ADMA Contributes to the Impaired Response to Erythropoietin in CKD-Anemia

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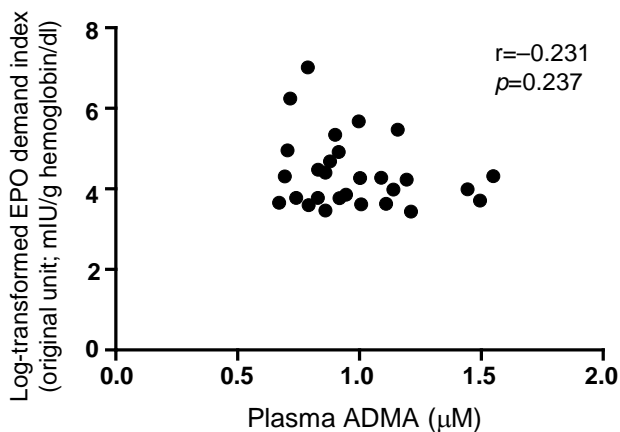Correspondence to Yosuke Nakayama

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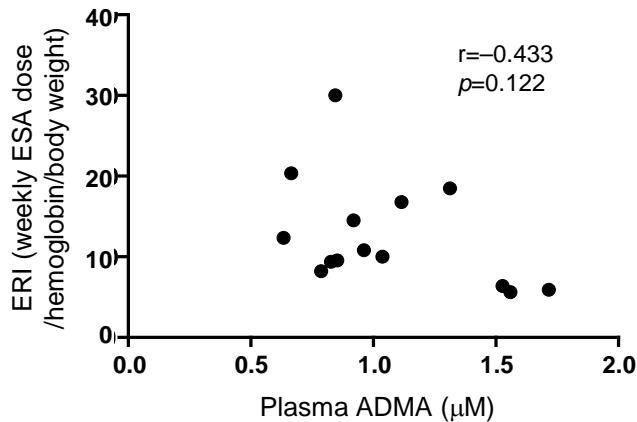
Supplemental Figure 1. Plasma ADMA levels showed no association with impaired response to erythropoietin in predialysis patients. (A) ERI in patients undergoing ESA therapy during more than 1 month without medications by oral iron supplement (n=14). (B) Log-transformed erythropoietin demand indices in patients who were not received ESA (n=28). Statistical significance was determined using Pearson correlation coefficient. ADMA; asymmetric dimethylarginine, ESA; erythropoietin stimulating agents, ERI; ESA resistance index.
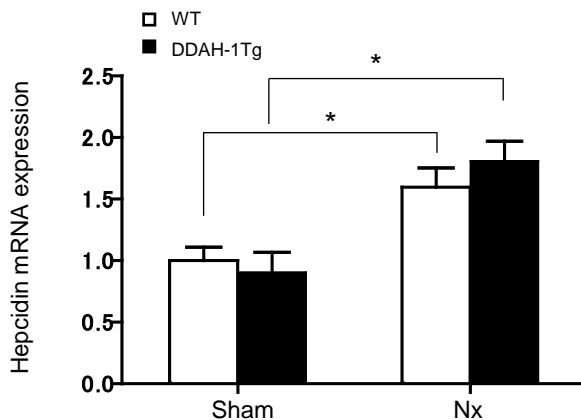
Supplemental Figure 2. mRNA expression of hepcidin was increased in the livers of mice 12 weeks after Nx. DDAH-1 overexpression failed to suppress hepatic hepcidin expression in Nx-mice. The mRNA expression of hepcidin relative to β-actin as an endogenous control. mRNA expression ratio was normalized to WT sham mice. Columns express means and error bars indicate S.E.M; WT mice, n=5; WT Nx-mice, n=5; DDAH-1 Tg mice, n=5; DDAH-1 Tg Nx-mice, n=8. Statistical significance was determined using a Mann Whitney test. *p<0.01. DDAH-1; dimethylaminohydrolase-1, Nx; 5/6 subtotal nephrectomy, Tg; transgenic, WT; wild type.
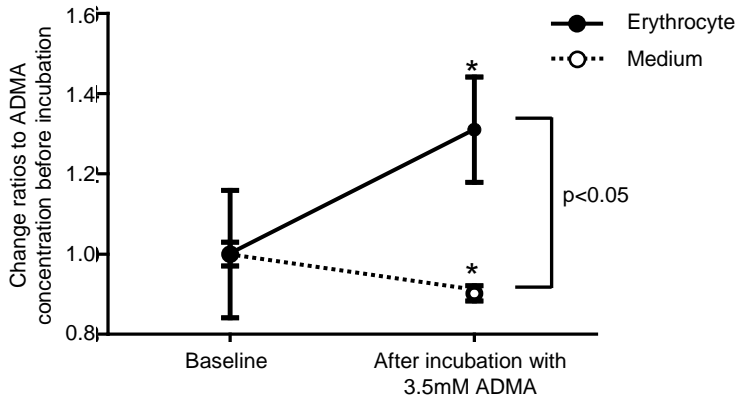
Supplemental Figure 3. *In vitro* experiment of the relation of plasma ADMA to erythrocyte ADMA. *In vitro* experiment was performed using healthy volunteer's blood (n=5). Blood was centrifuged at 1,800 *g* for 8 min at 4°C and plasma and buffy coat were removed, and then erythrocytes were obtained. After washing with PBS twice, erythrocytes were incubated with 3.5 mM ADMA for 14 hours at 37°C. Then, ADMA levels in the medium and erythrocyte were measured. Change ratios were the ratio of 14 hours to 0 hour (average±SD). *p<0.05 vs 0 hour. ADMA; asymmetric dimethylarginine, NaPi; sodium phosphate, PBS; phosphate buffered saline.

