SUPPLEMENTARY DATA

IMPACT OF THE NATRIURETIC PEPTIDE CLEARANCE RECEPTOR (NPRC) GENETIC POLYMORPHISM ON BLOOD PRESSURE IN TYPE 2 DIABETIC PATIENTS.

D2NG participating centers and investigators:

G Touchard, F Bridoux (Nephrology Department Poitiers), B Duly-Bouhanick, G Subra, F Tollis (Diabetology and Nephrology Department University Hospital of Angers), E Archambeaud-Mouveroux, C Leroux-Robert, Y Le Meur (Diabetology and Nephrology Department, University Hospital of Limoges) P Drouin, B Guerci, L Kessler (Diabetology and Nephrology Department University Hospital of Nancy), M Marre, F Mignon (Diabetology and Nephrology Department University Hospital of Bichat-Paris), P Lecomte, H Nivet, JM Halimi (Diabetology and Nephrology Department University Hospital of Tours), G Charpentier (Diabetology Department Hospital of Gilles de Corbeil), JF Pouget-Abadie, S Baron, A Sechet (Diabetology and Nephrology department Hospital of Niort), AM Chameau, J Yver (Diabetology and Nephrology department Hospital of Angoulème), JC Courrèges (Diabetology department Hospital of Narbonne), F Rachedi (Diabetology Department Hospital of Papeete).

Supplement : Functional study design

Patients

Following enrollment, patients continued their usual diet for 3 days (usual-sodium intake period). A restricted-salt diet consisting of a 50 % reduction of daily sodium intake was then administered to all participants for 10 days (restricted-salt diet period) after dietician counseling. For each patient, 24-hour urine collections were obtained during the consecutive last 3 days of each period, allowing 24-hour urinary sodium (UNa), albumin (Ualb) and creatinine (Ucreat) excretion determinations at the end of the usual vs restricted-sodium intake periods. The mean was calculated for each period. Seated BP was measured thrice at 3-min intervals using a semi -automated device (dinamap1846, critikon, Tampa, FL, USA) after 10 min seated rest. Systolic BP (SBP) and diastolic BP (DBP) levels were then calculated from the mean of the 3 consecutive measures. The schematic representation of the study design is presented in supplementary Figure 1.

Statistical analysis

In the functional study, variables before and after salt reduction in AA and non-AA participants were compared by Wilcoxon matched pairs signed rank test. Intergroup variations were compared using unpaired Mann-Whitney U test. Gene-environment interaction was estimated by comparing variation of variables between genotype groups using unpaired Mann-Whitney U test.

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Supplementary Table 1. Clinical and biological characteristics of type 2 diabetes patients in the DIABHYCAR and the DIAB2NEPHROGENE/SURDIAGENE (D2NG/SDG) studies

	DIABHYCAR	D2NG/SDG	*P* value
n	3,126	2,635	
Men : n (%)	2,488 (72.9)	1,634 (62.0)	<0.001
Age (years)	65.5±8.4	62.9±11.0	<0.001
Weight (kg)	81.5±14.3	84.3±17.0	<0.001
BMI (kg/m²)	29.3±4.6	30.4±5.8	<0.001
Current smoker: n (%)	449 (14.4)*	317 (12.3)†	<0.001
SBP (mmHg)	145.0±14.1	138.3±19.7	<0.001
DBP (mmHg)	82.1±8.5	76.0±11.0	<0.001
Duration of diabetes (years)	10.2±7.7	15.5±9.4	<0.001
Personal history of MI: n (%)	171 (5.5)	295 (11.1)	<0.001
Personal history of stroke: n (%)	121 (3.9)	114 (4.3)	0.3160
A1C (%)	7.9±1.8	7.7±1.6	0.006
Serum creatinine (µmol/l)	88 (26)	86 (361)	0.3743
eGFR (ml/min/1.73m²)	76 (26)	69 (38)	<0.001
UAlb (mg/l)	111 (200)	23 (106)	<0.001

Data are means ± SD or median (interquartile).
SBP, systolic blood pressure ; DBP, diastolic blood pressure personal history of MI, personal history of myocardial infarction; A1C, glycated hemoglobin; eGFR, estimated glomerular filtration rate by MDRD formula; Ualb, urinary albumin excretion.
Missing data for *433 and †66 patients, respectively.

