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Supplemental Data

Identification of Matrix Metalloproteinase-12 as a Candidate Molecule for Prevention and Treatment of Cardiometabolic Disease

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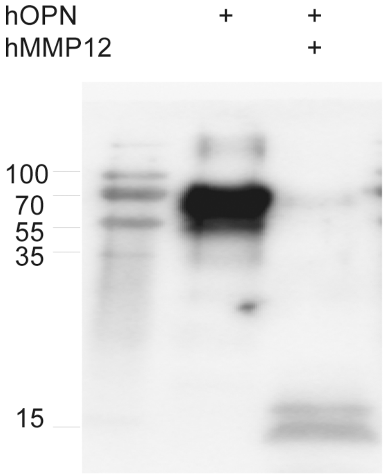

Supplementary Figure S1. Enzymatic cleavage of OPN by MMP12. 100μg of human full length OPN (PeproTech) were incubated with 50μg of recombinant *MMP12* (R&D) during 4 h. at 37°C. The products from the enzymatic reaction were loaded into a SDS-PAGE and western blot was performed as described in materials and methods. OPN was detected with anti-OPN rabbit polyclonal antibody (Novus). As it is shown in the figure, OPN is cleaved by *MMP12* (lane 3).

Supplementary Table S1. Human study baseline characteristics.

	Lean[A]	Obese[A]	P
Age (y)	44.14 + -2.12 (n = 21)	42 + -2.38 (n = 21)	0.50
BMI[B]	24.73 + -0.64 (n = 21)	46.42 + -1.53 (n = 21)	<0.001
WHR[C]	0.88 + -0.02 (n = 12)	0.93 + -0.02 (n = 21)	0.152
Glucose (mg/dl)	93.82 + -3.40 (n = 17)	91.86 + -2.02 (n = 21)	0.623
Insulin (μg/mL)	9.08 + -1.98 (n = 18)	19.60 + -2.60 (n = 21)	0.003
C-Peptide (ng/mL)	0.59 + -0.16 (n = 19)	3.96 + -0.31 (n = 21)	<0.001
HOMA IR[D]	2.23 + -0.47 (n = 18)	4.48 + -0.61 (n = 21)	0.006
Creatinine (mg/dl)	0.85 + -0.06 (n = 19)	0.88 + -0.03 (n = 21)	0,636
Triglycerides (mg/dl)	142.74 + -16 (n = 19)	176.67 + -17.96 (n = 21)	0,167
Cholesterol (mg/dl)	182.26 + -7.71 (n = 19)	218.57 + -9.39 (n = 21)	0.005
Apolipoprotein B (mg/dl)	95.37 + -6 (n = 19)	110.45 + -5.18 (n = 21)	0.065
hsCRP (mg/dl)	0.46 + -0.27 (n = 18)	0.79 + -0.12 (n = 21)	0.27
GOT (U/L)[E]	27.76 + -3.96 (n = 17)	27.50 + -1.60 (n = 20)	0.95
GPT (U/L)[F]	16.17 + -2.10 (n = 18)	39.23 + -4.70 (n = 21)	<0.001
γ-GT (U/L)[G]	28.12 + -5.80 (n = 17)	40.90 + -18.00 (n = 20)	0.081

[A]Data represented by mean ± SEM; [B]Body mass index; [C]Waist-to-hip ratio; [D]Insulin-resistance index; [E]Glutamic oxaloacetic transaminase; [F]Glutamic pyruvic transaminase; [G]γ-glutamyl transferase.

Supplementary Table S2. Data sets of 2 sample (case/control) microarray experiments included in the cardiovascular disease meta-analysis study.

