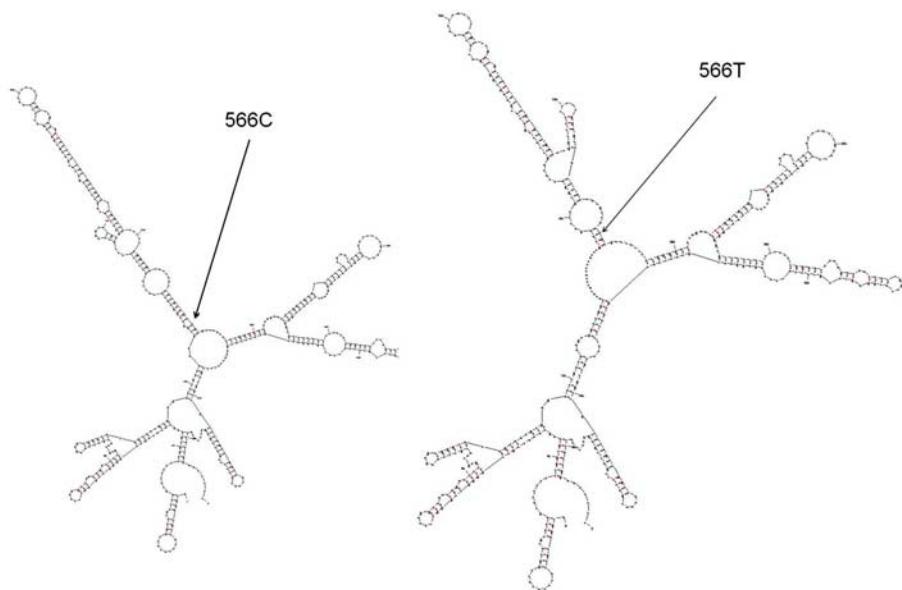
Structure of *KCNJ15* and SNPs used for haplotype analysis. Black blocks represent coding regions and white blocks are the untranslated regions. Three SNPs (rs2836252, rs2000457, rs2836266) were selected for haplotype estimation. Haplotypes with frequencies of less than 3% were excluded. The permutation *p* value was calculated by Haplovview ver. 4.1 with 10,000 permutations.

Figure S3. Statistical Power in the Danish Case-Control Study for Different Effect Sizes



A plot of the statistical power in the Danish case-control study for different effect sizes. The analyses are based on an additive model, a minor allele frequency of 0.81, a significance level of 0.05 and a disease prevalence of 8%.

Figure S4. Examples of KCNJ15 mRNA Folding Structures Predicted by MFOLD



KCNJ15 mRNA sequence carrying C566T synonymous SNP was used for secondary folding structure model building by the use of the computer program MFOLD. Note an obvious change of folding patterns between the 566C and the 566T *KCNJ15* mRNA.

Table S1. Basic Characteristics of Subjects

		N	Sex (M:F)	Age	BMI	Diabetes duration
Japanese screening sample	Case	Control	200	94:106	54.0±8.2	N.A.
		All case	149	68:81	59.9±9.7	23.1±2.1
		Lean case	96	46:50	59.8±9.9	21.4±1.7
		Lifelong lean case	36	13:23	56.1±11.6	20.4±1.4
Japanese replication sample A	Case	Control	500	353:147	37.3±6.3	N.A.
		All case	419	244:175	50.3±7.8	23.1±3.2
		Lean case	269	140:129	50.4±7.6	21.2±1.8
		Lifelong lean case	98	51:47	50.8±7.9	20.3±1.9
Japanese replication sample B	Case	Control	1,000	502:498	64.6±11.6	24.0±3.8
		All case	1,000	583:417	59.7±10.3	24.2±3.9
		Lean case	510	314:196	61.6±9.4	21.3±1.9
		Lifelong lean case	151	98:53	63.1±9.7	20.5±2.2
Danish replication sample	Case	Control	4,885	2264:2621	46.4±8.8	25.5±4.1
		All case	3,531	2097:1434	60.4±19.7	30.7±5.5
		Relative lean case	1,748	1105:643	61.3±10.0	26.4±2.6
		Absolute lean case	454	238:216	62.0±11.2	22.9±1.8

Japanese lean case was defined as the body mass index ≤ 24 at the time of sampling.

Japanese lifelong lean case was defined as the body mass index ≤ 24 for lifelong time.

Danish relative lean case was defined as the body mass index ≤ 30.0 as 50 percentile of type 2 diabetes mellitus cases

Danish absolute lean case was defined as the body mass index ≤ 25.0 as population based control samples 50 percentile

Values are means \pm standard deviation, NA: not available.