Supplementary Table 2. Pairwise linkage disequilibrium between the *NPR3* polymorphisms in the participants of the DIABHYCAR study

	rs9716700	rs1421811	rs12522446	rs6889608	rs700923	rs16890196	rs1173773	rs1173743	rs2270915
rs9716700	-	-0.85	-0.90	-0.91	0.09	0.10	0.11	-0.32	0.10
rs1421811	0.11	-	0.91	-0.90	-0.12	-0.10*	-0.09	0.31	-0.36
rs12522446	0.03	0.18	-	-0.95	0.08	0.12	-0.07†	0.30	0.00‡
rs6889608	0.07	0.19	0.04	-	-0.25	-0.19	-0.12	-0.12	0.02§
rs700923	0.01	0.00	0.00	0.01	-	1.00	0.89	-0.12	0.32
rs16890196	0.01	0.00	0.01	0.00	0.74	-	0.87	-0.29	0.40
rs1173773	0.01	0.00	0.00	0.00	0.48	0.34	-	-0.25	0.23
rs1173743	0.02	0.07	0.01	0.00	0.00	0.02	0.03	-	-0.89
rs2270915	0.01	0.02	0.00	0.00	0.08	0.14	0.03	0.18	-

In the upper-right triangle of the linkage disequilibrium (LD) matrix is given the extent of LD expressed in terms of D' which is the ratio of the unstandardized coefficient to its maximal/minimal value, while, in the lower-left triangle of this matrix, the LD is expressed in terms of r^2 statistics.
All D' and r^2 were highly significant ($p < 0.001$) except for *, p=0.004 ; †, p=0.160; ‡, p=0.825;§, p=0.319.

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Supplementary Table 3. Association between systolic blood pressure and SNPs of *NPR3* in patients from the DIABHYCAR study

			Genotype		
SNP	*n**/*n*†/*n*‡	A_1 A_1	A_1 A_2	A_2 A_2	*P* value §
		AA	AC	CC	
rs9716700	121/955/1,037	146.4±15.0	144.0±13.7	144.2±12.8	0.183
		CC	CG	GG	
rs1421811	498/1,448/1,166	143.7±13.4	144.3±13.1	144.4±13.2	0.592
		TT	CT	CC	
rs12522446	35/684/2,397	145.9±11.9	144.1±12.4	144.3±13.5	0.747
		GG	AG	AA	
rs700923	166/1,064/1,796	142.4±11.5	144.7±13.9	144.1±13.0	0.114
		GG	AG	AA	
rs16890196	98/906/2,030	144.0±13.0	144.6±13.8	143.0±11.7	0.346
		GG	GT	TT	
rs1173743	715/1,499/902	144.3±13.1	144.0±13.0	144.5±13.5	0.665

Data are means ± SD.

*/†/‡ / number of patient for A_1A_1/A_1A_2/A_2A_2 respectively.

§ unadjusted *P* values correspond to ANOVA statistics: A_1A_1 vs A_1A_2 vs A_2A_2.

Adjustment on age, sex and BMI did not modify the non significatnt of the association.

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Supplementary Table 4. Association between *NPR3* variants and SBP according to presence or absence of obesity in the DIABHYCAR study

