

## Appendix-II

### Spatiotemporal expression of matrix metalloproteinase-1 in progression of nonalcoholic steatohepatitis

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DOI: <http://nobleresearch.org/Doi/10.14312/2397-6845.2016-3>

#### Supplementary Table and Figures

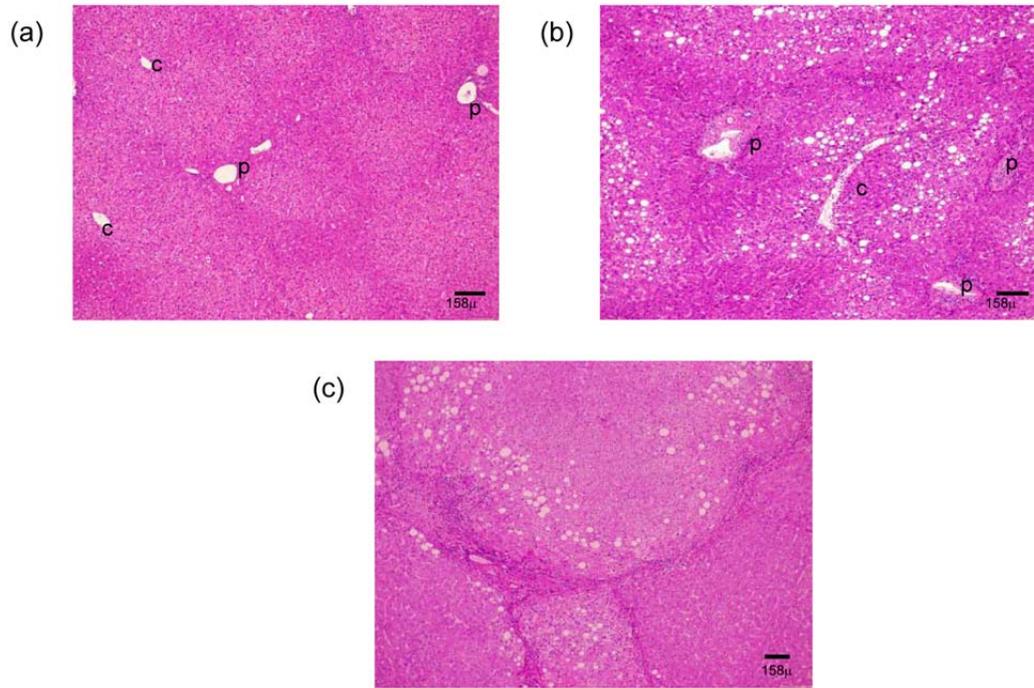
**Supplementary Table 1** Background of normal controls, early and advanced NASH patients. Pathological findings were diagnosed independently by three pathologists.

