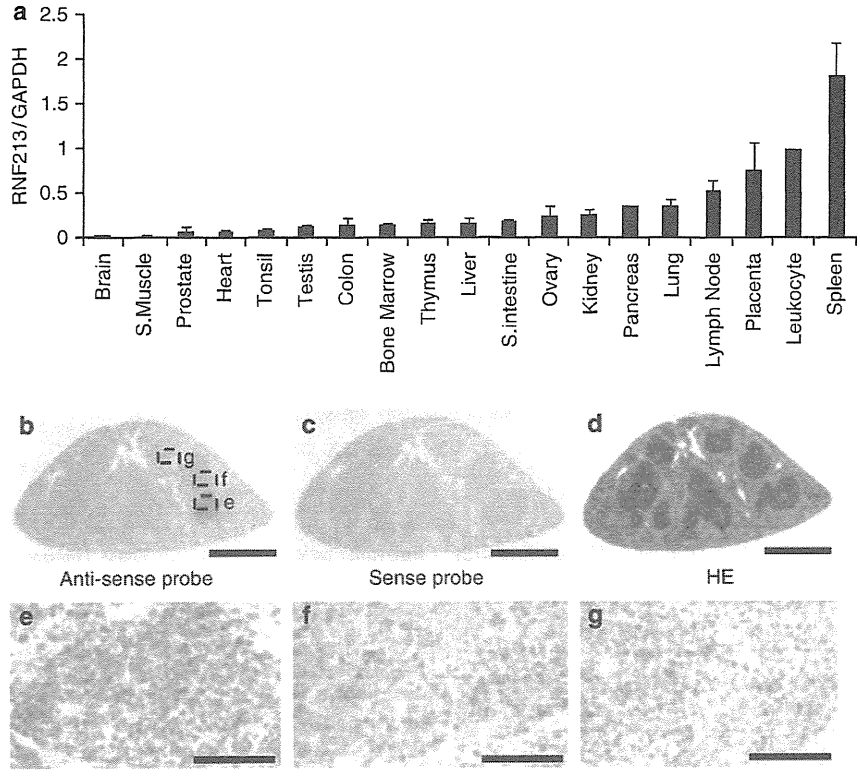

Figure 3 Expression of human RNF213 and murine Rnf213. (**a**) RT-PCR analysis of RNF213 mRNA in various human tissues. The expression levels of *RNF213* mRNA in various adult human tissues were evaluated by quantitative PCR using *GAPDH* mRNA as a control. The signal ratio of *RNF213* mRNA to *GAPDH* mRNA in each sample is shown on the vertical axis. (**b–g**) *In situ* hybridization (ISH) analysis of Rnf213 mRNA in mouse spleen. Specific signals for *Rnf213* mRNA were detected by ISH analysis with the anti-sense probe (**b**) but not with the sense probe (**c**). Hematoxylin–eosin staining of the mouse spleen (**d**). Signals for the *Rnf213* mRNA were observed in small mononuclear cells, which were mainly localized in the white pulps (dotted square, **e**) and partially distributed in the red pulps (dotted squares, **f** and **g**). Panels e, f and g show the high-magnification images of the corresponding fields in panel b. Scale bars, 1 mm (**b–d**) and 50 μm (**e–g**).

questions:[2,19] (i) why is MMD more prevalent in East Asia than in Western countries? The carrier frequency of p.R4859K in Japan is 1/72 (Table 2). In contrast, we found no p.R4859K carrier in 400 Caucasian controls (data not shown). Furthermore, no mutation was identified in five Caucasian patients with MMD after the full sequencing of *RNF213*. These results suggest that the genetic background of MMD in Asian populations is distinct from that in Western populations and that the low incidence of MMD in Western countries may be attributable to a lack of the founder *RNF213* mutation. (ii) Is unilateral involvement a subtype of MMD or a different disease?[2] We collected DNA samples from six patients with unilateral involvement and found a p.R4859K mutation in four of them (data not shown), suggesting that bilateral and unilateral MMD share a genetic background. (iii) Is pre-symptomatic diagnosis of MMD possible? In the present study, MMD never developed in the 15 mutation-negative family members in the 19 MMD families with the p.R4859K mutation (Table 3 and Supplementary Figure 1), suggesting the feasibility of presymptomatic diagnosis or exclusion by genetic testing.

How the mutant RNF213 protein causes MMD remains to be elucidated. The expression of *RNF213* was more abundant in a subset of leukocytes than in the brain, suggesting that blood cells have a function in the etiology of MMD. This observation agrees with a previous report that MMD patients have systemic angiopathy.[20] Recent studies have suggested that the postnatal vasculature can form through vasculogenesis, a process by which endothelial progenitor cell are recruited from the splenic pool and differentiate into mature endothelial cells.[21] Levels of endothelial progenitor cells in the peripheral blood are increased in MMD patients.[22] *RNF213* may be expressed in splenic endothelial progenitor cells and mutant *RNF213* might dysregulate the function of the endothelial progenitor cells. Further research is necessary to elucidate the role of *RNF213* in the etiology of MMD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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