		SBP (mmHg)			
SNP	Obesity status (*n**/*n*†/*n*‡)	A_1A_1	A_1A_2	A_2A_2	*P value*§
		AA	AG	GG	
rs9716700	Obese (49/384/809)	147.8±16.6	145.7±14.7	145.6±13.2	0.561
	Non obese (71/565/1,213)	145.5±14.0	142.9±12.8	143.3±12.2	0.280
		CC	CG	GG	
rs1421811	Obese (200/575/468)	145.2±14.3	145.9±13.6	145.6±13.7	0.815
	Non obese (296/861/689)	142.6±12.6	143.3±12.7	143.6±12.8	0.547
		TT	CT	CC	
rs12522446	Obese (8/286/950)	148.5±12.0	145.2±12.7	145.9±14.2	0.684
	Non obese (27/394/1,428)	145.1±11.9	143.2±12.0	143.2±12.9	0.757
		CC	CT	TT	
rs6889608	Obese (97/471/675)	145.1±12.4	146.4±13.9	145.3±14.0	0.389
	Non obese (132/681/1,036)	145.3±12.4	143.7±12.3	142.7±12.9	0.054
		GG	AG	AA	
rs700923	Obese (62/434/715)	142.7±12.2	146.3±15.3	145.5±13.1	0.147
	Non obese (102/624/1,070)	142.3±11.2	143.5±12.8	143.1±12.8	0.630
		GG	AG	AA	
rs16890196	Obese (34/375/800)	144.0±12.3	146.4±15.7	145.3±13.0	0.374
	Non obese (62/526/1,215)	142.6±11.4	143.4±12.2	143.2±13.0	0.872
		GG	GA	AA	
rs1173773	Obese (120/578/546)	141.8±14.0	145.9±14.5	146.4±12.9	0.004
	Non obese (210/810/828)	142.7±12.9	143.2±12.7	143.4±12.7	0.780
		GG	GT	TT	
rs1173743	Obese (278/593/373)	147.1±13.5	145.0±13.5	145.8±14.6	0.118
	Non obese (431/899/519)	142.4±12.6	143.5±12.8	143.6±12.6	0.299
		GG	GA	AA	
rs2270915	Obese (51/403/785)	149.0±16.3	145.7±14.3	145.6±13.4	0.226
	Non obese (88/581/1,168)	144.9±12.1	144.3±12.9	142.8±12.7	0.068

Data are means ± SD.

*/†/‡ / number of patient for A_1A_1/A_1A_2/A_2A_2 respectively.

§ *P* value from one-way ANOVA.

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Supplementary Table 5. Association between *NPR3* variants and SBP according to presence or absence of obesity in Europid-only participants of the DIAB2NEPHROGENE/SURDIAGENE studies

		SBP (mmHg)			
SNP	Obesity status (*n**/*n*†/*n*‡)	A_1A_1	A_1A_2	A_2A_2	*P* value§
		CC	CT	TT	
rs6889608	Obese (55/351/565)	139.6±21.6	140.9±20.3	139.6±10.1	0.649
	Non obese (60/334/591)	131.7±18.1	137.0±17.7	137.3±19.2	0.082
		GG	AG	AA	
rs1173773	Obese (105/409/456)	143.0±19.7	140.7±21.4	138.5±19.0	0.077
	Non obese (127/403/453)	137.9±18.3	136.5±18.8	137.2±18.7	0.729
		GG	AG	AA	
rs2270915	Obese (42/331/629)	139.2±20.0	142.0±20.6	138.8±19.9	0.066
	Non obese (48/327/639)	135.7±19.2	138.9±19.8	136.0±18.2	0.075

Data are means ± SD.
*/†/‡ / number of patient for A_1A_1/A_1A_2/A_2A_2 respectively.
§ *P* value from one-way ANOVA.

Supplementary Table 6. *NPR3* gene haplotype analysis of rs6889608 , rs1173773 and rs2270915 in the conjunct population : DIABHYCAR and Europid DIAB2NEPHROGENE-SURDIAGENE participants

	Haplotype		Haplotype frequency	SBP (mmHg)	*P* value
C/T*	G/A†	G/A‡			
T	A	A	0.395	141.3(140.3-142.3)	Reference
T	G	A	0.200	142.6(140.9-144.4)	0.235
C	A	A	0.133	143.2(141.2-145.2)	0.129
T	A	G	0.102	145.4(142.5-148.3)	0.016
C	G	A	0.067	142.2(138.7-145.8)	0.612
T	G	G	0.052	143.5(140.9-146.1)	0.129

*rs6889608, †rs1173773, ‡ rs2270915.
Comparisons were made using TAA as reference in THESIAS software and haplotype frequencies above 0.05 were considered taking more than 95% of chromosomes into account.