	Case No.	Age	Sex	BMI (kg/m <sup>2</sup> )	Steatosis	Inflammation	Ballooning	Fibrosis	AST (IU/L)	ALT (IU/L)	γGT (IU/L)	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)	BS (mg/dL)	HbA1c (%)	
Early	1	36	F	28.6	2	2	1	1	54	105	73	225	156	46	200	115	5.5	†,*
	2	37	M	25.9	3	1	2	1	65	122	39	247	169	53	177	104	5.8	†,*
	3	40	M	31.4	3	1	1	1	96	176	69	249	124	50	375	107	5.8	†,*
	4	40	M	26.5	2	2	1	1	26	68	64	201	121	40	113	118	6.1	†,*
	5	44	M	31.7	2	3	1	2	57	133	54	201	141	42	207	127	6.6	†
	6	49	F	27.0	2	2	2	1	55	77	48	213	129	62	148	118	6.8	†,‡
	7	61	M	28.1	1	1	1	1	48	46	34	155	86	46	171	97	7.9	†
	8	62	F	25.8	2	1	1	1	83	87	71	184	117	48	94	93	6.1	†,‡
	9	67	F	35.2	2	1	1	1	53	46	37	134	59	54	75	166	6.8	†,‡
	10	68	M	25.6	1	1	1	1	24	37	32	150	98	39	64	111	5.7	†,‡
Advanced	11	46	M	24.0	2	3	1	4	29	58	144	141	79	47	113	78	5.5	†,*
	12	50	M	30.1	2	3	2	4	48	31	320	124	74	41	67	369	10.2	†,‡,*
	13	59	F	22.4	2	2	2	4	105	107	186	208	133	41	171	184	8.9	†,*
	14	67	F	24.1	1	2	1	3	65	85	85	154	86	50	92	109	6.6	†,*
	15	75	M	27.6	2	1	1	4	42	29	132	156	196	24	89	205	9.0	†
	16	62	F	24.4	2	3	1	4	100	70	72	152	65	67	77	167	10.9	†
	17	78	M	21.0	1	3	1	4	22	13	114	147	84	31	160	93	4.6	†,‡
	18	71	M	27.2	2	1	1	4	54	20	10	77	33	34	46	87	4.3	‡
	19	62	F	19.9	2	2	2	4	43	22	27	37	-	-	-	67	-	‡
Control	1	64	M	21.8					29	26	202	231	179	47	76	109	5.7	†,‡
	2	71	F	20.2					16	12	33	119	63	36	98	98	5.4	†,‡
	3	71	M	25.0					63	73	52	233	156	41	100	109	5.2	†,‡
	4	84	F	19.6					12	6	12	165	90	60	73	109	5.0	†

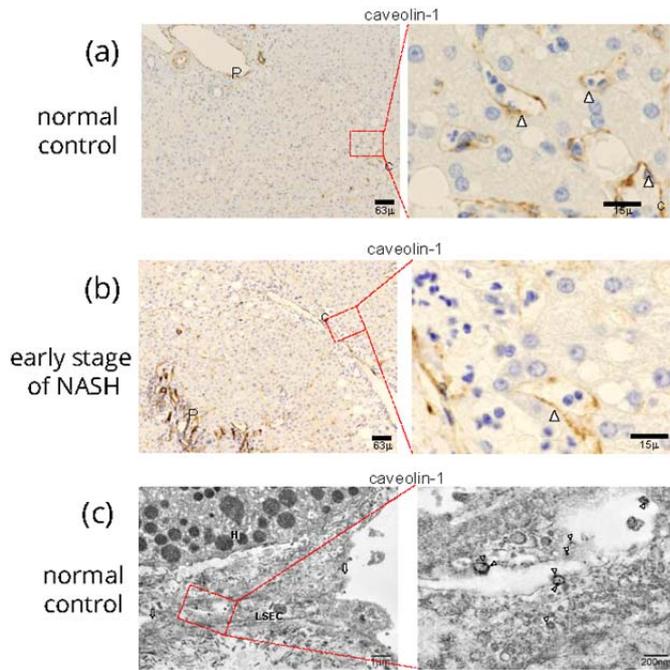
†, tissue: In this patient, liver tissues were analyzed for IHC and/or IEM of MMP-1.

‡, tissue: In this patient, liver tissues were analyzed for western blot.