	Data set 2 (GSE28829)	Data set 4 (GSE40231)	Data set 7 (GSE38574)	Data set 8 (GSE18443)	Data set 9 (GSE2372)	Data set 10 (GSE1560)
Number of genes	21,187	21,187	16,223	16,585	16,223	14,538
Platform	Affymetrix U133 Plus 2.0	Affymetrix U133 Plus 2.0	Affymetrix Mouse genome 430 2.0	Rosetta/Merck Mouse 44k 1.0	Affymetrix Mouse genome 430 2.0	Agilent 011978 Mouse Microarray G4121A
Species	Human	Human	Mouse	Mouse	Mouse	Mouse
Original Normalization	RMA log2 intensities	RMA intensities	MAS 5.0 raw intensities	log10 ratios	Signal value scaled to 500 by GCOS	log ratio
Additional preprocessing	Quantile-normalization	log2 + quantile normalization	log2 + quantile normalization	Quantile normalization	log2 + quantile normalization	Quantile normalization
Sample size (case/ control)	16/ 13	40/ 40	6 / 7	3/ 3	3/ 3	3/ 3

Supplementary Table S3. Data set of 1 sample (cases only) microarray experiments included in the cardiovascular disease meta-analysis study.

	Data set 1 (GDS3698)	Data set 3 (GSE21545)	Data set 5 (GSE2143)	Data set 6 (GSE23314)
Number of genes	15,109	21,187	11,765	20,158
Platform	Micro-CRIBI Human Oligo Array V_0 (Operon V2.0)	Affymetrix U133 Plus 2.0	Agilent – TQ Cardiovascular Human Oligonucleotide Array	Rosetta/Merck Human RSTA Affymetrix 1.0 microarray, custom CDF
Species	Human	Human	Mouse/Human	Human
Original Normalization	LOWESS, log2 (Ch1/Ch2)	RMA log2 signal intensity	Log2 ratio	RMA
Additional preprocessing	Quantile normalization	Quantile normalization	Quantile normalization	log2 + quantile normalization
Sample size (case/ control)	22 (cases)	126 (cases)	51 (cases)	113 (cases)

Supplementary Table S4. Fold change of the 8 candidate genes in a patient case/control microarray study (GSE28829) included in the cardiovascular disease meta-analysis.

	Fold change
MMP12/Mmp12	11.6
TREM2/ Trem2	2.0
MPEG1/Mpeg1	2.5[A]
GPR137B/ Gpr137b	1.8
SYK/Syk	1.7[A]
MAFB/Mafb	2.0[A]
PLTP/Pltp	2.8
RRM2/Rrm2	2.4[A]

[A] Mean of the fold change of all the probe sets present in the Affymetrix U133 Plus 2.0 chip.

S2 | AMOR ET AL. | MOL MED 22, 2016

Supplementary Table S5. Top 10 upregulated genes list from the GWAT microarray.

Gene name	Gene symbol	Fold change	Adjusted *p* value
ATPase, H + transporting, lysosomal V0 subunit D2	*Atp6v0d2*	40.0	2.38E-07
Olfactory receptor 111	*Olfr111*	26.9	6.71E-07
Transmembrane 4 L six family member 19	*Tm4sf19*	26.1	6.69E-06
Ring finger protein 128	*Rnf128*	23.7	8.60E-06
Regulator of G-protein signaling 1	*Rgs1*	22.3	1.26E-05
Glycoprotein (transmembrane) nmb	*Gpnmb*	21.8	1.15E-07
Integrin α X	*Itgax*	21.2	2.87E-07
Toll-like receptor 13	*Tlr13*	18.5	1.17E-06
Secreted phosphoprotein 1/ Osteopontin	*Spp1/OPN*	18.2	8.86E-05
Macrophage metalloelastase	*Mmp12*	16.9	5.45E-07

Supplementary Table S6. List of upregulated matrix metalloproteinases (MMPs) in the considered studies ordered by expression in the GWAT microarray.

	GWAT microarray (fold change)	Meta-analyses (pVal)	
	WT (HFD) versus WT (CD)	Cardiovascular disease	Type 2 diabetes
MMP12/Mmp12	16.9	0.00232	0.00077
MMP13/Mmp13	6.0	0.04091	-
MMP3/Mmp3	5.9	-	-
MMP2/Mmp2	4.0	-	-
MMP19/Mmp19	3.0	-	-
MMP27/Mmp27	2.3	-	-
MMP14/Mmp14	2.2	-	-
MMP9/Mmp9	-	-	0.00142
MMP7/Mmp7	-	-	0.00061