**ONLINE-ONLY SUPPLEMENTAL MATERIAL**

# *ACE* I/D polymorphism, plasma ACE levels and long-term kidney outcomes or all-cause death in patients with type 1 diabetes

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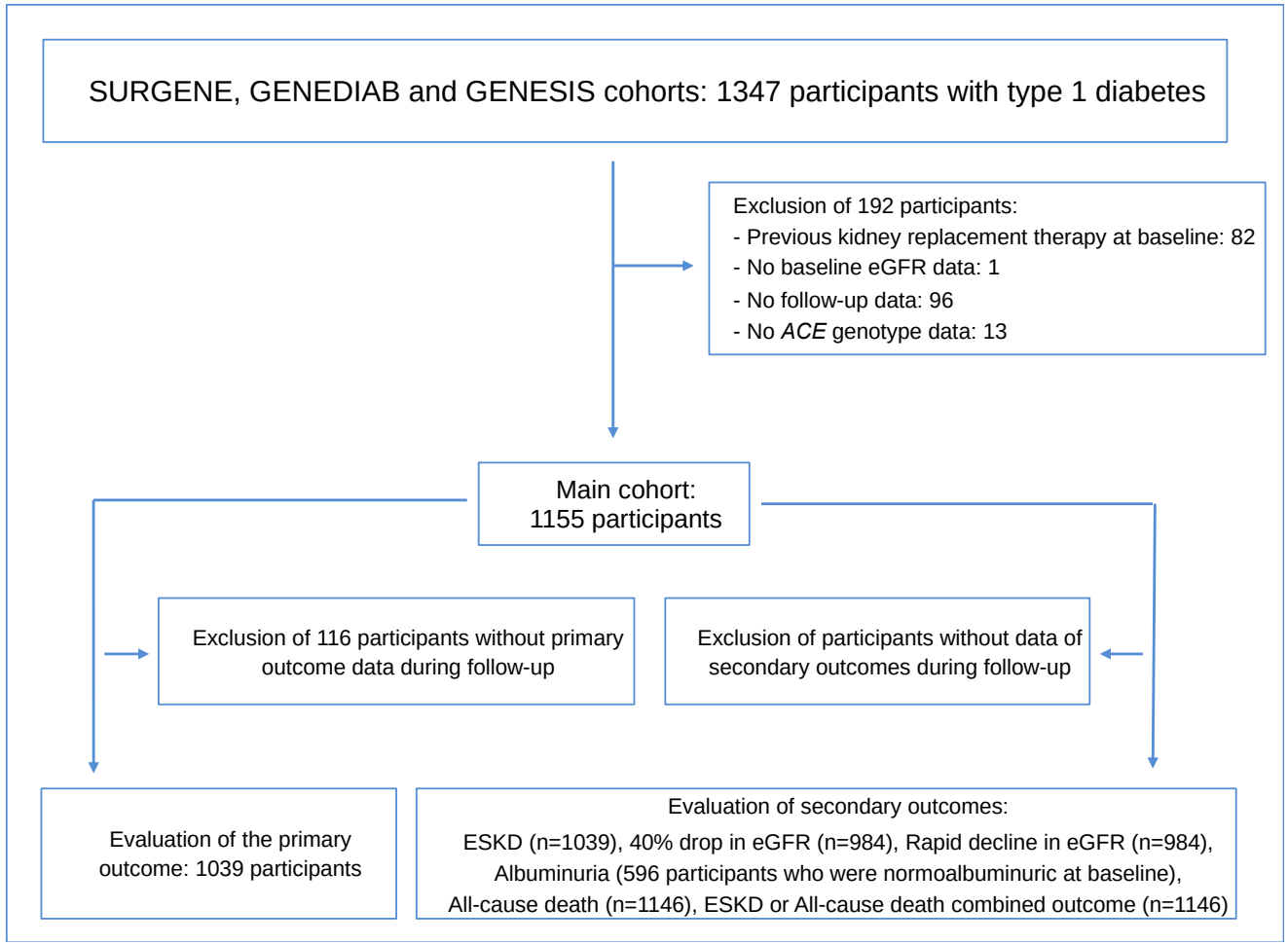
**Supplemental Figure S1:** Flow diagram of participants to the study



