Supplementary Online Content

Ma RCW, Tam CHT, Wang Y, et al. Genetic variants of the protein kinase C-β 1 gene and development of end-stage renal disease in patients with type 2 diabetes. *JAMA*. 2010;304(8):881-889.

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This supplementary material has been provided by the authors to give readers additional information about their work.

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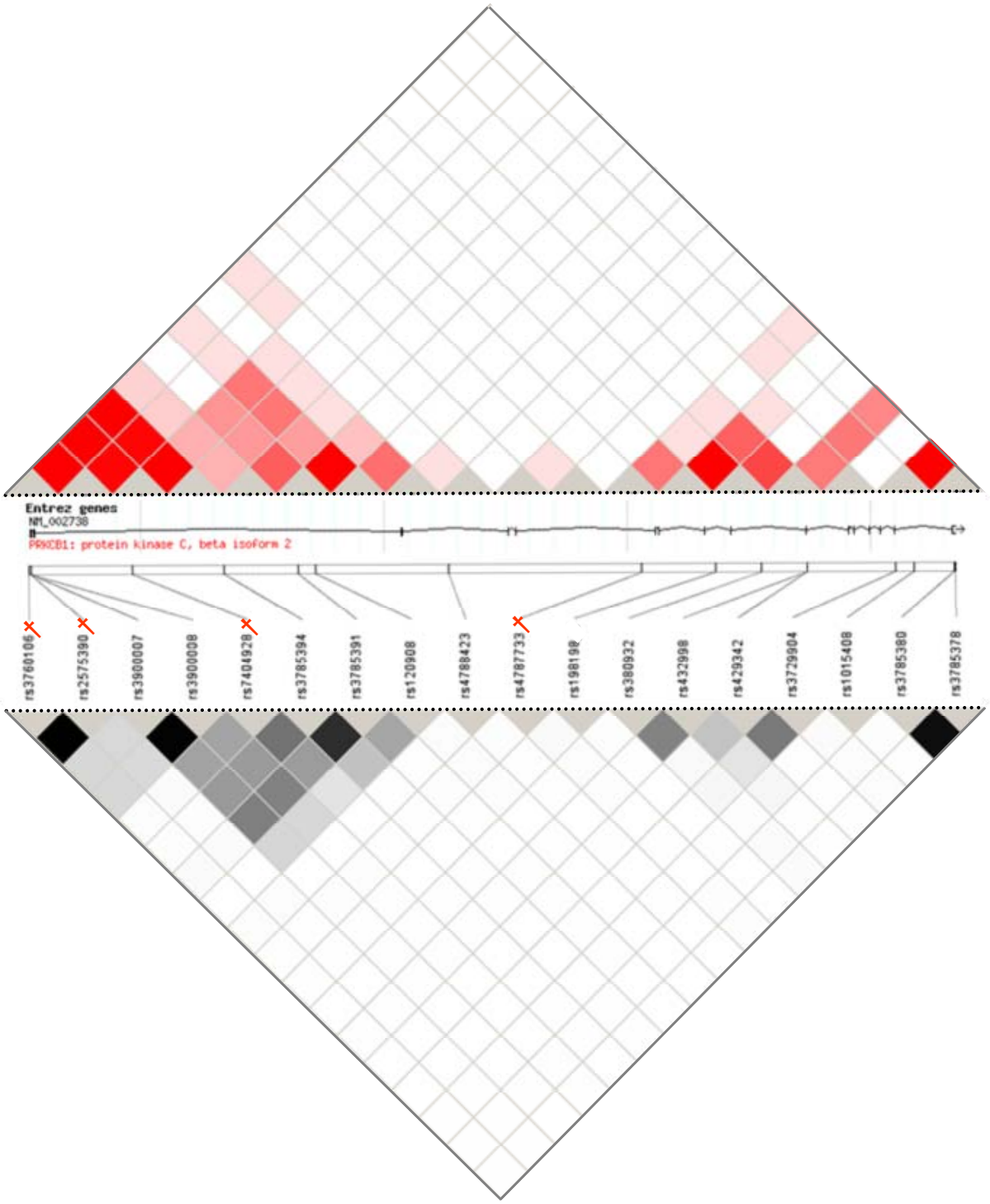
eFigure. Structure of *PRKCB1* gene and the location of SNPs genotyped. Shades of grey (white, shades of grey and black colour refer to $r^2 = 0$, $0 < r^2 < 1$ and $r^2 = 1$, respectively) and red (bright red and white refer to high and low |D'|, respectively) indicate the strength of pairwise LDs based on r^2 and |D'| respectively. "†"refer to SNP show significant association to ESRD endpoint in the present study.