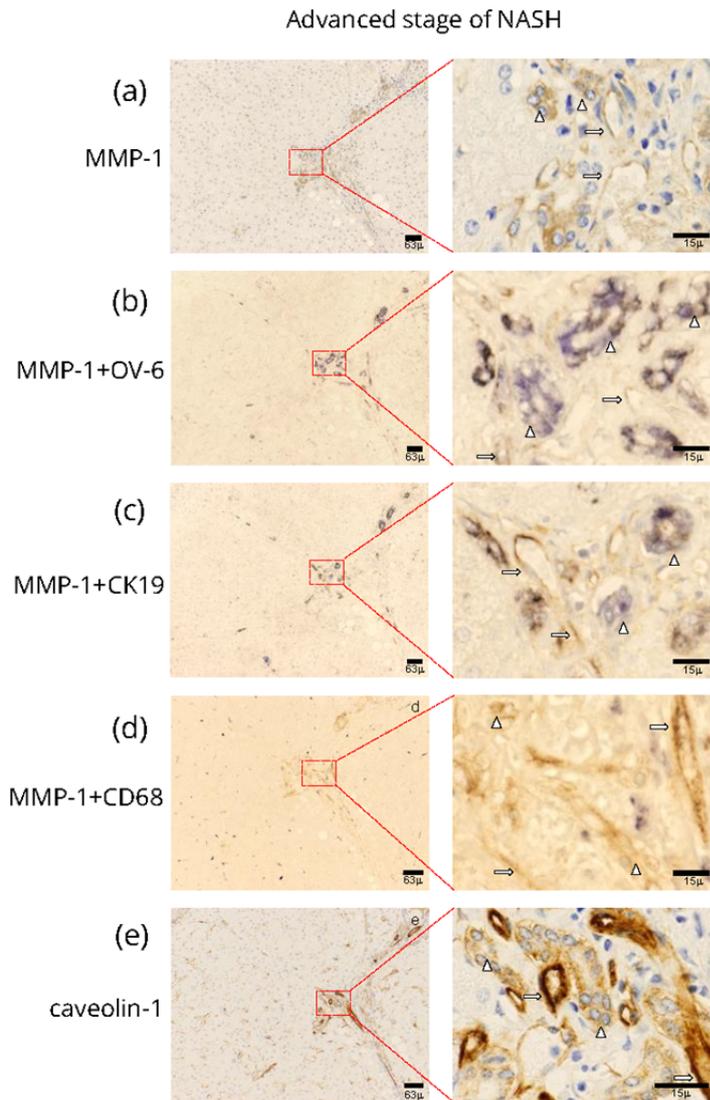
\*, tissue: In this patient, liver tissues were analyzed for MMP-1 dual staining.



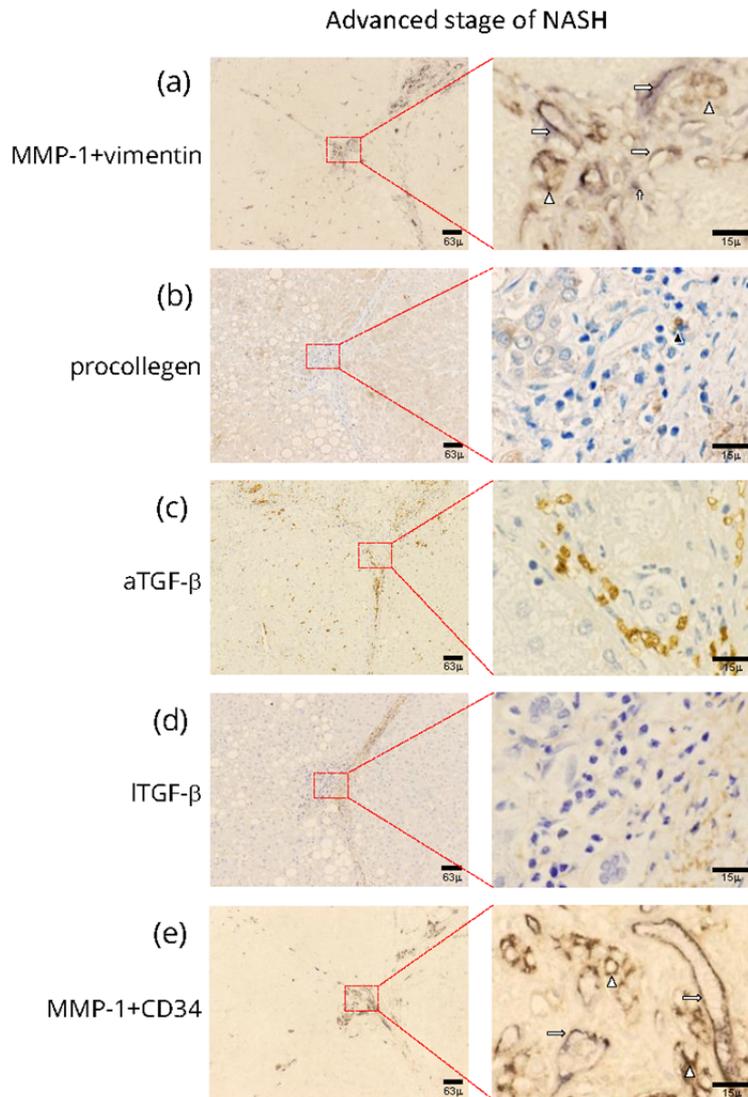
**Supplementary Figure 1** Histologic features of NASH stage in this study according to Brunt's grading and staging. (a) Normal control (Case No. 1); (b) Stage 1 grade 1 NASH liver (Case No. 10); (c) Stage 2 grade 3 NASH liver Case No. 17);  $\times 100$  magnification, Hematoxylin-eosine stain.



