

**Plasma adrenomedullin, allelic variations in the *ADM* gene, and risk for lower-limb amputation in people with type 2 diabetes**

**Supplemental Material**

- Page 2. Supplemental Table 1. Baseline characteristics of participants by the incidence of LLA and LLRV during follow-up
- Page 4. Supplemental Table 2. Minor and major LLA during follow-up in the SURDIAGENE cohort by baseline plasma MR-proADM
- Page 5. Supplemental Table 3. Correlations of baseline parameters with incident LLA
- Page 6. Supplemental Table 4. Prognostic value of baseline plasma MR-proADM for discrimination and classification of LLA risk during follow-up
- Page 7. Supplemental Table 5. Plasma MR-proADM at baseline by ADM genotype
- Page 8. Supplemental Table 6. ADM genotype by LLA incidence during follow-up
- Page 9. Supplemental Figure 1. A) Structure of the *ADM* gene and location of the SNPs. B) Linkage disequilibrium between the SNPs in the DIABHYCAR cohort
- Page 10. Supplemental Figure 2. Kaplan-Meier curves for the cumulative incidence of LLA during follow-up by *ADM* SNPs

**Supplemental Table 1. Baseline characteristics of participants by the incidence of LLA and LLRV during follow-up**

	LLA during follow-up			LLRV during follow-up		
	No	Yes	p	No	Yes	p
N (%)	4264 (97.5)	111 (2.5)	-	4171 (95.3)	204 (4.7)	-
Cohort: DIABHYCAR, n (%)	2928 (98.9)	34 (1.1)	<0.0001	2856 (96.4)	106 (3.6)	<0.0001
SURDIAGENE, n (%)	1336 (94.6)	77 (5.4)		1315 (93.1)	98 (6.9)	
Sex: male, n (%)	2885 (68)	98 (88)	<0.0001	2826 (68)	157 (80)	0.006
Age, y	66 ± 9	67 ± 9	0.14	65 ± 9	67 ± 9	0.03
Duration of diabetes, y	12 ± 9	15 ± 10	<0.0001	12 ± 9	14 ± 9	0.001
BMI, kg/m <sup>2</sup>	30.0 ± 5.3	30.0 ± 4.2	0.93	30.1 ± 5.3	28.9 ± 4.6	0.001
Systolic blood pressure, mmHg	141 ± 16	143 ± 16	0.14	141 ± 16	141 ± 17	0.85
Diastolic blood pressure, mmHg	79 ± 10	78 ± 11	0.18	79 ± 10	76 ± 12	<0.0001
Arterial hypertension, n (%)	2761 (65)	93 (84)	<0.0001	2692 (65)	162 (79)	<0.0001
Current tobacco smoking, n (%)	562 (13)	17 (16)	0.47	542 (13)	37 (168)	0.04
Previous myocardial infarction, n (%)	369 (8.6)	14 (12.2)	0.17	351 (8.4)	32 (15.7)	0.0009
Previous stroke, n (%)	189 (4.4)	8 (7.2)	0.16	181 (4.3)	16 (7.8)	0.02
Previous LLA, n (%)	48 (1.1)	24 (21.6)	<0.0001	58 (1.1)	14 (20.9)	<0.0001
MR-proADM, nmol/l*	0.41 [0.40]	0.81 [0.64]	<0.0001	0.41 [0.41]	0.56 [0.57]	<0.0001
HbA1c, %	7.8 ± 1.7	8.0 ± 1.8	0.34	7.8 ± 1.7	8.0 ± 1.8	0.06