**Supplemental Note S1. Clinical profile of excluded participants**

Patients with a history of kidney replacement therapy (hemodialysis, peritoneal dialysis or kidney transplantation, n=82) or without eGFR data (n=1) at baseline, those without kidney or mortality follow-up data (n=96), and those for whom *ACE* I/D genotyping was not available (n=13) were excluded from the analyses. Excluded participants were not a homogeneous group. Patients with a history of kidney replacement therapy at baseline had a younger age of onset of diabetes, a longer duration of diabetes, lower BMI and HbA1c, higher blood pressure, and were more often treated with anti-hypertensive drugs as compared to participants who remained in the analysis. Participants excluded for other reasons had clinical characteristics similar to those of the remaining participants. *ACE* genotype distribution was not significantly different in both groups: 35% DD, 48% ID and 17% II in excluded participants, and 35% DD, 51% ID and 14% II in remaining participants (p=0.62).

**Supplemental Table S1. Characteristics of participants at baseline by cohort membership**

| | SURGENE | GENEDIAB | GENESIS |
|---|---|---|---|
| N (%) | 336 | 337 | 482 |
| Sex (women), n (%) | 146 (43) | 144 (43) | 230 (48) |
| Age, years | 34 ± 13 | 44 ± 12 | 43 ± 12 |
| Age at diabetes diagnosis, years* | 18 (14) | 14 (12) | 14 (15) |
| Duration of diabetes, years | 15 ± 11 | 28 ± 10 | 26 ± 9 |
| Body mass index, $kg/m^2$ | 22.8 ± 3.1 | 23.7 ± 3.1 | 24.6 ± 3.7 |
| Systolic blood pressure, mmHg | 127 ± 16 | 138 ± 18 | 132 ± 19 |
| Diastolic blood pressure, mmHg | 72 ± 11 | 80 ± 11 | 75 ± 10 |
| Mean arterial pressure, mmHg | 91 ± 11 | 99 ± 12 | 94 ± 12 |
| HbA1c, % | 9.3 ± 2.3 | 8.7 ± 1.8 | 8.5 ± 1.3 |
| HbA1c, mmol/mol | 79 ± 25 | 71 ± 20 | 70 ± 14 |
| Total cholesterol, mmol/l† | 5.43 ± 1.34 | 5.73 ± 1.47 | - |
| Triglycerides, mmol/l*† | 0.91 (0.59) | 1.15 (0.87) | - |
| Serum creatinine, µmol/l | 82 ± 24 | 105 ± 56 | 92 ± 64 |
| eGFR, $ml/min/1.73m^2$ | 99 ± 22 | 77 ± 26 | 89 ± 30 |
| UAC, mg/l* | 7 (11) | 43 (449) | 16 (117) |

| | | | |
|---|---|---|---|
| UAC stages, n (%) | | | |
| Normoalbuminuria (<30 mg/l) | 277 (83) | 109 (32) | 264 (55) |
| Microalbuminuria (30 – 300 mg/l) | 35 (10) | 87 (26) | 107 (22) |
| Macroalbuminuria (>300 mg/l) | 24 (7) | 141 (42) | 111 (23) |
| Use of ACE inhibitors, n (%) | 29 (9) | 146 (43) | 178 (37) |
| Use of any antihypertensive drug, n (%) | 42 (13) | 183 (54) | 237 (49) |
| Classes of antihypertensive drugs, n | 0.21 ± 0.64 | 0.97 ± 1.08 | 0.80 ± 1.04 |
| Use of lipid-lowering drugs, n (%) | 0 (0) | 7 (2) | 43 (9) |
| Tobacco smoking, n (%)[‡] | 0 (0) | 163 (48) | 200 (41) |
| History of myocardial infarction, n (%) | 2 (0.6) | 23 (6.8) | 22 (4.6) |
| History of stroke, n (%) | 3 (0.9) | 10 (3.0) | 11 (2.3) |
| Plasma ACE levels, ng/ml[§] | 480 ± 168 | 461 ± 181 | - |