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eTable 1. Clinical characteristics and biochemical profile at baseline and allele frequencies at each polymorphic site of *PRKCB1* stratified according to the progression to ESRD and CKD for the replication stage in Chinese type 2 diabetic patients. Please refer to the methods section for detailed definition of ESRD and CKD

	Progression to ESRD in entire replication cohort			**Progression to CKD in subjects with young-onset diabetes**		
	No	**Yes**	**P-value**	**No**	**Yes**	**P-value**
N	3424	253		898	151	
Clinical characteristics						
Sex (male / female)	1610 / 1814	125 / 128	0.4630	434 / 464	77 / 74	0.5450
Age (years)	58.4 ± 12.5	66.6 ± 11.1	<0.0001	44.5 ± 8.8	53.1 ± 10.0	<0.0001
Age Onset (years)	51.8 ± 11.8	54.4 ± 12.7	0.0008	38.3 ± 5.9	39.1 ± 6.2	0.1205
Duration of diabetes (years)	6.7 ± 6.5	12.2 ± 8.1	<0.0001	6.2 ± 6.6	14.0 ± 9.2	<0.0001
Duration of follow-up (years)	7.8 ± 2.9	4.0 ± 2.8	<0.0001	8.3 ± 2.7	3.9 ± 2.9	<0.0001
BMI (kg/m^2)	25.1 ± 4.0	24.9 ± 3.6	0.3510	25.6 ± 4.5	25.6 ± 4.3	0.9972
Waist circumference (cm)						
Male	88.7 ± 9.5	88.4 ± 9.4	0.7710	88.8 ± 11.0	88.7 ± 10.8	0.9750
Female	83.8 ± 9.9	86.8 ± 10.2	0.0010	82.7 ± 10.7	86.3 ± 10.3	0.0070
HbA_{1C} (%)	7.5 ± 1.7	8.2 ± 1.9	<0.0001	7.6 ± 1.7	8.5 ± 2.0	<0.0001
Total cholesterol (mg/dL)	200.8 ± 42.5	208.5 ± 54.1	0.1340	196.9 ± 42.5	216.2 ± 54.1	0.0003
Triglycerides (mg/dL)	123.9 (88.5 – 177.0)	150.4 (106.2 – 221.2)	<0.0001	115.0 ± 88.5	132.7 ± 88.5	0.0050
HDL-cholesterol (mg/dL)	50.2 ± 15.4	46.3 ± 11.6	0.0014	50.2 ± 11.6	50.2 ± 19.3	0.9852
LDL-cholesterol (mg/dL)	119.7 ± 34.8	123.6 ± 42.5	0.6049	119.7 ± 34.8	131.3 ± 38.6	0.0016
Systolic blood pressure (mmHg)	135.5 ± 19.7	146.1 ± 21.3	<0.0001	127 ± 16.5	138 ± 18.6	<0.0001
Diastolic blood pressure (mmHg)	74.7 ± 10.4	74.1 ± 10.7	0.3835	74.4 ± 9.8	76.4 ± 10.7	0.0216
Hypertension (%)	68%	89%	<0.0001	47%	79%	<0.0001
Retinopathy (%)	25%	60%	<0.0001	17%	58%	<0.0001
Plasma creatinine (mg/dL)	1.05 (0.87 – 1.26)	1.53 (1.22 – 2.27)	<0.0001	0.96 (0.8 – 1.1)	1.14 (0.94 – 1.36)	<0.0001

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eTable 1 (continued). Clinical characteristics and biochemical profile at baseline and allele frequencies at each polymorphic site of *PRKCB1* stratified according to the progression to ESRD and CKD for the replication stage in Chinese type 2 diabetic patients. Please refer to the methods section for detailed definition of ESRD and CKD

