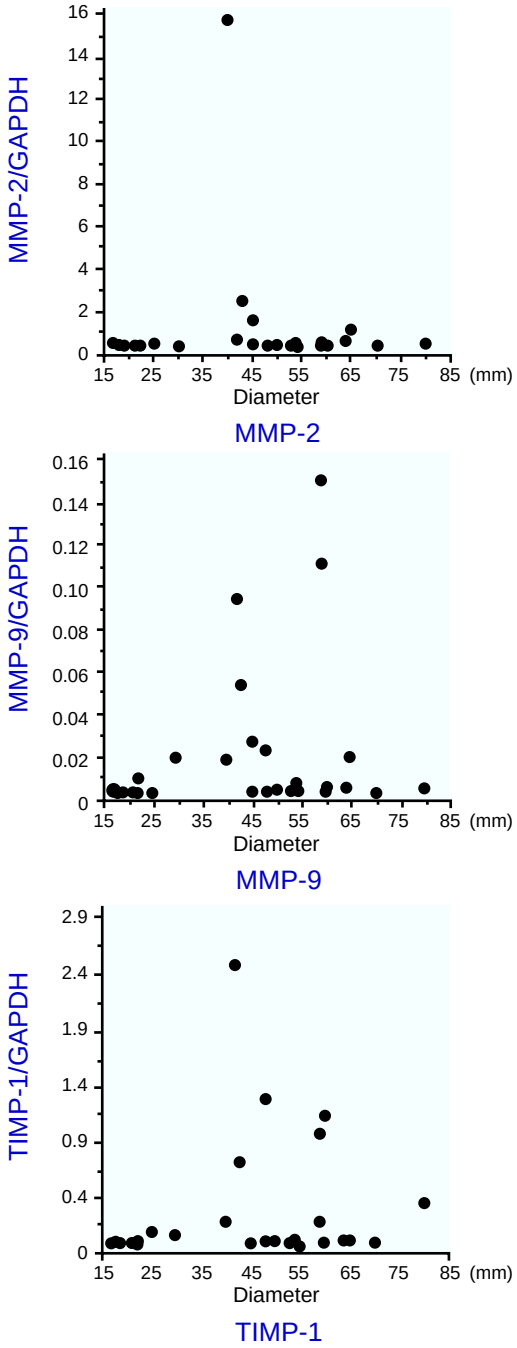

Fig. 4. Scatter diagrams show the expressions of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 mRNA in small abdominal aortic aneurysms (AAAs) [$n = 6$] and medium-large AAAs [$n = 14$] and in control specimens [$n = 10$]. The RNA amount is presented as a percentage to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

noted in 2 of the small AAAs. A higher expression of MMP-2 was also noted in 1 of the 3 aneurysms of the control group. On the other hand, MMP-9 showed lower or almost no expressions in all the specimens examined.

Discussion

This study analyzed the relationship between the development of AAAs and the expression of MMP-2, MMP-9 and TIMP-1 in the aortic walls. The AAAs were subclassified into 2 categories due to the diameter of AAAs; small type being 45 mm or less, and medium-large type being greater than 45 mm. This classification is based on a previous observation, which confirmed that AAAs with diameters greater than 45 mm showed a higher frequency of aneurysmal rupture (Kanaoka et al., 1999). Maeda et al. (1996) described 3 stages of AAA progression; development, growth and rupture. They corrected the diameter of AAAs followed by body height as a congenital factor and by age as an acquired factor, and found that small and medium-large AAAs corresponded well to the stages of growth and rupture, respectively.

The present study confirmed the highest expression of MMP-2 mRNA as well as protein levels among the 3 molecules. The expression was significantly higher in the small-diameter AAAs than in the controls, suggesting the involvement of MMP-2 in the growth of AAAs. This is partially consistent with the report by Freestone et al. (1995), who found that the production of MMP-2 protein was higher in the AAAs of 40 to 55 mm in diameter. Moreover, it is well known that MMP-2 is expressed only in small amounts in obstructive arteriosclerotic lesions (Freestone et al., 1995; Davis et al., 1998). Thus, it is conceivable that MMP-2 might play a crucial role in the development and growth, i.e., an early stage of AAAs.

Table 3. mRNA expressions of MMP-2, MMP-9 and TIMP-1 in the small and medium-large AAAs and controls

		MMP-2	MMP-9	TIMP-1
Controls	[10]	0.02 ± 0.03 *	0.001 ± 0.002 **	0.01 ± 0.03
Small AAAs	[6]	3.17 ± 6.04	0.03 ± 0.03 *	0.58 ± 0.98
Medium-large AAAs	[14]	0.10 ± 0.20	0.02 ± 0.05	0.29 ± 0.47

[ ], number of specimens
AAA, abnormal arotic aneurysm; MMP, matrix metalloproteinase; mRNA, messenger RNA; TIMP, tissue inhibitor of metalloproteinase.
*$P < 0.05$; **$P < 0.01$.