A)

r=−0.256
p=0.062

Plasma ADMA (μM) vs erythrocyte ADMA (nmol/g protein)

B)

r=0.155
p=0.597

Plasma ADMA (μM) vs erythrocyte ADMA (nmol/g protein)
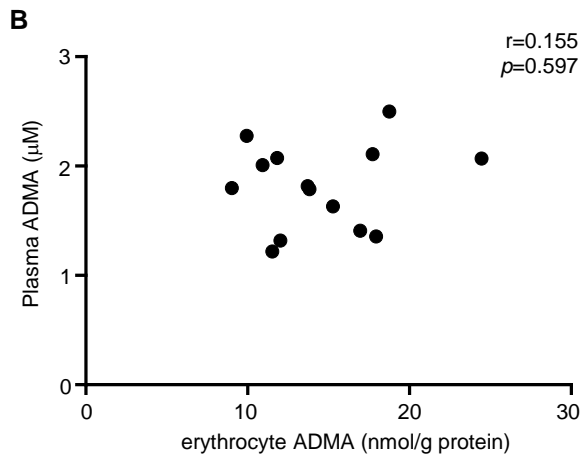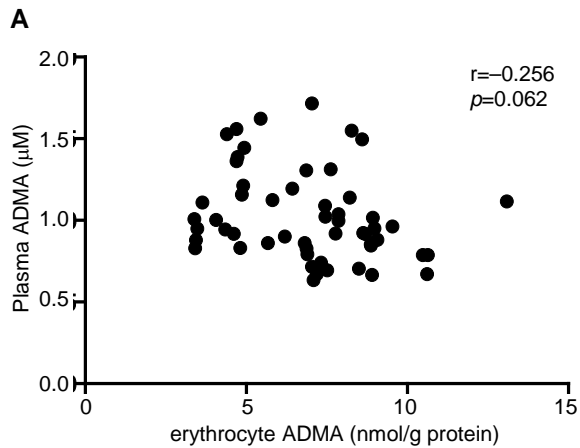
Supplemental Figure 4. Relationships between plasma ADMA and erythrocyte ADMA levels in predialysis patients and mice. **A)** No association is apparent in predialysis patients (n=54). **B)** No association is apparent in Nx-mice (n=14). Statistical significance was determined using Spearman correlation coefficients. ADMA, asymmetric dimethylarginine; Nx, 5/6 subtotal nephrectomy