Quantitative data expressed as mean ± SD or median (IQR)*. eGFR: estimated glomerular filtration rate. UAC: urinary albumin concentration. ACE: angiotensin-I-converting enzyme. [†]Total cholesterol and triglycerides were measured at baseline only in GENEDIAB and SURGENE participants. [‡]Current or former smokers. [§]Plasma ACE levels at baseline were measured in 44 SURGENE and 335 GENEDIAB participants.

**Supplemental Table S2. Characteristics of participants at baseline by the occurrence of primary outcome during follow-up**

| | Primary outcome | | p |
|---|---|---|---|
| | No | Yes | |
| N (%) | 844 (81) | 195 (19) | |
| Cohort membership, n (%) | | | |
| SURGENE | 298 (89) | 38 (11) | |
| GENEDIAB | 184 (69) | 81 (31) | <0.0001 |
| GENESIS | 362 (83) | 76 (17) | |
| Sex (women), n (%) | 364 (43) | 95 (49) | 0.17 |
| Age, years | 40 ± 13 | 41 ± 12 | 0.09 |
| Age at diabetes diagnosis, years* | 15 (14) | 14 (13) | 0.59 |
| Duration of diabetes, years | 23 ± 12 | 25 ± 10 | 0.01 |
| Body mass index, kg/m$^2$ | 23.9 ± 3.5 | 23.7 ± 3.4 | 0.48 |
| Systolic blood pressure, mmHg | 129 ± 17 | 142 ± 21 | <0.0001 |
| Diastolic blood pressure, mmHg | 74 ± 10 | 82 ± 12 | <0.0001 |
| Mean arterial pressure, mmHg | 92 ± 11 | 102 + 14 | <0.0001 |
| HbA1c, % | 8.6 ± 1.8 | 9.3 ± 2.1 | <0.0001 |
| HbA1c, mmol/mol | 71 ± 19 | 79 ± 23 | <0.0001 |
| Total cholesterol, mmol/l$^\dagger$ | 5.45 ± 1.28 | 6.11 ± 1.66 | <0.0001 |
| Triglycerides, mmol/l*$^\dagger$ | 0.91 (0.55) | 1.33 (0.84) | <0.0001 |
| Serum creatinine, µmol/l* | 81 (23) | 98 (80) | <0.0001 |

| | | | |
|---|---|---|---|
| eGFR, ml/min/1.73m$^2$ | 94 ± 23 | 73 ± 364 | <0.0001 |
| UAC, mg/l* | 10 (27) | 342 (1235) | <0.0001 |
| UAC stages, n (%) | | | |
| Normoalbuminuria (<30 mg/l) | 578 (68) | 38 (20) | |
| Microalbuminuria (30 – 300 mg/l) | 168 (20) | 28 (14) | <0.0001 |
| Macroalbuminuria (>300 mg/l) | 98 (12) | 129 (66) | |
| Use of ACE inhibitors, n (%) | 205 (24) | 102 (52) | <0.0001 |
| Use of any antihypertensive drug, n (%) | 269 (32) | 135 (69) | <0.0001 |
| Classes of antihypertensive drugs, n | 0.49 ± 0.85 | 1.32 ± 1.16 | <0.0001 |
| Use of lipid-lowering drugs, n (%) | 33 (4) | 12 (6) | 0.17 |
| Tobacco smoking, n (%)[‡] | 241 (29) | 69 (35) | 0.07 |
| History of myocardial infarction, n (%) | 27 (3.2) | 10 (5.3) | 0.20 |
| History of stroke, n (%) | 11 (1.3) | 8 (4.1) | 0.02 |
| ACE I/D genotype, n (%) | | | |
| DD | 294 (35) | 69 (36) | |
| ID | 389 (46) | 102 (52) | 0.07 |
| II | 161 (19) | 24 (12) | |
| Plasma ACE levels, ng/ml[§] | 450 ± 177 | 514 ± 179 | 0.004 |

