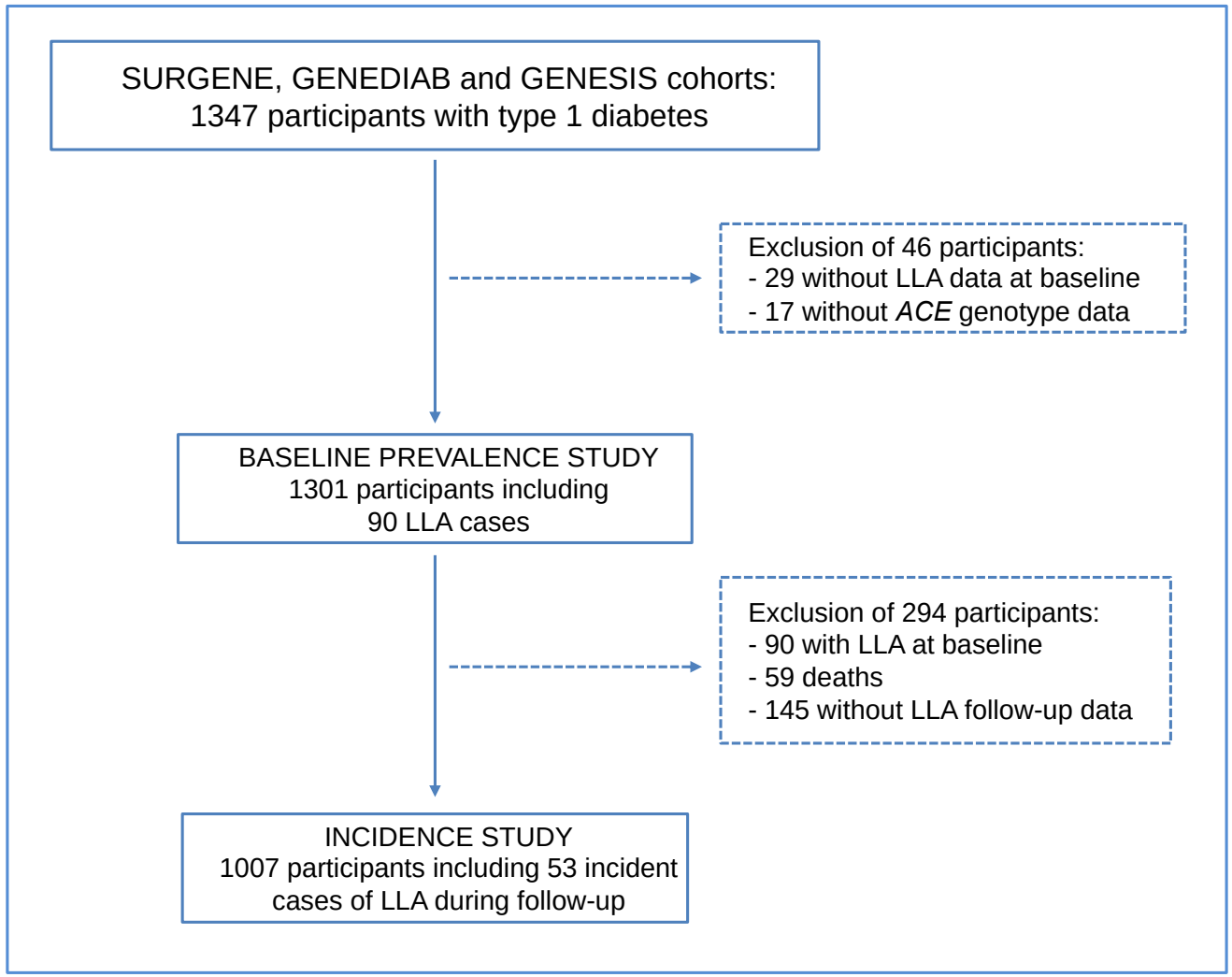
Association Between the Angiotensin-Converting Enzyme I/D Polymorphism and Risk of Lower-Limb Amputation in Patients with Long-Standing Type 1 Diabetes

Kamel Mohammedi, Yawa Abouleka, Charlyne Carpentier, Louis Potier, Severine Dubois, Ninon Foussard, Vincent Rigalleau, Jean-François Gautier, Pierre Gourdy, Guillaume Charpentier, Ronan Roussel, André Scheen, Bernard Bauduceau, Samy Hadjadj, François Alhenc-Gelas, Michel Marre, Gilberto Velho

SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Study Flow Chart



Supplemental Table 1. Characteristics of participants at baseline by cohort membership

