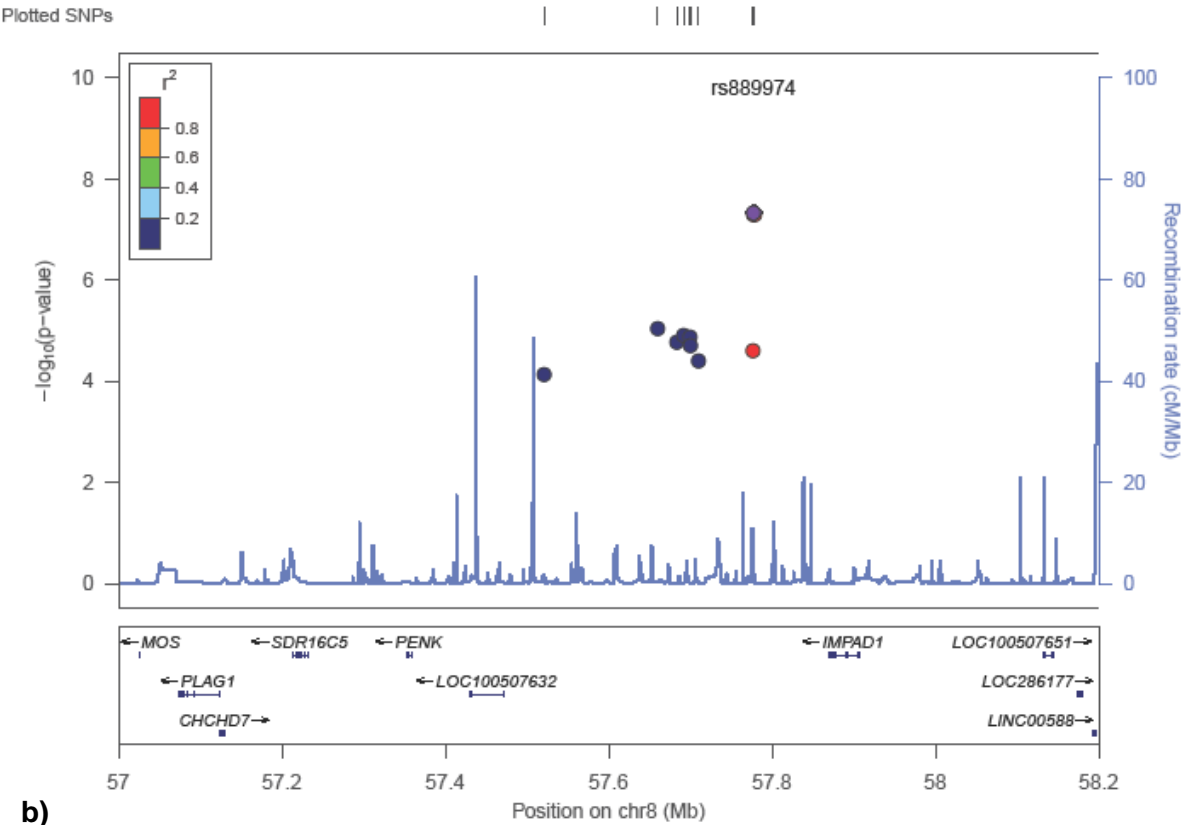


Supporting information

a)



b)