Table S2. Specific Primer Pairs for Pooled CE-SSCP

SNP ID	Sequence Forward primer	Sequence Reverse primer	Product (bp)	Annealing temperature ()	Temperature of SSCP ()
rs2070995	5'-ACTGCATGGGTGGAAAAGAC-3'	5'-GCTACC GGTCATCACAGAT-3'	163	63	20
rs2836154	5'-GATCAGAAGGTCCCAGTGG-3'	5'-GGGTTAGGAGAGAGGCCATCC-3'	221	66	25
rs743296	5'-TTCTGCACAGCACTTGCTT-3'	5'-CCAGCCTCCTGCAGAGTAAG-3'	303	61	25
rs461155	5'-TCTGCCTCAATAAGCCAACC-3'	5'-AGAAACACCCCTCGGTGAATG-3'	209	61	20
rs1541101	5'-TATTGCCAACAGCTGCCCTTCT-3'	5'-GAGCCATGGAGAAAAATGGA-3'	283	61	20
rs3787927	5'-GCTGGACTT GAGCACTTCC-3'	5'-CTGTCATCTTCCCACCCAGT-3'	195	66	25
rs666085	5'-AAGTCAAATGCCAAGGATGG-3'	5'-GGGAATGGTCAAGGCAAGT-3'	203	66	25
rs914162	5'-GTGGCAGGCTCTGTTCTAGG-3'	5'-GCTCAAGGCTGAATGGAGAG-3'	214	66	25
rs914172	5'-TGATCTGACCAACCCCTCCTC-3'	5'-GACAGGGAAGGAATTGCTCA-3'	251	61	25
rs2210277	5'-TATAGCAGGGTTGGCAGAC-3'	5'-TCTAACGCCCTCGGATGGAAA-3'	289	60	25
rs12149	5'-CTGCTCCCGATGCCCTCTA-3'	5'-GAGAAGAGAACGAGGCCAAGG-3'	217	63	20
rs398800	5'-AGCAAGGGATAAGCCTGTGA-3'	5'-GTACTTTGGGGCTCCTAGC-3'	194	63	20
rs462574	5'-GTGCCAACGTGTTCAAAGGT-3'	5'-CTCTCTGTTGCCCTTCAA-3'	265	63	20
rs11203151	5'-GATGGTGTGGCAGAGGAAT-3'	5'-GGCTGCTCAGAACTTTCCAC-3'	150	63	20
rs2838111	5'-GGCATCCAAATGTCAGCTT-3'	5'-GGTTTACCCAGAGTGTCCA-3'	269	66	20
rs2236692	5'-CACCTGGCAGGAAATAGCAT-3'	5'-CAGAACCCGAGTGGAAAGAA-3'	183	61	20
rs2839392	5'-GCAAAATGGACAGAGGGAAA-3'	5'-GTGAGAACACCTCCGCATC-3'	201	61	25
rs220159	5'-CTCCAATAAGGCCAGTTCA-3'	5'-AGAGCCTCGAACCGTAACAA-3'	184	61	20
rs225361	5'-GACCCCTGCTCAGAACACACC-3'	5'-TGCAGGAGAAAAACCCCTGAC-3'	163	63	20
rs2839497	5'-AGCAGGAGTCCGAGAAAACA-3'	5'-CCCACGAAGCCTACACATT-3'	260	63	20
rs11203201	5'-ACTTCTCCTCCGCTTCTTC-3'	5'-CAGCGTCCACCAATCTTAAC-3'	152	63	20
rs1893592	5'-TTGAAAACGATCCCCATTAA-3'	5'-GCCACTTTCTGCTGTGTA-3'	244	59	25
rs884339	5'-TAAGCTGGACTTGTCTATGC-3'	5'-GGGTCTGAGTGGAGGCTGGTA-3'	195	63	25
rs1788448	5'-ATTTCCAGCAATCTCGCACT-3'	5'-TTTAGCCACACCATGTGCTC-3'	242	61	25
rs869725	5'-GGTGTACCGAGGGCTACAA-3'	5'-CTCTCTCAGCCCTGGAGGT-3'	290	63	30
rs2839629	5'-CTCTCTGCAATGGGTGCTT-3'	5'-CGAAATCTTAGAGGGGGTCA-3'	166	63	20
rs234784	5'-CCATGGGCTCAGCTGATCT-3'	5'-TTCTTCCCCAGGAAGGTTT-3'	151	57	25
rs870137	5'-CCACCCAGCACCTCATAAAC-3'	5'-AGCCTGTGGAATGAGGTAC-3'	218	66	25
rs229351	5'-TGTGAGAGGCAGAAATGCAA-3'	5'-GAATGAGGCTGCCTGAGAAC-3'	177	61	30
rs162385	5'-CCCAGGAACACTGAAGGAAGTG-3'	5'-TGAATGCGTCATTGTTAGGG-3'	150	59	25
rs229341	5'-TAGGGAGTGAGCCTCTCTG-3'	5'-CTTCCGGTGTCAATTGCTCTC-3'	224	63	25
rs762400	5'-CCTCAGTGTATTCCCATTGGT-3'	5'-ACGGCGAACACGGTAATGAG-3'	170	63	30
rs1107204	5'-TCTTCCTCCCTCAGTCCTCA-3'	5'-TTGGGTTCCAGACTTGCAG-3'	155	61	30
rs11701801	5'-CTGTGAGGGGAAGAGGGAGTG-3'	5'-CTAAGCTGTGACAGGCACCA-3'	180	63	25
rs915770	5'-TTGCCTCTGTGACGTCTCTC-3'	5'-CAAGAAAGGGCAACTCCAAG-3'	185	61	20
rs915876	5'-GAAAGGCCTCTGAAACAGC-3'	5'-AGAGAGGCTCGTCATCAT-3'	192	61	30
rs1051981	5'-CATGAATGCTCTGCCTCTT-3'	5'-GCTGCAAATTCCCTGCTTT-3'	232	61	20
rs2073435	5'-TTCTCTGATGAAGCAGAACAGCA-3'	5'-TGGTCTGTGACCCAGCAATA-3'	238	63	30
rs2026880	5'-GAGGCCTTCAGTCATGTTCA-3'	5'-CTGTGCTCGAACACAGGAA-3'	181	59	25
rs1800522	5'-AACGATGGCCATGATTCTGT-3'	5'-GGAGGTGTCTTCAGCAC-3'	199	63	25
rs1057034	5'-CCTACAACTGGTCCAGCAC-3'	5'-AAACCCCAGGGCCTACAGT-3'	161	66	20
rs11911937	5'-ATGCTCGTGGGGAGAAG-3'	5'-GCTCTGGCCTAGGGAGTAGG-3'	166	66	25
rs458178	5'-CCAGGCTGATCTCAAACCTCC-3'	5'-CTGGCATGTGCAATTACACC-3'	208	66	25
rs2838624	5'-GCTGCCGTCACTGAAGCTA-3'	5'-GGAGAAACAAACGCTGGAAG-3'	162	63	25
rs1883039	5'-AGCTGATCGATGTGACGATG-3'	5'-CAAAAAGTCCAAGCAGCTC-3'	219	63	30
rs235312	5'-GGACGAAGACCCGACCTTACA-3'	5'-AGCAGCCATCAAATTGGAC-3'	215	63	20
rs235280	5'-AGAAAATGCACCAAGGACAG-3'	5'-ACCGTAGCGTAGGTTGAGA-3'	161	66	20
rs760458	5'-GGAAGAAAGGACAGCCACAG-3'	5'-GGTTCTGCCCTCATCAAATC-3'	230	63	30
rs928311	5'-GACTTGTGCTTGGGATG-3'	5'-AACACAAGCCACCAGGACTC-3'	179	63	30
rs10854467	5'-CCGGAAAGTCAGACACCTTTT-3'	5'-TTCTACGACTTGCCCTGGT-3'	245	63	30