	SURGENE		GENEDIAB		GENESIS	
	Data	Missing data (n)	Data	Missing data (n)	Data	Missing data (n)
N	338		444		519	
Male sex, n (%)	190 (56)	0	249 (56)	0	269 (52)	0
Age (years)	34 ± 13	0	43 ± 12	0	43 ± 11	0
Tobacco smoking, n (%)	NA	NA		2		0
Former	-	-	90 (20)		56 (11)	
Current	-	-	124 (28)		159 (31)	
Duration of diabetes (years)	15 ± 11	0	28 ± 10	1	26 ± 9	1
Body mass index (kg/m2)	23 ± 3	0	24 ± 3	3	24 ± 4	5
Systolic blood pressure (mmHg)	127 ± 16	3	138 ± 19	0	131 ± 20	15
Diastolic blood pressure (mmHg)	73 ± 11	3	80 ± 11	0	76 ± 10	15
HbA1c (%)	9.3 ± 2.3	2	8.6 ± 1.8	32	8.5 ± 1.3	0
HbA1c (mmol/mol)	79 ± 25		71 ± 20		70 ± 14	
Total cholesterol (mmol/l)[§]	5.4 ± 1.3	33	5.7 ± 1.4	5	NA	
eGFR (mL/min/1.73m^2)	99 ± 22	4	72 ± 29	0	86 ± 33	5
Urinary albumin concentration (mg/l)*	7 (3, 14)	3	42 (8, 472)	4	19 (7, 169)	11
History of microvascular disease, n (%)						
Diabetic kidney disease	58 (17)	6	266 (60)	4	241 (47)	9
Non-proliferative retinopathy	94 (28)	0	85 (19)	0	323 (62)	0
Proliferative retinopathy	35 (10)	0	359 (81)	0	196 (38)	0
Peripheral diabetic neuropathy	NA	NA	297 (67)	0	191 (37)	51
History of macrovascular disease, n (%)						

Myocardial infarction	2 (0.6)	1	33 (7)	1	23 (4)	0
Stroke	3 (0.8)	0	17 (4)	4	14 (3)	4
Peripheral artery disease	NA	NA	77 (17)	0	48 (9)	59
Treatments, n (%)						
Antihypertensive drugs	42 (12)	0	246 (57)	12	262 (50)	0
ACE inhibitors	29 (9)	0	148 (43)	17	191 (37)	0
Lipid-lowering drugs	8 (3)	37	38 (8)	0	43 (8)	0
ACE I/D genotype, n (%)		0		0		0
II	56 (17)		82 (18)		83 (16)	
ID	167 (49)		215 (49)		245 (47)	
DD	115 (34)		147 (33)		191 (37)	
Plasma ACE (ng/ml)*‡	468 (350, 598)	95	437 (330, 566)	0	NA	NA

Quantitative data presented as mean ± SD or as *median (25^{th}–75^{th} percentiles) for those with skewed distribution.
£Incident lower-limb amputation during follow-up considered in participants without history of LLA at baseline
§Data available in a subset of participants (N=744)
‡ Data available in a subset of participants (N=687)
NA: Data not available as not collected in SURGENE (smoking, neuropathy and PAD) or in GENESIS (total cholesterol and plasma ACE)

Supplemental Table 2. Characteristics of participants at baseline by *ACE* I/D genotype

	***ACE* I/D genotype**		**P**
	XD	**II**	
N (%)	1080 (83)	221 (17)	
Cohort membership, n (%)			0.58
SURGENE	282 (26)	56 (25)	
GENEDIAB	362 (34)	82 (37)	
GENESIS	436 (40)	83 (38)	
Male sex, n (%)	587 (54)	121 (55)	0.94
Age (years)	40 ± 13	41 ± 14	0.26
Tobacco smoking, n (%)			
Former	121 (11)	25 (11)	0.61
Current	240 (22)	43 (19)	
Duration of diabetes (years)	24 ± 11	25 ± 11	0.39
Body mass index (kg/m2)	24 ± 3	23 ± 3	0.13
Systolic blood pressure (mmHg)	133 ± 19	132 ± 18	0.46
Diastolic blood pressure (mmHg)	77 ± 11	75 ± 11	0.20
HbA1c (%)	8.8 ± 1.8	8.7 ± 1.7	0.65
HbA1c (mmol/mol)	73 ± 20	72 ± 18	
Total cholesterol (mmo/L)[§]	5.6 ± 1.3	5.7 ± 1.6	0.54
eGFR (mL/min/1.73m^2)	84 ± 31	86 ± 30	0.42
Urinary albumin concentration (mg/l)*	15 (6, 156)	12 (6, 65)	0.13
History of microvascular disease, n (%)			