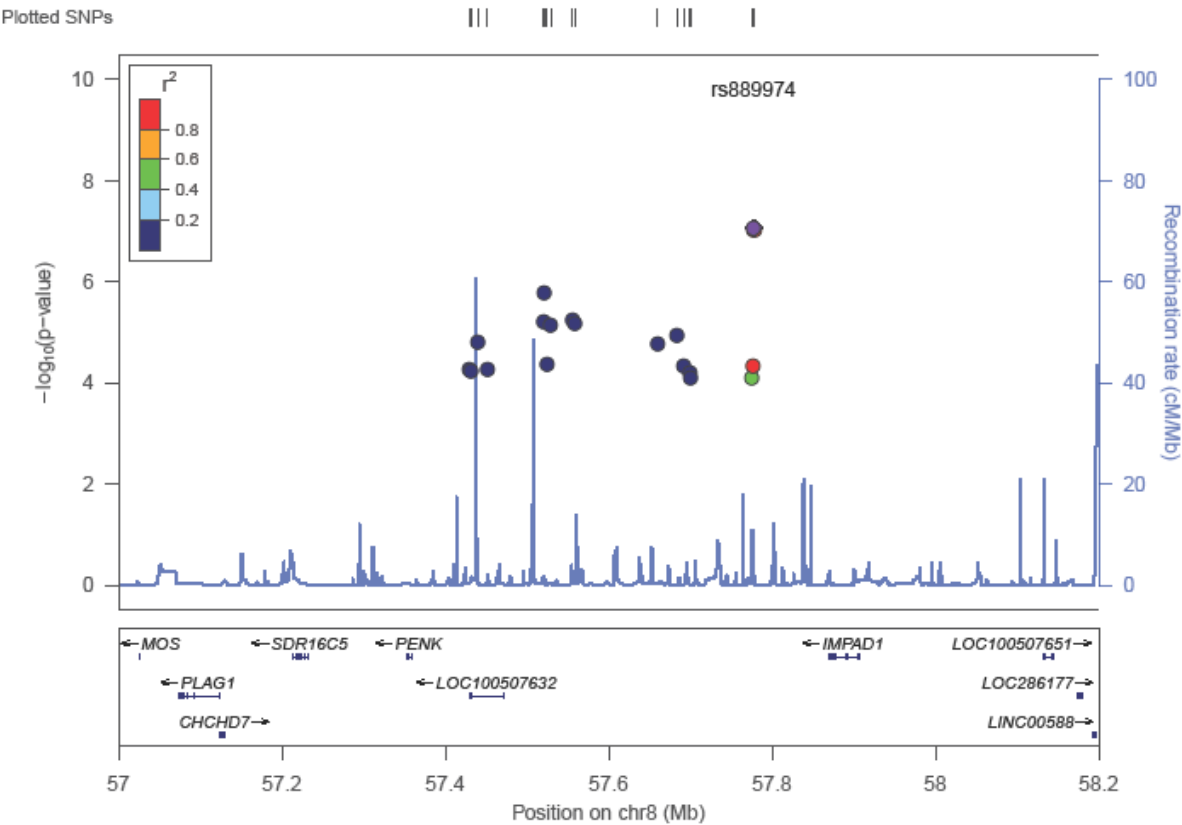


Figure S1: Conditional analyses on a) rs1012178 (top variant $P=4.666^{-21}$) and b) rs17760660 ($P=7.78^{-20}$) genotype. The purple Diamond indicates the lead SNP in addition to all identified SNPs within different degrees of perfect LD ($r^2 \geq 0.80\%$ (red), 0.8-0.6 (orange), 0.6-0.4 (green), 0.4-0.2 (light blue) and ≤ 0.2 , respectively) at this locus. After adjusting for a) rs1012178 the previously observed associations below genome-wide level disappeared, including a complete loss of association for rs17760660. Likewise, no further associations with P-values $< 5 \times 10^{-8}$ were observed after conditioning on b) rs17760660, but rs1012178 still remained associated with fasting plasma pro-ENK concentration ($P=5.3 \times 10^{-05}$) and was used in further analyses.