rs2236445	5'-AAAAGCACCTTCTGCACACA-3'	5'-ATCGTCAGGAGGCATTGAG-3'	184	63	20
rs2255761	5'-GGCCATACTGCCATCCCTA-3'	5'-CATCAGGACACCCTCTGC-3'	199	63	20
rs2297291	5'-ATGGCTGATTCCAGCTCACT-3'	5'-AGAGCTGAGCAGAGGCCACTC-3'	171	63	30
rs9306142	5'-TGCCTAAAATCGAGCTAGGG-3'	5'-CTTCCTCAGGACCACTCCA-3'	228	61	20 or 25
rs1736421	5'-GAGGGGCAGGGAGATACTTC-3'	5'-AAGATGCCAACATCCAGGAG-3'	218	63	25
rs13047753	5'-TGTGCAGCCTGTTCTGTTC-3'	5'-TCAGAACTGCTCCCTTCGAT-3'	197	61	25
rs12626197	5'-GGTCTGCGGTACGAAGTCAG-3'	5'-GTTCTCTGTCGCGACCGTA-3'	183	66	30
rs9637215	5'-GAAGGGAGCTCACAGGATTG-3'	5'-GGCAGGCAGCAGTTGAGTT-3'	236	66	30
rs2280957	5'-CTATGGCTCTGCTCCCAAAG-3'	5'-GGGTGAGGGAGAGACTAGCA-3'	248	63	30
rs2839171	5'-CCAAAAGTTCTTAAGGGAAA-3'	5'-AGGGACACAAATCCAAACCA-3'	185	59	20
rs2839181	5'-AAATGCAGCTAGTCCCCCTCA-3'	5'-GTGTCAGTCGGGAAATTGT-3'	196	63	20
rs2249057	5'-GCACCTCTCGTGTTGAAGAT-3'	5'-TGGAACTGCTTGCTTACCC-3'	217	61	25
rs2839302	5'-AGCAAGCAGCTGTTTGACA-3'	5'-AGAGTGTGGTGTGGGAGCAT-3'	155	63	20
rs1107065	5'-TCCTCTCTGCAGGAACAGT-3'	5'-AGCCTCCCACTCACAGACTC-3'	250	63	25
rs9722	5'-CCGTTAAACAGCCTTGGA-3'	5'-ACTACTGCCTGCCACGAGTT-3'	213	61	20