HbA1c, mmol/mol	62 ± 18	64 ± 20	0.34	62 ± 18	64 ± 20	0.06
Total cholesterol, mmol/l	5.47 ± 1.19	5.20 ± 1.28	0.02	5.46 ± 1.19	5.49 ± 1.35	0.75
HDL cholesterol, mmol/l	1.28 ± 0.38	1.16 ± 0.36	0.001	1.29 ± 0.38	1.217 ± 0.34	<0.0001
Triglycerides, mmol/l*	1.76 [1.30]	1.77 [1.11]	0.69	1.75 [1.29]	1.85 [1.58]	0.06
Plasma copeptin, pmol/l*	7.07 [7.22]	9.43 [12.63]	<0.0001	7.06 [7.17]	8.53 [9.52]	0.0008
Plasma creatinine, µmol/l*	87 [30]	97 [45]	<0.0001	87 [30]	95 [37]	<0.0001
eGFR, ml/min/1.73 m <sup>2</sup>	74 ± 20	65 ± 26	<0.0001	74 ± 20	67 ± 23	<0.0001
UAC, mg/l	60 (128)	152 (453)	<0.0001	60 [129]	97 [333]	<0.0001
UAC stages: Normoalbuminuria, n (%)	622 (15)	17 (15)		606 (15)	33 (16)	
Microalbuminuria, n (%)	2678 (63)	44 (40)	<0.0001	2623 (63)	99 (49)	<0.0001
Macroalbuminuria, n (%)	958 (22)	50 (45)		936 (22)	72 (35)	
Use of antiplatelet or anticoagulation drugs, n (%)	1238 (29)	62 (56)	<0.0001	1185 (28)	115 (56)	<0.0001
Use of lipid lowering drugs, n (%)	1138 (27)	15 (14)	0.001	1098 (26)	55 (27)	0.87
Use of blood pressure lowering drugs, n (%)	2761 (65)	93 (84)	<0.0001	2691 (65)	163 (80)	<0.0001
Use of ACE-I or ARB, n (%)	1003 (24)	63 (57)	<0.0001	988 (234)	78 (38)	<0.0001
Use of diuretics, n (%)	1262 (30)	56 (50)	<0.0001	1235 (30)	83 (41)	0.001
Use of insulin, n (%)	804 (19)	52 (47)	<0.0001	786 (19)	70 (34)	<0.0001

Quantitative data expressed as mean ± SD or median [IQR]\*. Statistics are Student's t test, \*Wilcoxon test, Pearson's chi-squared test or Fisher's exact test. LLA: lower limb amputation. LLRV: lower limb revascularization. eGFR, estimated glomerular filtration rate. UAC: urinary albumin concentration. ACE-I: angiotensin converting enzyme inhibitor. ARB: angiotensin receptor blocker. p<0.05 was significant.

**Supplemental Table 2. Minor and major LLA during follow-up in the SURDIAGENE cohort by baseline plasma MR-proADM**

	Crude Model		Adjusted Model 1		Adjusted Model 2	
	Hazard Ratio (95% C.I.)	P	Hazard Ratio (95% C.I.)	P	Hazard Ratio (95% C.I.)	P
Minor LLA						
T3 vs T1	5.52 (2.86 – 11.72)	<0.0001	4.29 (2.03 – 9.79)	<0.0001	3.78 (1.78 – 8.64)	0.0004
T2 vs T1	2.18 (1.05 – 4.87)	0.04	1.93 (0.90 – 4.39)	0.09	1.79 (0.83 – 4.08)	0.14
T3 vs T2	2.53 (1.49 – 4.42)	0.0005	2.22 (1.24 – 4.08)	0.007	2.11 (1.18 – 3.89)	0.01
Log[MR-proADM]	2.98 (1.82 – 4.50)	<0.000	4.06 (1.67 – 9.64)	0.002	2.33 (0.92 – 5.74)	0.07
Major LLA						
T3 vs T1	4.64 (2.49 – 9.29)	<0.0001	2.46 (1.20 – 5.33)	0.01	2.29 (1.11 – 4.99)	0.02
T2 vs T1	2.23 (1.14 – 4.61)	0.02	1.77 (0.89 – 3.70)	0.11	1.65 (0.82 – 3.47)	0.16
T3 vs T2	2.08 (1.26 – 3.52)	0.004	1.39 (0.78 – 2.52)	0.26	1.39 (0.77 – 2.53)	0.27
Log[MR-proADM]	1.90 (1.47 – 2.46)	<0.0001	1.58 (1.11 – 2.28)	0.01	1.42 (1.00 – 2.05)	0.05

Minor LLA: transmetatarsal amputation. Major LLA: transtibial or transfemoral amputation. Hazards ratio (HR) for LLA computed by Cox proportional hazards survival regression analysis for tertiles (T) of plasma MR-ProADM and for 1 SD of log[MR-ProADM]. Model 1: adjusted for sex, age, BMI, duration of diabetes, arterial hypertension, tobacco smoking, HbA1c, total cholesterol, HDL-cholesterol, eGFR, UAC, use of insulin, ACE-I or ARB, diuretics, antiplatelet or anticoagulation drugs and lipid lowering drugs at baseline. Model 2: Model 1 plus adjustment for previous history of LLA at baseline. Number of participants with/without minor LLA by tertiles of MR-proADM: 2/467 (T1), 9/458 (T2) and 25/447 (T3). Number of participants with/without major LLA by tertiles of MR-proADM: 4/469 (T1), 13/462 (T2) and 24/446 (T3).