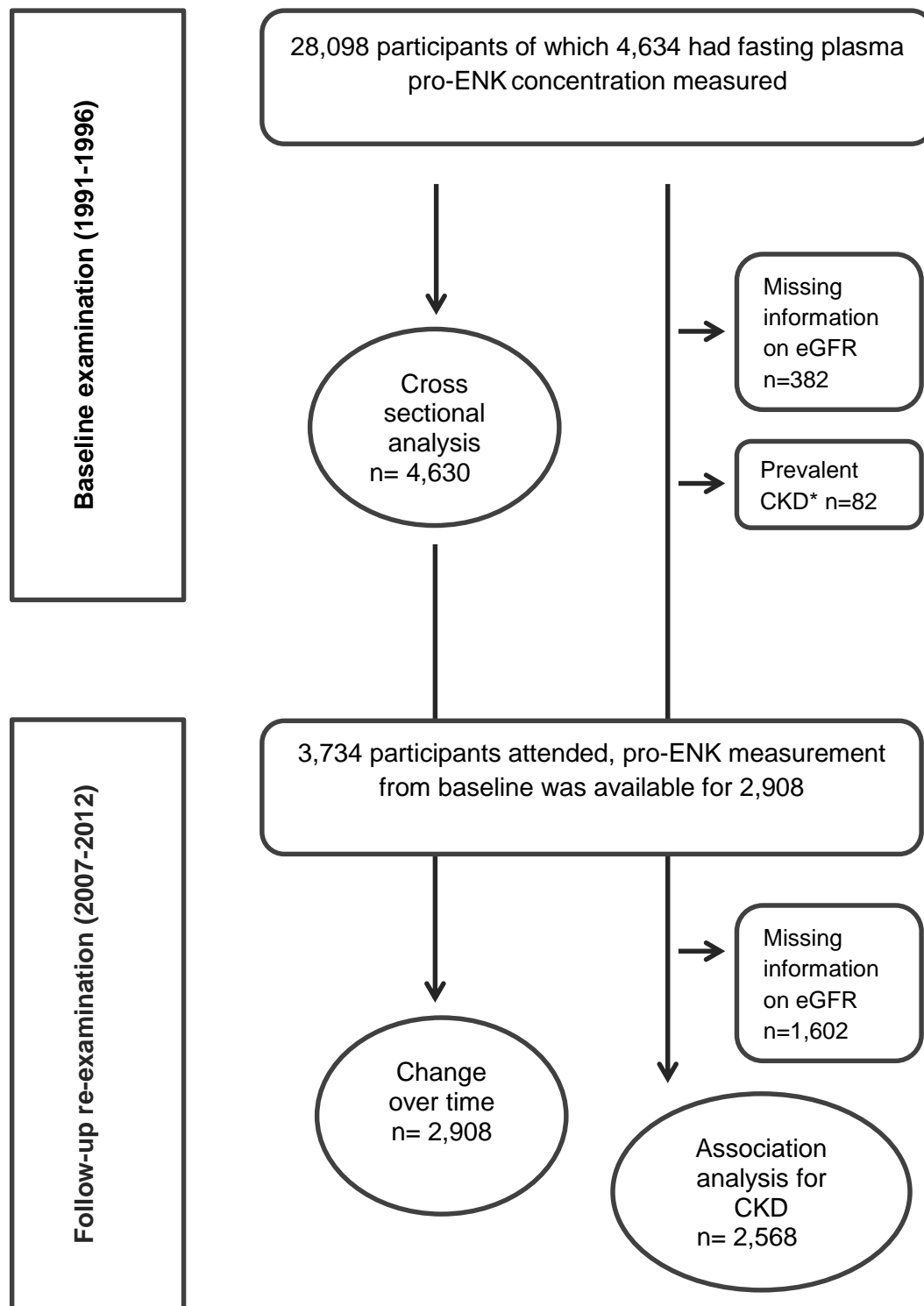


Figure S2: Participants included in association analyses for pro-ENK concentration and future CKD (eGFR < 60/mL/min² based on equation by Inker et al. 2012)

Table S1 Cross-sectional associations between pro-ENK concentration and baseline characteristics multivariate adjusted

	n	Fasting Plasma pro-enkephalin concentration			P-trend ¹	P-trend ²	P-trend ³	P-trend ⁴	P-trend ⁵	P-trend ⁶	P-trend ⁷
		Low	Medium	High							
Age (years)	4634	57.08 (0.152)	57.56 (0.152)	58.63 (0.152)	<0.000001	/	/	/	/	/	/
BMI (kg/m²)	4630	26.69 (0.099)	25.80 (0.098)	24.96 (0.099)	<0.000001	/	0.005158	<0.000001	0.014483	<0.000001	<0.000001
Waist (cm)	4629	87.65 (0.255)	84.85 (0.254)	83.00 (0.255)	<0.000001	0.000019	0.000054	<0.000001	0.002791	<0.000001	<0.000001
SBP (mmHg)	4634	144.42 (0.459)	141.58 (0.458)	141.33 (0.460)	0.000002	0.019607	0.011960	0.000249	0.001302	<0.000001	<0.000001
DBP (mmHg)	4634	88.36 (0.237)	87.07 (0.236)	86.78 (0.237)	0.000003	0.060197	0.051434	0.000050	0.010587	<0.000001	<0.000001
Glucose (mmol/L)*	4616	6.04 (0.039)	5.71 (0.039)	5.56 (0.039)	<0.000001	<0.000001	<0.000001	/	<0.000001	<0.000001	<0.000001
Creatinine (μmol/L)	4541	81.80 (0.370)	84.73 (0.368)	88.96 (0.370)	<0.000001	<0.000001	<0.000001	<0.000001	<0.000001	<0.000001	0.031277
Cystatin C (mg/L)	4310	0.75 (0.004)	0.78 (0.004)	0.83 (0.004)	<0.000001	<0.000001	<0.000001	<0.000001	<0.000001	/	0.090540
eGFR CKD-EPI 2012	4252	93.55 (0.302)	90.04 (0.302)	85.40 (0.306)	<0.000001	<0.000001	<0.000001	<0.000001	<0.000001	<0.000001	/