Table S3. Specific Primer Pairs for the Variation Screening

Region	Forward primer	Reverse primer
Promoter	5'-CAGAGATCTTCATTAGAACGCT-3'	5'-ATTGGTTCTTCTGGATCATGGA-3'
Exon 1a	5'-TCCATGATCCAGAACCAAAT-3'	5'-CTGAGAAAGGAGACCATGATGTCAGC-3'
Exon 2& exon1b	5'-CTGGATGCCACTAGAACCTGTG-3'	5'-TGTCCCCTCATTAAGTAAAATCCC-3'
Exon 1c	5'-ATGAGGGCAGCTGGCGGTGGAC-3'	5'-CCAGAAATCTCCTTAAGGTC-3'
Exon 3	5'-GAACCTTACCCATTCTGTGCAC-3'	5'-GAAAGAGAGCAGCTGGCACTCAGC-3'
Exon 4 (section 1)	5'-AGAATTCTCCTTCTTGTGACTTC-3'	5'-CAGAGAGCTGGCACTGAATCAA-3'
Exon 4 (section 2)	5'-TTGATTCAAGTGCCAGCTCTG-3'	5'-GCTTGAGGGTTAACCTGGATGGC-3'
Exon 4 (section 3)	5'-GCCATCCAGGTTAACCTGCAAGC-3'	5'-CATTGAGGGTTAACCTGGATGGC-3'
Exon 4 (section 4)	5'-ACAACGTGGAAACCTACACAAATG-3'	5'-TGAACAGACAACAATTACTCTCAAG-3'
Exon 4 (section 5)	5'-CTTGAGAGTAATTGTTCTCAGTCA-3'	5'-ATGTATGCAGGGCTGAATATTCA-3'

Table S4. Specific Primers for Quantitative Real-Time PCR

Gene name	Direction	Sequence
Human KCNJ15	Sense	5'-GGAATGTCCTCATGCCATCT-3'
	Antisense	5'-TTCTGCTTGGTGTGACTGC-3'
Rat kcnj15	Sense	5'-CCGTTCCATCACAGAGGAGT-3'
	Antisense	5'-GCTTTTGGGTCTTGCAATC-3'
Rat kir6.2	Sense	5'-GCCATGCTGTCCCAGAAAGGG-3'
	Antisense	5'-GGCCAGGGGACATTCTCTGT-3'
Rat glut2	Sense	5'-ATGTCAGAAGACAAGATCACCGGA-3'
	Antisense	5'-CCCGAGCCACCCACCAAAGAAT-3'
Rat preproinsulin	Sense	5'-TGCCCCGGCTTTGTCAAAC-3'
	Antisense	5'-CTCCAGTGCCAAGGTCTGAA-3'
Human & rat β -actin	Sense	5'-CGCACCACTGGCATTGTCAT-3'
	Antisense	5'-TTCTCCTTGATGTCACGCAC-3'
GFP	Sense	5'-ACGTAAACGGCCACAAGTTC-3'
	Antisense	5'-AAGTCGTGCTGCTTCATGTG-3'

Table S5. Estimated Allele Frequencies by Pooled CE-SSCP and Allele and Genotype Frequencies Determined by Individual Typing