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Supplementary Table 7. Clinical and biological response to salt reduction in the sodium restriction functional study (additional data)

Variable	Salt diet	AA		G carriers	Genotype effect	Global genotype effect
		(n = 7)		(n = 7)	(*P* value)	(*P* value)
Weight (kg)	Usual	87.5 ± 11.5		89.7 ± 15.7	0.848	0. 8182
	Low	86.2 ± 10.8		88.5 ± 14.9	0.848	
Treatment effect (*P* value)		0.063		0.1148		
Global treatment effect (*P* value)			0.0131			0.7491*
HR (min^{-1})	Usual	65 ± 9		64 ± 22	0.848	0. 8542
	Low	65 ± 5		64 ± 18	0.949	
Treatment effect (*P* value)		0.999		0.8658		
Global treatment effect (*P* value)			0.925			0.8480*
DBP (mmHg)	Usual	79.3 ± 6.7		73.3 ± 6.2	0.0409	0.3952
	Low	70.4 ± 8.0		73.3 ± 7.7	0.4062	
Treatment effect (*P* value)		0.018		0.999		
Global treatment effect (*P* value)			0.048			0.0845*
Diuresis (ml/24h)	Usual	2200 (1883)		1777 (404)	0.4057	0.8182
	Low	3183 (1836)		1642 (387)	0.2774	
Treatment effect (*P* value)		0.6002		0.3980		
Global treatment effect (*P* value)			0.9721			0.3706*
Serum creatinine (µmol/l)	Usual	89 (24)		94 (37)	0.4057	0.8182
	Low	87 (28)		105(14)	0.3056	
Treatment effect (*P* value)		0.4990		0.4990		
Global treatment effect (*P* value			0.3305			0.6540*

Data are mean +/- SD or median (interquartile). HR, heart rate ; DBP, diastolic blood pressure. *P* values within each genotype are treatment effect (Wilcoxon rank test). *P* values within each salt diet are genotype effect (Mann-Whitney U test) **P* values for estimated genotype-treatment interaction (Mann-Whitney U test). Treatment effect means effect of diet sodium intervention.

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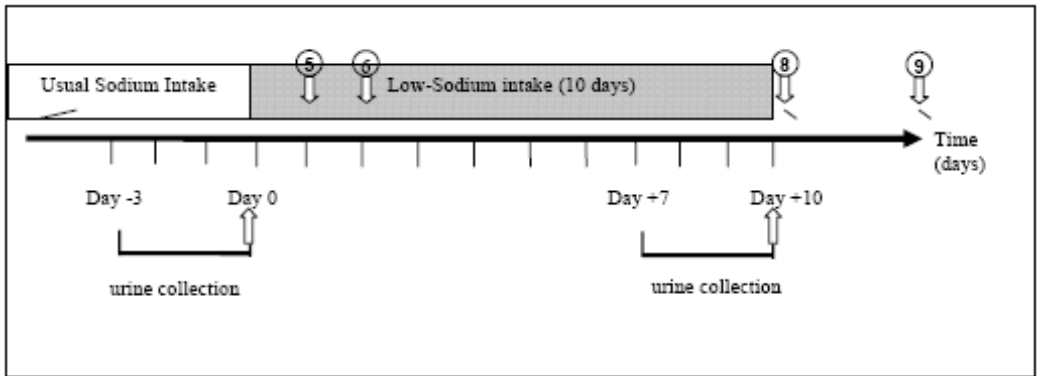
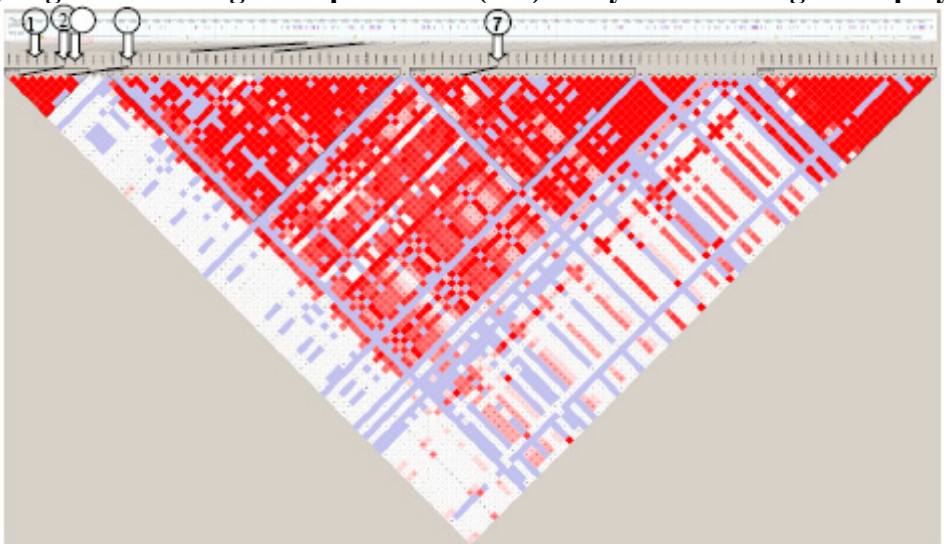
Supplementary Figure 1. Organisation of the functional study of sodium intake restriction



Arrows represent BP measure.