Primary outcome: ESKD or a 40% drop in eGFR during follow-up. Quantitative data expressed as mean ± SD or median (IQR)*. Qualitative data expressed as number of patients (and %). eGFR: estimated glomerular filtration rate. UAC: urinary albumin concentration. ACE: angiotensin-I-converting enzyme. [†]Total cholesterol and triglycerides were measured at baseline only in GENEDIAB and SURGENE participants: 119 incident cases and 482 non-incident cases of the primary outcome. [‡]Current or former smokers. [§]Plasma ACE levels were measured at baseline in 97 incident cases and 211 non-incident cases of the primary outcome. Statistics are Fisher's exact test, ANOVA, or Wilcoxon tests. P<0.05 was significant.

**Supplemental Table S3. Use of antihypertensive and lipid-lowering drugs at baseline by *ACE* I/D genotype and primary outcome during follow-up**

| | ACE genotype | | | Primary outcome | | |
|---|---|---|---|---|---|---|
| | **XD** | **II** | **p** | **No** | **Yes** | **p** |
| N | 954 | 201 | | 844 | 195 | |
| ACE inhibitors | 297 (31) | 56 (28) | 0.40 | 205 (24) | 102 (52) | <0.0001 |
| Angiotensin II receptor antagonists | 22 (2.3) | 2 (1.0) | 0.41 | 11 (1.3) | 9 (4.6) | 0.006 |
| Diuretics | 109 (11) | 23 (11) | 0.99 | 57 (7) | 51 (26) | <0.0001 |
| Calcium channel blockers | 140 (15) | 23 (11) | 0.27 | 87 (10) | 54 (28) | <0.0001 |
| Adrenergic beta blockers | 51(5) | 10 (5) | 0.99 | 31 (4) | 22 (11) | <0.0001 |
| Adrenergic alpha blockers | 15 (1.6) | 2 (1.0) | 0.75 | 6 (0.7) | 7 (3.6) | 0.005 |
| Alpha-2 adrenergic receptor agonists | 28 (2.9) | 3 (1.5) | 0.34 | 15 (1.8) | 13 (6.7) | 0.0006 |
| Statins | 33 (3.5) | 9 (4.5) | 0.53 | 27 (3.2) | 10 (5.1) | 0.20 |
| Fibrates | 8 (0.84) | 0 | 0.36 | 6 (0.7) | 2 (1.0) | 0.65 |

Data expressed as number of participants (and %). The XD genotype represents the combined data of ID and DD genotype carriers of rs1799752. Primary outcome: ESRD or a 40% drop in eGFR during follow-up. Statistics are Fisher's exact test.

**Supplemental Table S4. Primary outcome during follow-up by *ACE* I/D genotype in individual cohorts**

| | Outcomes | | Incidence Rate | Adjusted Model | | |
|---|---|---|---|---|---|---|
| | No, n (%) | Yes, n (%) | (95% C.I.) | Hazard Ratio (95% C.I.) | p | p for interaction* |
| SURGENE | | | | | | |
| II genotype | 52 (93) | 4 (7) | 15.6 (8.4 – 29.0) | 1 | | |
| XD genotype | 246 (88) | 34 (12) | 30.1 (23.8 – 37.9) | 1.81 (0.65 – 6.54) | 0.27 | |
| GENEDIAB | | | | | | 0.54 |
| II genotype | 43 (81) | 10 (19) | 11.7 (6.3 – 21.8) | 1 | | |
| XD genotype | 141 (67) | 71 (33) | 17.4 (13.6 – 22.1) | 2.43 (1.18 – 5.78) | 0.01 | |
| GENESIS | | | | | | |
| II genotype | 66 (87) | 10 (13) | 4.7 (1.8 – 12.7) | 1 | | |
| XD genotype | 296 (82) | 66 (18) | 6.6 (4.8 – 9.3) | 2.97 (1.49 – 6.55) | 0.001 | |