**Supplemental Table 3. Correlations of baseline parameters with incident LLA**

Covariates	Stepwise regression		Univariate correlation	
	Cumulative R <sup>2</sup>	p	R <sup>2</sup>	p
Previous LLA	0.096	<0.0001	0.089	<0.0001
MR-proADM	0.133	<0.0001	0.075	<0.0001
Sex: male	0.169	<0.0001	0.024	<0.0001
Use of ACE-I or ARB	0.177	0.003	0.053	<0.0001
HbA1c	0.185	0.005	0.0007	0.41
Macroalbuminuria	0.192	0.008	0.029	<0.0001
Use of diuretics	–	0.11	0.020	<0.0001
Cohort: SURDIAGENE	–	0.11	0.063	<0.0001
Total Cholesterol	–	0.13	0.006	0.01
eGFR	–	0.19	0.030	<0.0001
Tobacco smoking	–	0.20	0.0005	0.46
Use of lipid lowering drugs	–	0.27	0.011	0.0008
Use of antiplatelet or anticoagulation drugs	–	0.39	0.032	<0.0001
HDL cholesterol	–	0.44	0.014	0.0001
Arterial hypertension	–	0.70	0.019	<0.0001
Use of insulin	–	0.81	0.042	<0.0001
BMI	–	0.85	0.0001	0.70
Use of anti-hypertensive drugs	–	0.89	0.019	<0.0001
Duration of diabetes	–	0.92	0.011	0.0008
Age	–	0.95	0.002	0.13

Continuous parameters were normalized as Z-scores of log transformed data for these analyses.

**Supplemental Table 4. Prognostic value of baseline plasma MR-proADM for discrimination and classification of LLA risk during follow-up**

	<b>Estimate</b>	<b>95% Confidence Interval</b>	<b>p</b>
Harrell's c index for the basic model	0.738	0.684 – 0.791	–
Change in Harrell's c index for the basic model plus log[MR-proADM]	0.046	0.012 – 0.081	0.009
Relative IDI	0.109	0.051 – 0.161	<0.001
Categorical NRI	0.748	0.509 – 1.077	<0.0001

IDI: integrated discrimination improvement. NRI: net reclassification improvement. Basic model of traditional risk factors: sex, age, duration of diabetes, systolic and diastolic blood pressure, HbA1c, total cholesterol, HDL-cholesterol, eGFR, urinary albumin concentration and tobacco smoking at baseline. Relative (IDI) and categorical NRI tests were performed for 5-year risk of LLA associated with the basic model plus log[MR-proADM] compared to the basic model alone.  $p < 0.05$  was significant.

**Supplemental Table 5. Plasma MR-proADM at baseline by ADM genotype**

SNP	N	MR-proADM (nmol/l)	p
rs4399321			
AA	1798	0.60 ± 0.01	0.0007
AG	1900	0.63 ± 0.01	
GG	467	0.65 ± 0.02	
rs11042725			
CC	1180	0.60 ± 0.01	0.002
CA	2066	0.62 ± 0.01	
AA	977	0.63 ± 0.01	
rs7944706			
GG	1357	0.62 ± 0.01	0.004
GA	2076	0.63 ± 0.01	
AA	823	0.59 ± 0.01	
rs2957692			
AA	1441	0.60 ± 0.01	0.002
AG	1913	0.63 ± 0.01	
GG	843	0.62 ± 0.01	
rs2957717			
CC	1893	0.62 ± 0.01	0.59
TC	1850	0.63 ± 0.01	
TT	427	0.58 ± 0.02	

Data expressed as mean ± SEM. Comparisons between genotypes are ANCOVA adjusted for cohort membership, sex, age, duration of diabetes, HbA1c and eGFR. Statistics performed with log[MR-proADM].  $p \leq 0.05$  is significant.