Supplementary Figure 2. Linkage disequilibrium (LD) analysis of *NPR3* genetic polymorphism



LD for western European ancestry population (from Centre d'Etude du Polymorphisme Humain [CEU]) is displayed by standard color schemes: red color for very strong LD (LOD = 2 D' = 1), white color for no LD (LOD<2, D'<1), pink, red (LOD = 2 D'<1), and blue (LOD<2 D' = 1) for intermediate LD.
Numbers circled represent genotyped SNPs: 1, rs9716700; 2, rs1421811; 3, rs12522446; 4, rs6889608; 5, rs700923; 6, rs16890196; 7, rs1173773;8, rs1173743; 9, rs2270915. LD blocks were defined using Haploview.

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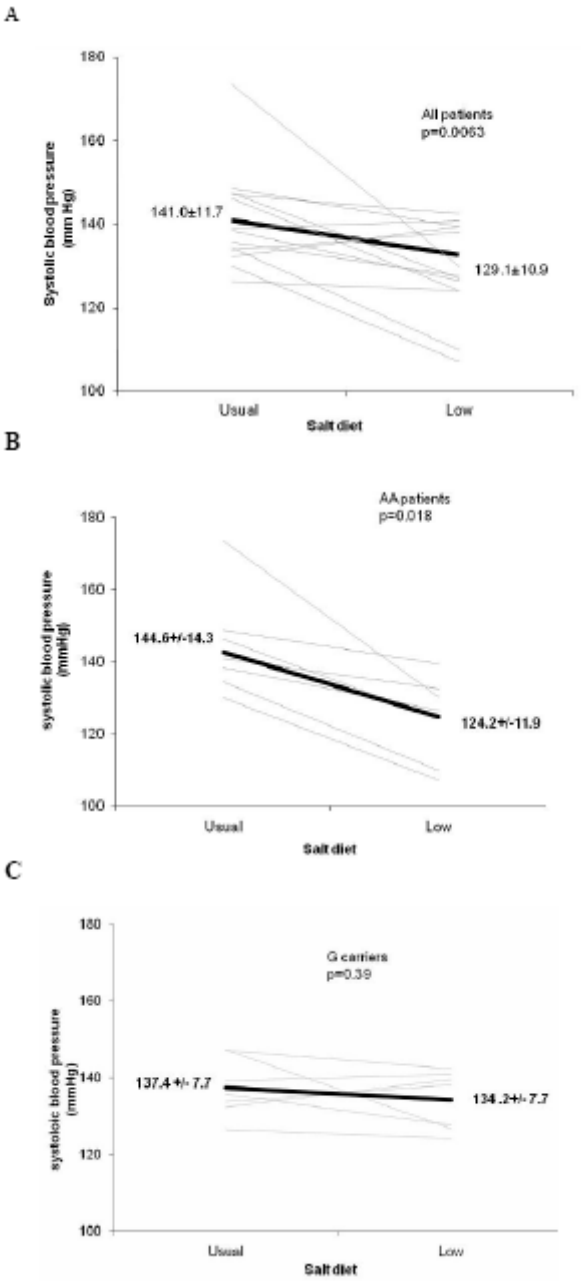
Supplementary Figure 3. **Change in systolic blood pressure (mmHg) induced by salt reduction according to rs2207915 *NPR3* polymorphism.**

Data are presented individually and mean is in bold font; * Salt reduction effect (Wilcoxon signed-rank test); †, genotype effect (Mann-Whitney U test); ‡ estimated salt reduction-genotype interaction (Mann-Whitney U test).

A: all patients (*P=0.006; † P =0.51; ‡ P =0.006); **B**: AA homozygote; * P =0.0117; **C**: G carriers * P =0.3980.



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Supplementary Figure 4. **Local plot of the height association test results ($log^{10}(P)$) along the *NPR3* gene.**
Imputed genotypes using HapMap CEU data in the DIABHYCAR population