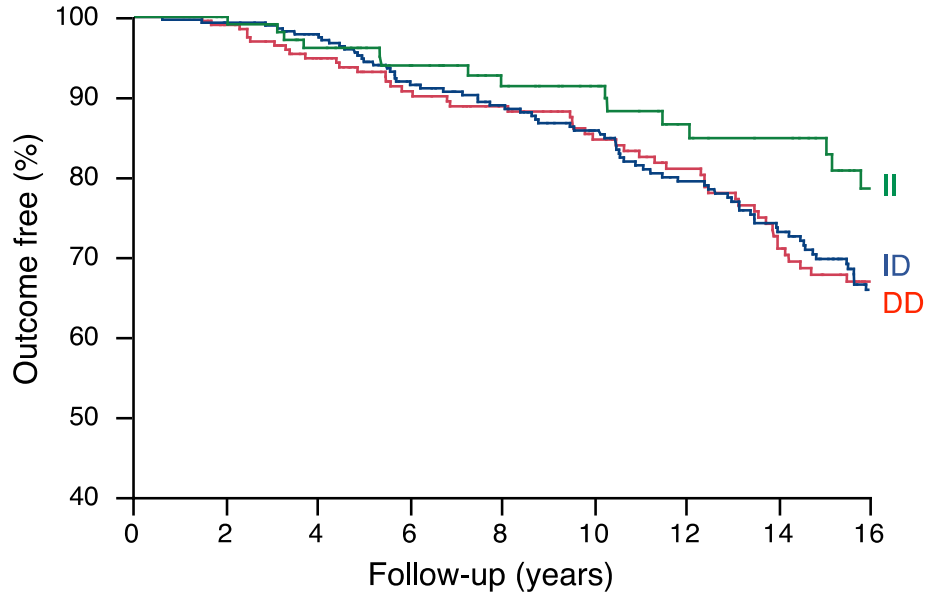
Primary outcome: ESRD or a 40% drop in eGFR during follow-up. Data expressed as number of participants (and %). Incidence rates expressed per 1000 persons-years. Hazard ratios with 95% confidence intervals (CI) for the XD versus the II genotype of rs1799752 computed by Cox regression analysis, adjusted for sex, age, duration of diabetes, MAP, HbA1c, eGFR, and use of ACE inhibitors and antihypertensive drugs at baseline. The XD genotype represents the combined data of ID and DD genotype carriers. Genotypes were in Hardy-Weinberg equilibrium. *Statistical significance for the interaction between *ACE* I/D genotype and cohort membership. P<0.05 was significant.

**Supplemental Table S5. Prognostic performance of *ACE* I/D genotype for primary outcome**

| Statistical test | Estimate | 95% Confidence Interval | p |
|---|---|---|---|
| Harrell's c-statistic for model 1 | 0.844 | (0.815 – 0.874) | |
| Change in Harrell's c-statistic for model 1 plus *ACE* I/D genotype | -0.0004 | (-0.0006 – 0.005) | 0.89 |
| IDI | 0.012 | (0.001 – 0.021) | 0.02 |
| NRI | 0.154 | (0.007 – 0.279) | 0.04 |

Model 1: cohort membership, sex, age, duration of diabetes, MAP, HbA1c, eGFR, and use of ACE inhibitors and antihypertensive drugs at baseline. Integrated discrimination improvement (IDI) and net reclassification improvement (NRI) tests were performed for 10-year risk of primary outcome (ESKD or a 40% drop in eGFR during follow-up) associated with model 1 plus *ACE* I/D genotype versus model 1 alone. P<0.05 was significant.

**Supplemental Figure S2:** Kaplan-Meier outcome-free curves for the incidence of albuminuria during follow-up by *ACE* I/D genotype (rs1799752)




| Number of participants at risk | | | | | |
|---|---|---|---|---|---|
| II | 113 | 97 | 72 | 51 | 36 |
| ID | 281 | 265 | 206 | 158 | 99 |
| DD | 202 | 176 | 138 | 108 | 75 |

**List of Contributors to SURGENE, GENEDIAB, and GENESIS studies**

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