Diabetic kidney disease	480 (45)	85 (40)	0.15
Non-proliferative retinopathy	413 (38)	89 (40)	0.64
Proliferative retinopathy	489 (45)	101 (46)	
Peripheral diabetic neuropathy	408 (38)	82 (37)	0.60
History of macrovascular disease, n (%)			
Myocardial infarction	54 (5.0)	4 (1.8)	0.03
Stroke	31 (2.9)	3 (1.4)	0.25
Peripheral artery disease	109 (10.1)	16 (7.2)	0.33
Treatments, n (%)			
Antihypertensive drugs	470 (44)	80 (37)	0.05
ACE inhibitors	342 (32)	62 (28)	0.30
Lipid-lowering drugs	73 (7.0)	16 (7.3)	0.88
Plasma ACE (mg/ml)*‡	470 (359, 599)	356 (269, 445)	<0.0001

Quantitative variables are presented as mean ± SD or as *median (25^{th}–75^{th} percentiles) for those with skewed distribution. Comparisons of qualitative and quantitative variables were performed using χ^2 and ANOVA tests, respectively. Wilcoxon test was used to compare variables with skewed distribution. $p<0.05$ was considered as significant.

§Data available for 744 participants.

‡Data available for 687 participants.

Supplemental Table 3. Incidence of minor and major lower-limb amputations during follow-up by *ACE* I/D genotype

	Lower-limb amputation			Hazard ratio (95% CI) for minor LLA vs absent	P	Hazard ratio (95% CI) for major LLA vs absent	P
	Absent, n	**Minor, n (%)**	**Major, n (%)**				
***ACE* genotype**							
II	181	2 (1.1)	3 (1.6)	1	-	1	-
XD	817	35 (4.0)	23 (2.6)	3.63 (1.08 – 22.65)	0.03	1.88 (0.62 – 8.28)	0.29

Hazard ratios (with associated 95% CIs) were computed by Cox model for XD (DD or ID) versus II genotype, adjusted as in model 2: cohort membership, sex, age, history of tobacco smoking, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin concentration, estimated glomerular filtration rate, use of ACE inhibitors and use of lipid-lowering drugs. $p<0.05$ was considered as significant.

Supplemental Table 4. Prevalence of lower-limb amputation by *ACE* I/D genotype in users and non-users of ACE inhibitors at baseline

	Non users of ACE inhibitors				Users of ACE inhibitors				
	LLA		Odds ratio (95% CI)	P	LLA		Odds ratio (95% CI)	P	*P for interaction
	No, n	Yes, n (%)			No, n	Yes, n (%)			
***ACE* genotype**									
II	152	5 (3.2)	1	-	53	9 (14.5)	1	-	
XD	652	71 (9.8)	5.29 (1.80 – 15.54)	0.002	286	56 (16.4)	1.3 (0.60 – 3.11)	0.46	0.08
Plasma ACE tertiles									
1st tertile	155	24 (13.4)	1	-	30	10 (25)	1	-	
2d tertile	134	20 (13.0)	1.14 (0.50 – 2.64)	0.75	49	19 (28)	1.30 (0.47 – 3.57)	0.61	
3d tertile	122	13 (9.6)	0.87 (0.33 – 2.31)	0.78	71	20 (22)	0.86 (0.32 – 2.30)	0.76	0.55

Odds ratios computed by logistic regression analyses for the prevalence of lower-limb amputation (LLA) at the end of follow-up by *ACE* genotype (XD vs II) or tertiles of plasma ACE distribution (2d and 3d tertiles vs 1st tertile). Adjustment model: cohort membership, sex, age, history of tobacco smoking, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin concentration, estimated glomerular filtration rate, and use of lipid-lowering drugs. *P for interaction between *ACE* genotype or plasma ACE tertiles and use of ACE inhibitors. $p<0.05$ was considered as significant.

Supplemental Table 5. Prognostic performance of *ACE* I/D genotype for the stratification of risk of lower-limb amputation

Statistical test	Estimate	95% CI	p
Harrell's c-statistic for basic model	0.791	0.752 – 0.828	
Change in Harrell's c-statistic for basic model plus *ACE* I/D genotype	0.005	-0.005 – 0.014	0.35
IDI	0.008	-0.001 – 0.024	0.22
Continuous NRI	0.161	0.023 – 0.303	0.02

Basic model: cohort membership, sex, age, history of tobacco smoking, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin concentration, estimated glomerular filtration rate, use of ACE inhibitors and use of lipid-lowering drugs.
Harrell's c-statistic, integrated discrimination improvement (IDI) and net reclassification improvement (NRI) tests were performed for the prevalence of lower-limb amputation at the end of follow-up associated with basic model plus *ACE* I/D genotype versus basic model alone.
$P<0.05$ was considered as significant.