SNP ID	Pooled sample				Individual sample		
	All case	Lean case	Control		All case (%)	Lean case (%)	Control (%)
rs743296	Ilele	n.s.	$P = 0.039$		Allele	$P = 0.042$	$P = 0.013$
	C	66.2%	68.4%	58.9%	C	218 (75.7)	143 (78.6)
	G	33.8%	31.6%	41.1%	G	70 (24.3)	70 (21.4)
				Genotype	$P = 2.7 \times 10^{-3}$	$P = 4.2 \times 10^{-4}$	
				CC	88 (61.1)	61 (67.0)	77 (44.3)
				CG	42 (29.2)	21 (23.1)	84 (48.3)
				GG	14 (9.7)	9 (9.9)	13 (7.5)
rs869725	Allele	$P = 0.036$	n.s.		Allele	$P = 0.037$	n.s.
	T	67.6%	67.6%	59.2%	T	181 (63.7)	118 (64.1)
	C	32.4%	32.4%	40.8%	C	103 (36.3)	66 (35.9)
				Genotype	n.s	n.s	
				TT	59 (41.5)	37 (40.2)	74 (37.4)
				TC	63 (44.4)	44 (47.8)	91 (46.0)
				CC	20 (14.1)	11 (12.0)	33 (16.7)
rs162385	Allele	$P = 0.042$	n.s.		Allele	n.s	n.s.
	A	49.7%	51.2%	56.7%	A	146 (51.4)	96 (53.3)
	G	50.3%	48.8%	43.3%	G	138 (48.6)	84 (46.7)
				Genotype	n.s	n.s	
				AA	38 (26.8)	25 (27.8)	52 (26.5)
				AG	70 (49.3)	46 (51.1)	103 (52.6)
				GG	34 (23.9)	19 (21.1)	41 (21.1)
rs2297291	Allele	n.s.	$P = 0.035$		Allele	n.s	n.s.
	A	61.3%	62.7%	52.5%	A	164 (57.3)	105 (57.7)
	G	38.7%	37.3%	47.5%	G	122 (42.7)	77 (42.3)
				Genotype	n.s	n.s	
				AA	43 (30.1)	30 (33.0)	50 (26.5)
				AG	78 (54.5)	45 (49.5)	91 (48.1)
				GG	22 (15.4)	16 (17.6)	48 (25.4)
rs2280957	Allele	n.s.	$P = 0.026$		Allele	$P = 0.043$	$P = 0.019$
	G	75.7%	73.5%	81.9%	G	216 (76.1)	134 (71.3)
	A	24.3%	26.5%	18.1%	A	68 (23.9)	54 (28.7)
				Genotype	n.s	$P = 8.8 \times 10^{-3}$	
				GG	76 (53.5)	45 (47.9)	128 (64.0)
				GA	64 (45.1)	44 (46.8)	64 (32.0)
				AA	2 (1.4)	5 (5.3)	8 (4.0)

Lower p value based on genotype frequencies with recessive or dominant model

Lean case was defined as the body mass index ≥ 24 at the time of sampling.

Lifelong lean case was defined as the body mass index ≥ 24 for lifelong time.

Table S6. Clinical Characteristics Associated with C566T

	Japanese panel [#]			Danish panel		
	CC	CT	TT	CC	CT	TT
N (men/women)	492	53	3	5690 (2824/2866)	93 (46/47)	0 (0/0)
Age (years)	49.4±8.8	50.0±8.2	46.5±10.0	46.2 ± 7.9	44.7 ± 8.1	-
BMI at enrollment	23.2±3.2	22.7±2.9	20.8±1.6	26.2 ± 4.6	25.6 ± 3.7	-
Maximal BMI	26.4±4.0	50.0±3.4	46.5±1.2*	N.A.	N.A.	-
Insulin deficiency	40.4 ^{##}	59.3 ^{##,**}	66.7 ^{##,**}	49.5 ± 70.4 ^{###}	64.8 ± 51.3 ^{###}	-
HOMA-IR	2.7±2.0	2.9±3.7	-	4.8 ± 5.5	4.2 ± 2.5	-

*: C/T + T/T p value < 0.05 compared with CC.

**: T/T p value < 0.05 compared with CC.

#: Cases followed up more than 10 years

##: Insulin deficiency was defined as require insulin therapy or HOMA-β < 10 within 10 years after diagnosis

###: HOMA-B (fasting plasma insulin (U/ml) × 20 / (fasting plasma glucose (mmol/l) -3.5) data at enrollment was documented.

HOMA-IR: fasting insulin (μU/ml) x fasting glucose (mg/dl) / 405.

Values are means ± S.D