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Plasma fibronectin concentration in inbred mouse strains

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Dear Sir,

Several observations indicate that the concentration of fibronectin in plasma is a determinant of platelet thrombus formation. Injured arterioles of mice engineered to have <2% or 50% normal plasma fibronectin concentration develop less stable platelet thrombi and occlude more slowly than arterioles of mice with normal plasma fibronectin concentration (1,2). Fibronectin concentration is also a strong determinant of platelet thrombus build-up in the parallel flow chamber assay (3–5). The concentration of fibronectin varies widely in humans, from 230 to 650 μg/ml (6). High concentrations have been associated with venous thromboembolism (7, 8) and with coronary artery disease in some but not all populations (9–12). The latter association suggests that there is genetic interaction between fibronectin concentration in plasma and other determinants of cardiovascular disease. In order to relate studies in mice to observations in humans, we sought evidence for a similarly wide concentration range of plasma fibronectin in various genetic strains of mice.

Rabbit antibodies to purified human plasma fibronectin were purified by affinity chromatography on immobilized human fibronectin. These antibodies recognized mouse fibronectin with high titer, and only fibronectin was detected by the antibodies in Western blots of mouse plasma proteins. A portion of the antibodies was labeled with biotin. Fibronectin from mouse plasma was isolated by affinity chromatography on gelatin as previously described (13). The protein was >95% pure by electrophoresis and used as a standard, assuming a 1 mg/ml solution to have an optical density (280 nm, 1 cm) of 1.3. Pooled citrated plasma from Swiss family mice was purchased (Pel-Freez, Rogers, AK) and served as reference plasma. The reference plasma, antibodies and purified mouse fibronectin standard were used to measure fibronectin concentration in the plasma by three different immunoassays.

The concentration of fibronectin in the reference plasma was estimated to be about 240 μg/ml by Western blotting of diluted plasma in comparison to known amounts of purified fibronectin, 173 +/− 43 μg/ml (mean +/− SD) by a sandwich ELISA in which fibronectin was captured by immobilized anti-fibronectin and captured antigen was quantified by biotinylated anti-fibronectin followed by streptavidin-alkaline phosphatase (MPD, http://www.jax.org/phenome, Tomasini1 protocol), and 234 +/− 79 μg/ml in a competitive ELISA in which soluble fibronectin competed with immobilized fibronectin for binding of soluble anti-fibronectin. In addition, approximately 200 μg protein bound to gelatin-agarose from 1 ml of reference plasma and was shown to be nearly pure fibronectin by electrophoresis. The concentrations estimated by 4 methods, therefore, were consistent and centered on about 210 μg/ml. This value is 2.5-fold lower than concentrations previously reported for rodent plasma fibronectin (14–16). The reasons for the differences between our values and values reported in previous studies are unclear.

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Small portions of citrated plasma from 358 individual 9-week old mice from 24 strains from 7 genetic families were provided by Jackson Laboratory (Bar Harbor, ME). Similar plasma samples were used by Jackson Laboratory to search for strain-specific differences in prothrombin time, partial thromboplastin time, and fibrinogen (17) as part of the Mouse Phenome Project (MPD, Peters1). These plasma samples were assayed for fibronectin concentration by the sandwich ELISA (Table 1). The results are posted (MPD, Tomasini1) and can be viewed using the various tools at the website. The concentration for all animals was 200 +/− 54 μg/ml (mean +/− SD). There were no differences between males (199 +/− 66 μg/ml) and females (202 +/− 42 μg/ml). The 70 individuals from 5 strains of the Swiss family had a concentration of 179 +/− 46 μg/ml, which is similar to the concentration measured in the pooled reference plasma by sandwich ELISA as described above.

The variation in fibronectin concentration among mouse strains was similar to the variation reported for humans, ranging from 121 +/− 45 μg/ml for male SWR/J mice to 414 +/− 115 μg/ml for male SPRET/EiJ mice. Statistical analyses were performed on MPD based on unpaired t-tests between the mean values for each pair of strains. There are significant differences between male SWR/J and SPRET/EiJ mice ($p < 0.01$). One-Way Anova analysis with both parametric and non-parametric post-tests revealed significant differences ($p < 0.001$) between SPRET/EiJ or Balb/cByJ males with high concentration and males of 3 strains SWR/J, C57BL/6J, or AKR/J with low concentration. Other differences ($p < 0.05$) were seen between pairs of strains with intermediate concentrations. These differences would need to be tested prospectively with more animals to assess significance.