List of Contributors to SURGENE, GENEDIAB, and GENESIS studies

Clinical investigators at baseline by alphabetical order of enrollment centers

All cities are in France, except for Liège, in Belgium:

Albert Fournier, Jean-Daniel Lalau (*Centre Hospitalier Universitaire d'Amiens*); Béatrice Bouhanick, Line Godiveau, Michel Marre, Vincent Rohmer (*Centre Hospitalier Universitaire d'Angers*); Jean-Raymond Attali, Patrick Miossec (*Assistance Publique des Hôpitaux de Paris, Hôpital de Bondy*); Henri Gin, Vincent Rigalleau (*Centre Hospitalier Universitaire de Bordeaux*); Isabelle Cerf, Guillaume Charpentier, Isabelle Petit, Jean-Pierre Riveline (*Centre Hospitalier de Corbeil-Essonne*); Bertrand Godeau, Zoubida Kahal, Dominique Simon (*Assistance Publique des Hôpitaux de Paris, Hôpital de Créteil*); Daniel Cordonnier, Serge Halimi (*Centre Hospitalier Universitaire de Grenoble*); Pierre-Jean Lefebvre, Nicolas Paquot, André Scheen, Laurent Weekers (*Centre Hospitalier Universitaire de Liège*); Pierre Fontaine, Gaëtan Prevot (*Centre Hospitalier Régional Universitaire de Lille*); François Berthezene, Fabrice Bonnet, Maurice Laville, Jean-Pierre Fauvel, Charles Thivolet (*Assistance Publique des Hôpitaux de Lyon*); Bertrand Dussolle, Philippe Vague (*Assistance Publique des Hôpitaux de Marseille*); Patrick Giraud (*Clinique Pont de Chaume, Montauban*); Jacques Bringer, Florence Galtier, Michel Rodier (*Centres Hospitaliers Universitaires de Montpellier et Nîmes*); Pierre Drouin, Laurent Dusselier, Thérèse Crea, Bruno Guerci, Michèle Kessler (*Centre Hospitalier Universitaire de Nancy*); Lucy Chaillous, Bernard Charbonnel (*Centre Hospitalier Universitaire de Nantes*); Hamid Boukersi, Françoise Defrance, Etienne Larger, Michel Marre, Ronan Roussel (*Assistance Publique des Hôpitaux de Paris, Hôpital Bichat-Claude Bernard*); Gérard Slama, Agnès Sola (*Assistance Publique des Hôpitaux de Paris, Hôpital de l'Hôtel Dieu*); André Grimaldi, Agnès Heurtier, Caroline Sert (*Assistance Publique des Hôpitaux de Paris, Hôpital de La Pitié Salpétrière*); Jean-Pierre Grunfeld (*Assistance Publique des Hôpitaux de Paris, Hôpital Necker*); Ahmed Bouallouche, Jean-François Gautier, Pierre-Jean Guillausseau, Hervé Leblanc, Philippe Passa (*Assistance Publique des Hôpitaux de Paris, Hôpitaux Saint-Louis et Lariboisière*); Samy Hadjadj, Richard Maréchaud, Anne Muller, Florence Torremocha (*Centre Hospitalier Universitaire de Poitiers*); Jacques Chanard, Jean-Paul Melin (*Centre Hospitalier Universitaire de Reims*); Hubert Allannic, Jean-Yves Poirier (*Centre Hospitalier Universitaire de Rennes*); Christian Charasse (*Centre Hospitalier de Saint-Brieuc*); Bernard Bauduceau, Lyse Bordier, Hervé Mayaudon (*Hôpital d'Instruction des Armées Bégin, Saint-Mandé*); Marie-Pierre Arpin-Bott (*Centre Hospitalier Universitaire de*

*Strasbour*g); Hélène Hanaire, Pierre Gourdy, Henri Sackmann, Jean-Pierre Tauber (*Centre Hospitalier Universitaire de Toulouse*); Odile Verier-Mine (*Centre Hospitalier de Valenciennes*)

Associated laboratories

Yves Gallois (*Laboratoire de Biochimie Universitaire, Centre Hospitalier Universitaire d'Angers*); François Alhenc-Gelas (*Centre de Recherche des Cordeliers, INSERM, Université de Paris, Sorbonne Université, Paris*)

Statistical Analyses

Kamel Mohammedi (*CHU et université de Bordeaux*); Gilberto Velho (*Centre de Recherche des Cordeliers, INSERM, Université de Paris, Sorbonne Université, Paris*).