Both small and medium-large AAAs showed higher expressions of MMP-9 than controls. When the medium-large AAAs were divided into 2 subgroups by size, the mean expression ratio of MMP-9 mRNA was higher in the medium-type AAAs than in the large-type AAAs, although the difference was not significant. Similar results were reported by McMillan et al. (1997), who divided AAAs into 3 groups; small (less than 49 mm), medium

Table 4. Correlation of MMP-2, MMP-9 and TIMP in the small, medium-large and total AAAs and controls

	Small AAA	Medium-large AAA	AAA	Controls
MMP-2 and MMP-9	–0.20 [6]	0.62 [15]*	0.46 [21]*	0.06 [10]
MMP-2 and TIMP-1	0.26 [6]	0.42 [14]	0.30 [20]	0.39 [10]
MMP-9 and TIMP-1	0.77 [6]	0.75 [15]**	0.70 [21]**	0.52 [11]

[ ], number of specimens
AAA, abnormal arotic aneurysm; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.
Spearman's correlation coefficient by rank (*$P < 0.05$, **$P < 0.01$).

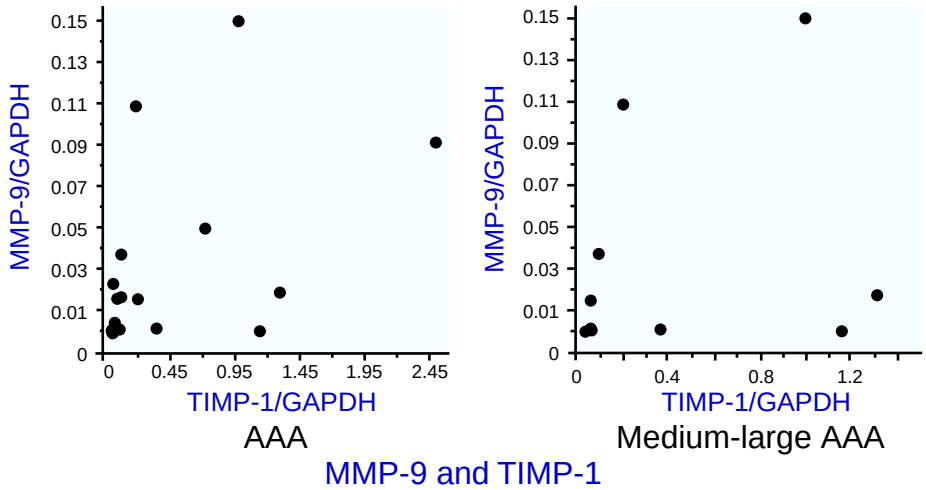

Fig. 5. Correlations between matrix metalloproteinase (MMP)-9 and tissue inhibitor of metalloproteinase (TIMP)-1. There is a significant correlation between TIMP-1 and MMP-9 in AAAs [$n = 21$, $r = 0.70$, $P < 0.01$] (**left**), especially in medium-large AAAs [$n = 15$, $r = 0.75$, $P < 0.01$] (**right**). The RNA amount is presented as a percentage to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

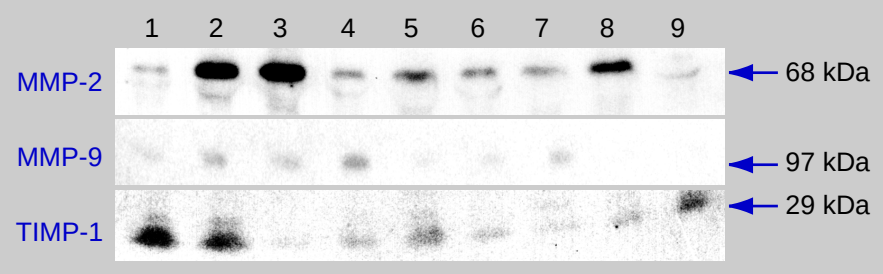

Fig. 6. Western blot analysis for the expression of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 in amall-diameter abdominal aortic aneurysms (AAAs) (lanes 1–3), medium-large-diameter AAAs (lanes 4–6) and in control specimens (lanes 7–9): $n = 3$ per group.

(50–69 mm) and large (more than 70 mm). They found a significantly higher expression in the medium type than in the small or large type. In other words, the MMP-9 expression obviously decreased in the large AAAs. Moreover, immunohistochemistry confirmed a diffuse distribution of MMP-9-positive cells in the entire aneurysmal wall, in contrast to mainly intimal localization of MMP-2- and TIMP-1-positive cells. In spite of the expression ratio of MMP-9 being lower than that of MMP-2, the results indicated that MMP-9 might influence the disorder of elastin metabolism and participate in the growth stage of AAAs, as suggested by McMillan et al. (1997).

It is of interest that the MMP-2 and MMP-9 expressions well correlated in the medium-large AAAs, but not in the small AAAs. The simultaneous lower expressions of MMP-2 and MMP-9 could imply that the gene expression might be regulated by the aneurysmal stroma or the cell-stromal interaction in the remodeled aneurysmal wall.

TIMP-1 has been shown to inhibit the activity of MMPs and to prevent the degeneration of elastic fibers, as well as development and rupture of AAAs (Birkedal-Hansen et al., 1993; Allaire et al., 1998). In the present study, the TIMP-1 expression significantly correlated with MMP-9 in the medium-large AAAs, suggesting that TIMP-1 might inhibit MMP-9 in a reactive or protective manner. In fact, there was no significant correlation between the expressions of TIMP-1 and MMP-9 in the small AAAs and control specimens. Although there was no statistical correlation between the expressions of TIMP-1 and MMP-2, there was a tendency for the TIMP-1 expression to increase along with the MMP-2 expression.

In conclusion, this study demonstrated that MMP-2 and MMP-9 play crucial roles in the development or growth of AAAs, and that TIMP-1 inhibits their proteinase activity. The precise roles of other MMP and TIMP family molecules, such as TIMP-2, await further clarification.

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