	Progression to ESRD in entire replication cohort			**Progression to CKD in subjects with young-onset diabetes**		
	No	**Yes**	**P-value**	**No**	**Yes**	**P-value**
AER (μg/min)	18.2 (7.3 - 73.7)	389.1 (85.1 - 1441.6)	<0.0001	12.1 (6.5 - 43.9)	148.7 (35.8 - 685.5)	<0.0001
eGFR (min/ml per 1.73 m^2)	103.1 ± 30.5	64.6 ± 32.7	<0.0001	123.0 ± 27.8	98.4 ± 27.5	<0.0001
Treatment						
Lipid lowering	18%	26%	<0.0001	12%	25%	<0.0001
Blood pressure anti-hypertensive	41%	65%	<0.0001	20%	44%	<0.0001
ACE inhibitor	26%	45%	<0.0001	16%	47%	<0.0001
Oral glucose lowering	71%	59%	<0.0001	67%	69%	0.6560
Insulin treatment	18%	44%	<0.0001	16%	45%	<0.0001
Allele frequencies						
rs3760106 (T/C)	0.074 / 0.926	0.074 / 0.926	0.9634	0.075 / 0.925	0.095 / 0.905	0.2350
rs2575390 (G/C)	0.077 / 0.923	0.074 / 0.926	0.8355	0.080 / 0.920	0.098 / 0.902	0.3016
rs7404928 (C/T)	0.352 / 0.648	0.330 / 0.670	0.3282	0.358 / 0.642	0.348 / 0.652	0.7306
rs4787733 (G/A)	0.116 / 0.884	0.126 / 0.874	0.4969	0.110 / 0.890	0.096 / 0.904	0.4786

Data are shown as N, mean ± SD or median (interquartile range).

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eTable 2. Genotype distributions of *PRKCB1* SNPs and hazard ratio of *PRKCB1* polymorphisms for risk of CKD for the validation study in young onset Chinese type 2 diabetic patients.

			CKD	No CKD	Additive		Dominant		Recessive	
SNP	**Location**	**Risk / non-risk allele**	**RR / RN / NN genotype frequencies**	**RR / RN / NN genotype frequencies**	**P**	**HR (95% C. I.)**	**P**	**HR (95% C. I.)**	**P**	**HR (95% C. I.)**
rs3760106	Promoter	T / C	0.007 / 0.176 / 0.818	0.016 / 0.117 / 0.867	0.0386	1.49 (1.02 - 2.17)	**0.0171**	**1.68 (1.1 - 2.57)**	0.7611	0.74 (0.1 - 5.3)
rs2575390	Promoter	G / C	0.007 / 0.182 / 0.811	0.016 / 0.128 / 0.856	0.0496	1.46 (1 - 2.12)	**0.0240**	**1.62 (1.07 - 2.47)**	0.754	0.73 (0.1 - 5.25)
rs7404928	Intron	T / C	0.444 / 0.417 / 0.139	0.413 / 0.459 / 0.129	0.9611	1.01 (0.79 - 1.28)	0.3198	0.79 (0.5 - 1.26)	**0.4797**	**1.12 (0.81 - 1.55)**
rs4787733	Intron	A / G	0.815 / 0.179 / 0.007	0.795 / 0.190 / 0.014	**0.8412**	**1.04 (0.71 - 1.51)**	0.3163	2.77 (0.38 - 20.42)	0.9052	0.98 (0.64 - 1.48)

Hazard ratios refer to the risk-conferring alleles. *P* values were calculated from Cox proportional hazard regression adjusted for conventional risk factors (sex, age and duration of diabetes, and the use of drugs (yes/no)) at baseline. R-risk alleles, N-non-risk alleles.

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eTable 3. Clinical characteristics of type 2 diabetic patients from the Shanghai cohort