Data presented as mean (SE); general linear model adjusted for ¹age (years) and sex (basic); ² basic and BMI (kg/m²); ³ basic and fatmass (kg); ⁴ basic and glucose

concentration(mg/dl); ⁵ basic and lean mass (kg); ⁶ basic and cystatin C concentration(mg/L); ⁷ basic and eGFR based on EPI-CKD 2012⁴¹ (mL/min/1.73m²); * whole blood

fasting blood concentrations were transferred into plasma value by multiplying with the factor 1.1; SBP= Systolic blood pressure; DBP= Diastolic blood pressure

Table S2 Baseline characterizes depending on availability of fasting plasma pro-ENK concentration at MDCS baseline (1991-1994)

	pro-ENK concentration unavailable at baseline (n=1460)	pro-ENK concentration available at baseline (n=4634)
Male (%)	41.2	42.5
Age (years)	56.64 (5.71)	57.74 (5.98)
Weight (kg)	73.88 (14.17)	73.75 (13.55)
BMI (kg/m²)	25.79 (4.13)	25.76 (3.95)
Waist (cm)	85.06 (13.30)	83.96 (12.91)
SBP (mmHg)	138.77 (18.59)	142.21 (19.15)
DBP (mmHg)	86.46 (9.29)	87.16 (9.49)
Fasting plasma glucose (mmol/L)¹	5.46 (1.26)	5.74 (1.56)
Cystatin C (mg/L)	0.77 (0.15)	0.78 (0.15)
Creatinine (μmol/L)	88.01 (16.67)	84.15 (16.16)
Use of anti-hypertensive medication (%)	15.1	17.0

Data presented as mean (SD); SBP= Systolic blood pressure; DBP= Diastolic blood pressure ;¹ Whole blood fasting concentrations were transferred into plasma value by multiplying with the factor 1.1;

Table S3 Equations for estimated glomerular filtration rate (eGFR)*

Gender	Creatinine (μmol/L)	Cystatin C (mg/L)	eGFR equation
CKD-EPI-2012			
Female	≤ 62	≤ 0.8	$130 \times (\text{creatinine} \times 0.0113 / 0.7)^{-0.248} \times (\text{cystatin C} / 0.8)^{-0.375} \times 0.995^{\text{Age}}$
Female	≤ 62	> 0.8	$130 \times (\text{creatinine} \times 0.0113 / 0.7)^{-0.248} \times (\text{cystatin C} / 0.8)^{-0.711} \times 0.995^{\text{Age}}$
Female	> 62	≤ 0.8	$130 \times (\text{creatinine} \times 0.0113 / 0.7)^{-0.601} \times (\text{cystatin C} / 0.8)^{-0.375} \times 0.995^{\text{Age}}$
Female	> 62	> 0.8	$130 \times (\text{creatinine} \times 0.0113 / 0.7)^{-0.601} \times (\text{cystatin C} / 0.8)^{-0.711} \times 0.995^{\text{Age}}$
Male	≤ 80	≤ 0.8	$135 \times (\text{creatinine} \times 0.0113 / 0.9)^{-0.207} \times (\text{cystatin C} / 0.8)^{-0.375} \times 0.995^{\text{Age}}$
Male	≤ 80	> 0.8	$135 \times (\text{creatinine} \times 0.0113 / 0.9)^{-0.207} \times (\text{cystatin C} / 0.8)^{-0.711} \times 0.995^{\text{Age}}$
Male	> 80	≤ 0.8	$135 \times (\text{creatinine} \times 0.0113 / 0.9)^{-0.601} \times (\text{cystatin C} / 0.8)^{-0.375} \times 0.995^{\text{Age}}$
Male	> 80	> 0.8	$135 \times (\text{creatinine} \times 0.0113 / 0.9)^{-0.601} \times (\text{cystatin C} / 0.8)^{-0.711} \times 0.995^{\text{Age}}$

*modified based on Inker et al. ¹; factor 0.0113 was used to convert creatinine concentration from mg/dL into μmol/L