Supplemental Table 1. Univariate and stepwise multiple regression analyses of erythrocyte ADMA levels in predialysis patients

Variable	Univariate regression analysis		Stepwise multiple regression analysis	
	r	*p*	Adjusted β (95% CI; lower, upper)	*p*
Age, years	0.039[a]	0.778		
BMI, kg/m^2	0.097[a]	0.495		
Systolic BP, mmHg	0.145[a]	0.296		
Diastolic BP, mmHg	−0.023[a]	0.868		
Hemoglobin, g/dl	**−0.411[a]**	**0.002**	**−0.269 (−0.613, −0.066)**	**0.016**
Hematocrit, %	**−0.380[a]**	**0.005**		
RBC, x 10^4/μl	−0.158[a]	0.252		
Mean corpuscular volume, fl	**−0.321[a]**	**0.018**		
Mean corpuscular hemoglobin, pg/cell	**−0.370[a]**	**0.006**		
Total protein, g/dl	−0.212[a]	0.125		
Albumin, g/dl	−0.186[a]	0.178		
LDL-cholesterol, mg/dl	0.078[a]	0.577		
BUN, mg/dl	**0.337[a]**	**0.013**		
Serum Creatinine (mg/dl)	**0.428[b]**	**0.001**		
eGFR, ml/min/1.73m^2	**−0.443[b]**	**0.001**		
LDH, IU/l	0.153[a]	0.285		
Mean of erythrocyte fragility, %	0.078[a]	0.633		
CRP, mg/dl	−0.061[b]	0.720		
Ferritin, ng/ml	−0.156[b]	0.258		
Serum iron (mg/dl)	**−0.357[a]**	**0.008**		
TSAT, %	**−0.275[a]**	**0.044**	−0.211 (−0.086, 0.000)	0.050
NT-proBNP	**0.575[b]**	**<0.001**	**0.500 (0.401, 1.009)**	**<0.001**
Plasma ADMA, μM	−0.256[a]	0.062		
Erythrocyte ADMA, nmol/g protein	-	-		

a, Pearson's correlation coefficient, b, Spearman's rank correlation coefficient. Stepwise multiple regression analysis was performed to input age, sex and significantly correlating variables by the univariate correlation after logarithmic transformation of NT-proBNP levels. Adjusted r^2 for this model is 0.424. Bold values are statistically significant ($p < 0.05$ in the univariate analysis, $p < 0.05$ in stepwise regression analysis). * The values of 17 patients were below the detection limit ($p < 0.01$). ADMA; asymmetric dimethylarginine, BMI; body mass index, BP; blood pressure, BUN; blood urea nitrogen, CRP; C-reactive protein, eGFR; estimated glomerular filtration rate, Hb; hemoglobin, LDH; lactate dehydrogenase, NT-proBNP; N-terminal pro-B-type natriuretic peptide, TSAT; transferrin saturation.

Supplemental Table 2. Stepwise multiple regression analyses of logarithm of NT-proBNP in predialysis patients

Variable	Stepwise multiple regression analysis	
	Adjusted β (95% CI; lower, upper)	P
Erythrocyte ADMA, nmol/g protein	0.559 (0.227, 0.539)	<0.001
Albumin, g/dl	−0.264 (−1.078, -0.080)	0.024
Age, years	0.230 (0.000, 0.050)	0.046

Stepwise multiple regression analysis was performed after logarithmic transformation of NT-proBNP levels. Input variables are age, sex, current smoking history, BMI, systolic BP, diastolic BP, hemoglobin, total protein, albumin, LDH, LDL-cholesterol, eGFR, TSAT and erythrocyte ADMA. Variables that are not shown in the table were excluded from the model. Adjusted R^2 for this model is 0.439. ADMA; asymmetric dimethylarginine, NT-proBNP; N-terminal pro-B-type natriuretic peptide.