**Supplementary Figure 2** IHC and IEM: (a) IHC of caveolin-1 expression in normal control liver (Case No. 1). Caveolin-1 was observed on the walls of hepatic artery capillary venule and portal vein in the portal tracts of normal liver. The high magnification image in the inset shows caveolin-1 expression in hepatic sinusoidal lining cells around pericentral zone 3; (b) Caveolin-1 in early stage NASH (Case No. 10). In an early stage of NASH, caveolin-1 expression was similar to expression in control liver tissues. White arrows indicate sinusoidal lining cells with caveolin-1 expression in an early stage of NASH liver (brown).  $\times 100$  magnification. Column denotes high magnification (400). P, portal tract; C, central vein; (c) IEM expression of caveolin-1 in normal control liver (Case No. 1). Immunogold particles indicating caveolin-1 are found in non-coated vesicles in liver sinusoidal endothelial cells around zone 2. Arrowheads indicate caveolin-1 reaction products. Arrow denotes sinusoidal endothelial fenestrae. Scale bar shows magnification: H, hepatocyte; LSEC, liver sinusoidal endothelial cell. The scale bar shows magnification: H, hepatocyte; LSEC, liver sinusoidal endothelial cell.



**Supplementary Figure 3** IHC expressions of advanced NASH liver (stage 2, grade 4) using serial sections (Case No. 17): (a) IHC expression of MMP-1 (brown); (b) Dual expression of MMP-1(brown) and OV-6 (blue); (c) IHC dual expression of MMP-1 (brown) and CK-19 (blue); (d) IHC dual expression of MMP-1 (brown) and CD68 (blue); (e) IHC expression of caveolin-1 (brown). The expression of MMP-1 is localized on sinusoidal lining cell in steatosis with features of significant cell injury or inflammation. Expression of MMP-1 increased proportionally with increase in hepatic progenitor cells and development of fibrosis. White arrowheads mark the hepatic progenitor cell. White arrows indicate proliferative capillary arteries or sinusoidal lining cells. Brown, immunoperoxidase staining was used for MMP-1. Blue, alkaline phosphatase staining was used for OV-6, cytokeratin 19, and CD68. Column denotes high magnification.



**Supplementary Figure 4** IHC expressions of advanced NASH liver (stage 2, grade 4) using serial sections (Case No. 17): (a) IHC dual expression of MMP-1(brown) and vimentin (blue); (b) IHC expression of type 1 procollagen (brown); (c) IHC expression of aTGF- $\beta$  (brown); (d) IHC expression of ITGF- $\beta$  (brown); (e) IHC dual expression of MMP-1 (brown) and CD34 (blue). Expression of MMP-1 is localized in sinusoidal lining cells in steatosis with features of significant cell injury or inflammation. Black arrowheads mark vimentin or CD34-positive cells. White arrows indicate proliferative capillary arteries or sinusoidal lining cells. Brown, immunoperoxidase staining was used for MMP-1, latent TGF- $\beta$ , active TGF- $\beta$ , type 1 procollagen. Blue, alkaline phosphatase staining was used for CD34. Column denotes high magnification.