**Supplemental Table 6. ADM genotype by LLA incidence during follow-up**

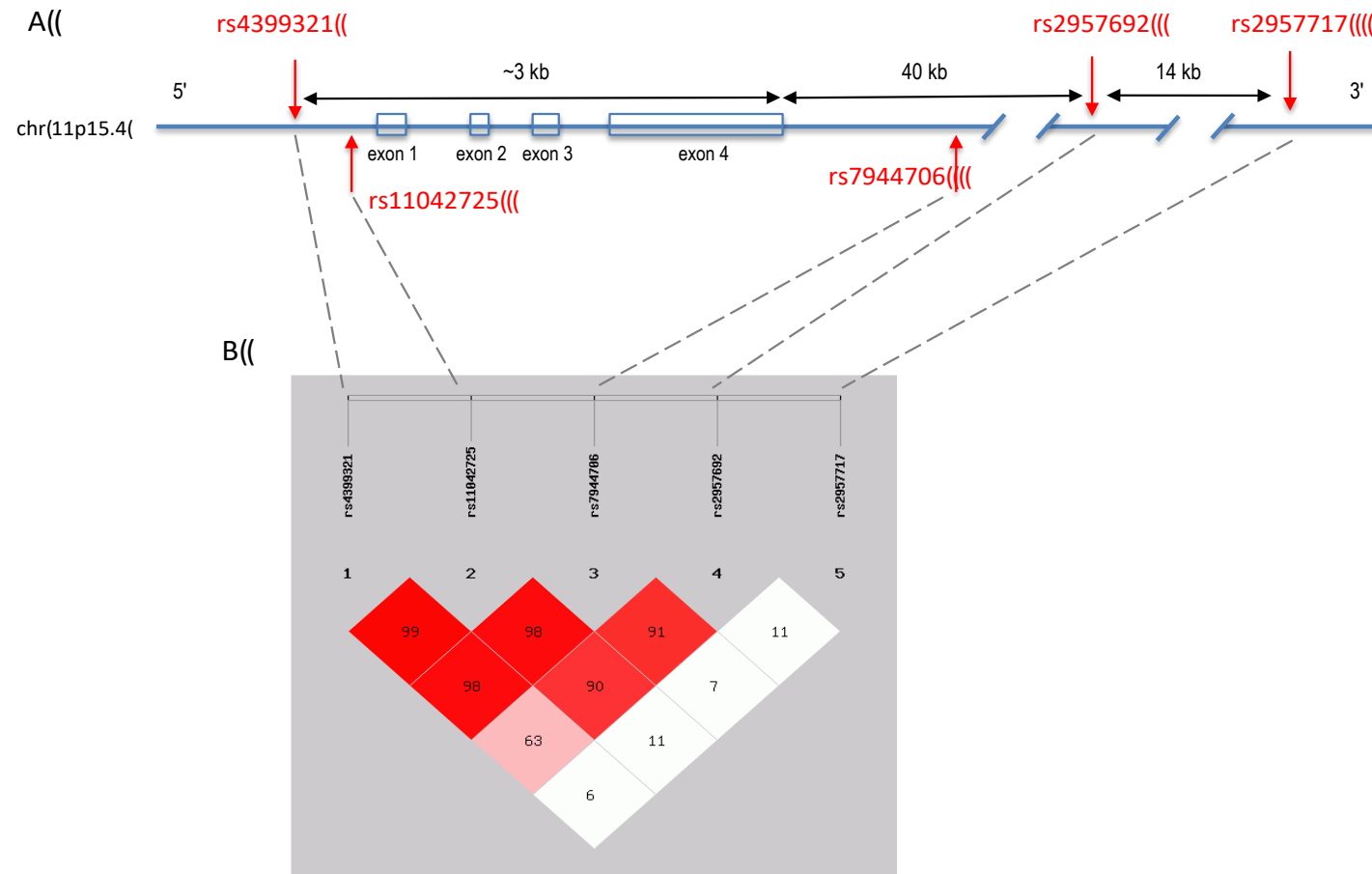
SNP	Incident LLA	
	No	Yes
rs4399321		
AA	1762 (0.434)	36 (0.340)
AG	1846 (0.455)	54 (0.509)
GG	451 (0.111)	16 (0.151)
MAF	0.339	0.406
rs11042725		
CC	1153 (0.280)	27 (0.246)
CA	2018 (0.491)	48 (0.436)
AA	942 (0.229)	35 (0.318)
MAF	0.474	0.536
rs7944706		
GG	1313 (0.317)	44 (0.400)
GA	2030 (0.490)	46 (0.418)
AA	803 (0.194)	20 (0.182)
MAF	0.438	0.391
rs2957692		
AA	1410 (0.345)	31 (0.290)
AG	1868 (0.457)	45 (0.421)
GG	812 (0.199)	31 (0.290)
MAF	0.427	0.500
rs2957717		
CC	1852 (0.456)	41 (0.380)
TC	1799 (0.443)	51 (0.472)
TT	411 (0.101)	16 (0.148)
MAF	0.323	0.384

Data expressed in number of cases and genotype frequency (1=100%).

SNPs are sorted in 5' to 3' order. MAF: minor allele frequency (1=100%).



**Supplemental Figure 1.** A) Structure of the *ADM* gene and location of the SNPs. B) Linkage disequilibrium between the SNPs in the DIABHYCAR cohort



**Supplemental Figure 2.** Kaplan-Meier curves for the cumulative incidence of LLA during follow-up by *ADM* SNPs. Cox proportional hazards survival regression analyses adjusted for cohort membership, sex, age, duration of diabetes, arterial hypertension, tobacco smoking, HbA1c, total cholesterol, HDL-cholesterol, eGFR, UAC, use of insulin, ACE-I or ARB, diuretics, antiplatelet or anticoagulation drugs and lipid lowering drugs at baseline.