		Shanghai
N		1892
Clinical characteristics		
	Sex (male / female)	980 / 912
	Age (years)	61.2 ± 12.6
	Age Onset (years)	54.1 ± 11.8
	Duration of diabetes (years)	7.2 ± 6.9
	BMI (kg/m^2)	24.1 ± 3.5
	HbA_{1C} (%)	9.2 ± 2.4
	Total cholesterol (mg/dL)	189.2 ± 46.3
	Triglycerides (mg/dL)	132.7 (88.5 – 194.7)
	HDL-cholesterol (mg/dL)	46.3 ± 23.2
	LDL-cholesterol (mg/dL)	119.7 ± 38.6
	Systolic blood pressure (mmHg)	136.3 ± 18.7
	Diastolic blood pressure (mmHg)	81.3 ± 10.0
	Hypertension (%)	78%
	Retinopathy (%)	22%
	Creatinine (mg/dL)	0.86 ± 0.52
	eGFR (min/ml per 1.73 m^2)	102.4± 35.4
Treatment		
	Lipid lowering	-
	Blood pressure anti-hypertensive	-
	ACE inhibitor	-
	Oral glucose lowering	77%
	Insulin treatment	20%
Allele frequencies		
	rs3760106 (T/C)	0.057/ 0.943
	rs2575390 (G/C)	0.061/ 0.939
	rs7404928 (C/T)	-
	rs4787733 (G/A)	0.125 / 0.875

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eTable 4. Functional annotation of the implicated genetic variants in PRKCB1

SNP	Physical position on Chr 16	Location in PRKCB	H3K27me3 binding signal in normal human renal epithelial cell defined by ChIP-chip[1]	CTCF binding signal in human embryonic kidney 293 cells defined by ChIP-chip[1]	Conserved sequence in promoter region defined by cisRED[2]	Transcriptional regulatory modules predicted by PReMod[3]	CpG Island	Predicted influence of the SNP on PRKCB function
rs3760106	23753296	Promoter (1.7kb upstream of transcription start)			Lies within the conserved sequence of PRKCB promoter	60 bp away from the predicted regulatory module (chr16:23752889-23753233), which consists TCF-4, Sox-5, Ik-2 and Elk-1 predicted by PReMod.		As this SNP shares the same LD block with rs2575390, it might mark the change in PRKCB promoter activity by rs2575390.
rs2575390	23754255	Promoter (0.6kb upstream of transcription start)	Lies within a H3K27me3 binding hotspot		Lies within the conserved sequence of PRKCB promoter	30 bp away from the predicted regulatory module (chr16:23754284-23755200), which consists cap, Sp1 and E47 (for transcription initiation) predicted by PReMod.		Histone H3 trimethylated at lysine 27 (H3K27me3) normally repress transcription. This SNP might lead to the change of H3K27me3 deposition in the promoter of PRKCB. As a result, the binding of the transcirption initiation complex to the promoter is influenced.

1. Sabo PJ, Hawrylycz M, Wallace JC, et al. Discovery of functional noncoding elements by digital analysis of chromatin structure. *Proc Natl Acad Sci U S A*. 2004;101(48):16837-16842.
2. Robertson G, Bilenky M, Lin K, et al. cisRED. *Nucleic Acids Res*. 2006;34(Database issue):D68-D73.
3. Ferretti V, Poitras C, Bergeron D, Coulombe B, Robert F, Blanchette M. PReMod. *Nucleic Acids Res*. 2007;35(Database issue):D122-D126.

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SNP	Physical position on Chr 16	Location in PRKCB	H3K27me3 binding signal in normal human renal epithelial cell defined by ChIP-chip[1]	CTCF binding signal in human embryonic kidney 293 cells defined by ChIP-chip[1]	Conserved sequence in promoter region defined by cisRED[2]	Transcriptional regulatory modules predicted by PReMod[3]	CpG Island	Predicted influence of the SNP on PRKCB function
rs7404928	23796340	Intron 1		In the LD block containing this SNP, there is a CTCF binding hotspot				It might indicate the alteration of the DNA sequence in its LD block modifying the CTCF binding. CTCF is a regulatory insulator blocking the enhancer from binding to the cis-regulatory element.
rs4787733	24005375	Intron 3	Lies within the region detected with moderate signal of H3K27me3					This SNP might indicate the alteration of H3K27me3 deposition in the region, which normally blocks the enhancer from binding to the cis-regulatory element and repress transcription.

1. Sabo PJ, Hawrylycz M, Wallace JC, et al. Discovery of functional noncoding elements by digital analysis of chromatin structure. *Proc Natl Acad Sci U S A*. 2004;101(48):16837-16842.
2. Robertson G, Bilenky M, Lin K, et al. cisRED. *Nucleic Acids Res*. 2006;34(Database issue):D68-D73.
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