The value for the reference plasma varied with a coefficient of variation (CV, standard deviation divided by the mean) of 0.25 for the 33 different times the plasma was tested over an 18-month period. Samples from individual mice of a given strain were assayed within a short period of time. To address the possibility that strain differences may be due to when a particular strain was tested, the sandwich ELISA was performed on pools composed of equal volumes of plasma from each of the males or females from 13 strains that had been tested individually. The strains were selected to represent the full range of mean individual values presented in Table 1. As shown in Figure 1 of the Supplemental Data, fibronectin concentrations of the strain and sex-specific pools correlated with the mean fibronectin concentrations of the individuals that composed the pools (slope = 0.86, $r = 0.84$, $p < 0.0001$, $n = 26$).

The strain that was used for knock-out of fibronectin was C57BL/6J (14), a strain with among the lowest concentrations of fibronectin. Based on 140 +/− 17 μg/ml for wild-type male individuals of this strain (Table 1), plasma of C57BL/6J mice heterozygote for the knock-out would be predicted to have a fibronectin concentration of about 70 μg/ml. Indeed, the concentration of fibronectin in citrated plasma of 3 such heterozygous male mice, generously provided by Dr. Denisa Wagner of Harvard Medical School, was 77 μg/ml (individual values of 86, 89, and 56 μg/ml). Such low concentrations are poorly supportive of thrombus formation in flow chamber assays (3,18), consistent with the long occlusion times found in fibronectin heterozygote knock-out mice after ferric chloride injury of arterioles (2).

We found marked variations among individuals within certain strains (noted in SDs in Table 1).To learn whether in-strain variability was unique to fibronectin, we calculated the CVs of measurements of fibronectin and fibrinogen from values posted on MPD, Tomasini1 and Peters1, respectively. As depicted in Figure 2 of the Supplemental Data, the range in CVs for fibronectin concentration varied from 0.08 to 0.56 in different strains and was similar to the range in CVs (0.03 to 0.53) for fibrinogen concentration, thus suggesting that intrinsic variations in the concentrations of both plasma proteins exist among individuals in certain strains. There was no strain-dependent correlation between CVs for fibronectin and CVs for

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fibrinogen (r= −0.01, n=24), as well as no correlation between the concentrations of fibronectin and fibrinogen (r=0.38, p=0.1, n=24). Thus, there does not appear to be a single factor, e.g., concentration of a cytokine, that accounts for either differences or variabilities of the two proteins in various strains.

This survey of plasma fibronectin concentration in diverse mouse strains can serve as a reference in strain selection for mouse models of diseases, such as thrombosis, in which plasma fibronectin may be an important component of the genetic background. In addition, it suggests that variation in fibronectin concentration may be in part genetic and opens the possibility for identification of pathways and genetic regulatory domains responsible for control of concentration by Quantitative Trait Locus analysis of F2 animals from crosses of strains with high and low concentration and for discovery of genetic interaction between fibronectin and other determinants of disease. Data on many relevant parameters are posted on MPD for the same strains. We encourage the hemostasis/thrombosis community to add to this data set.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

List of inbred mice arranged by genetic family, assayed for fibronectin (FN) concentration in plasma. The number of individuals per strain assayed is presented along with the mean concentration of plasma fibronectin (ND, not done).

Family	Strain	n		mean mouse plasma FN µg/ml (+/− SD)	
		male	*female*	male	female
Swiss	BUB/BnJ	8	8	166 (34)	203 (35)
	FVB/NJ	8	ND	166 (19)	ND
	SJL/J	8	7	161 (58)	179 (42)
	SWR/J	7	8	121 (46)	144 (35)
	RIII/s/J	9	7	197 (60)	270 (88)
C57	C57BL/6J	7	5	140 (17)	188 (42)
	C57L/J	8	7	162 (33)	165 (25)
	C58/J	8	8	192 (33)	209 (48)
	C57BL/10J	ND	8	ND	226 (52)
	C57BR/cdJ	8	8	164 (23)	138 (44)
Bagg-Albino	A/J	9	8	161 (39)	208 (43)
	PL/J	7	10	220 (44)	186 (54)
	Balb/cByJ	8	7	352 (197)	227 (36)
	AKR/J	6	8	134 (29)	166 (35)
	Balb/cJ	8	8	189 (39)	176 (65)
Castle	LP/J	8	13	209 (85)	190 (58)
	129S1/SvImJ	8	8	255 (107)	262 (81)
	BTBRT+tf/J	8	7	230 (124)	274 (67)
J/NZ	KK/HiJ	8	8	159 (31)	205 (23)
	NZW/LacJ	8	8	220 (18)	183 (29)
Wild	CAST/EiJ	8	8	212 (38)	200 (65)
	SPRET/EiJ	8	8	414 (115)	310 (39)
	MOLF/EiJ	7	6	171 (47)	194 (22)

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Family	Strain	n		mean mouse plasma FN µg/ml (+/− SD)	
		male	*female*	**male**	**female**
CC Little's DBA	DBA/2J	9	8	196 (35)	191 (32)

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