



## Supplementary files

### **The impact of human platelet antigen allele on antiplatelet antibodies and cryoglobulins in patients with primary immune thrombocytopenia and hepatitis C virus-associated immune thrombocytopenia.**

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## **Online Supplement**

## **Supplementary Tables**

**Supplementary Table 1. Odds ratio of HPA15b versus HPA15a for detection of antiplatelet antibodies in the whole ITP patients.**

	OR	95% CI	P
Anti-GPIIb/IIIa Ab	1.146	0.656-2.002	0.632
Anti-GPIa/IIa Ab	1.679	0.951-2.963	0.073
Anti-GPIb/IX Ab	2.452	1.089-5.521	0.027
Anti-GPIV Ab	3.841	1.166-12.658	0.019
Anti-HLA Class I Ab	1.939	1.058-3.551	0.031

GP, glycoprotein; Ab, antibody; OR, odds ratio; CI, confidence interval

**Supplementary Table 2. Odds ratio of HPA3b versus HPA3a for detection of anti-GPIIb/IIIa antibodies and cryoglobulins in the whole ITP patients.**

Characteristic	Odd	95% CI	P
Cryoglobulin IgG	1.506	0.827-2.744	0.180
Cryoglobulin IgA	1.966	1.007-3.838	0.046
Cryoglobulin IgM	1.905	1.034-3.511	0.038
Anti-GPIIb/IIIa Ab	1.317	0.753-2.303	0.333

GP, glycoprotein; Ab, antibody; OR, odds ratio; CI, confidence interval

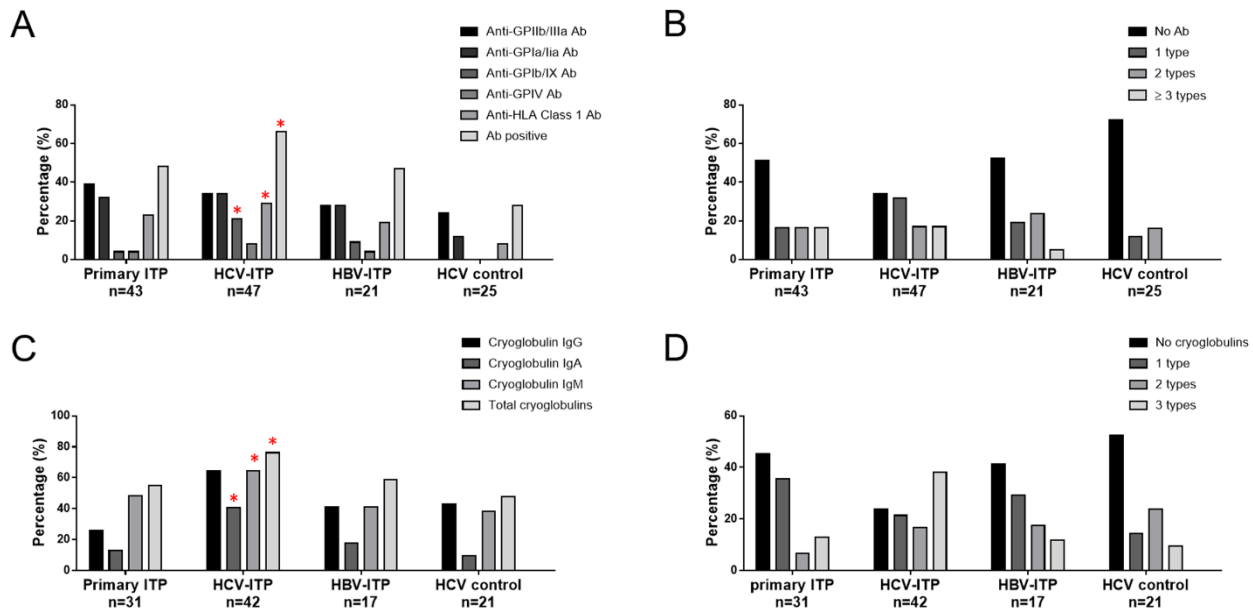
**Supplementary Table 3. The correlation between antiplatelet antibodies and total cryoglobulins in the HCV-ITP patients.**

		Total cryoglobulins	
		positive	negative
Anti-GPIIb/IIIa Ab	positive	13	1
	negative	19	9
Anti-GPIa/IIa Ab	positive	12	3
	negative	20	7
Anti-GPIb/IX Ab	positive	8	1
	negative	24	9
Anti-GPIV Ab	positive	4	0
	negative	28	10

HCV, hepatitis C virus; ITP, immune thrombocytopenia; GP, glycoprotein; Ab, antibody

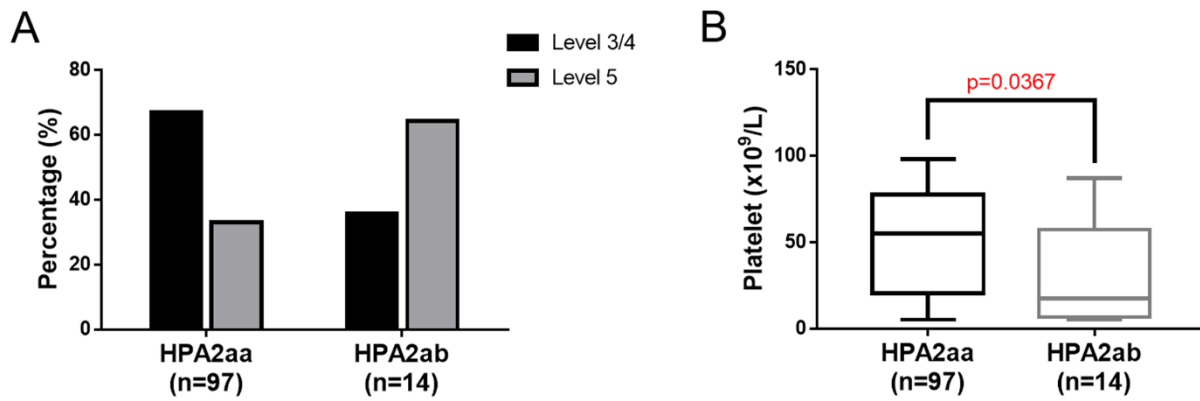
## Supplementary Figures

### Supplementary Figure 1



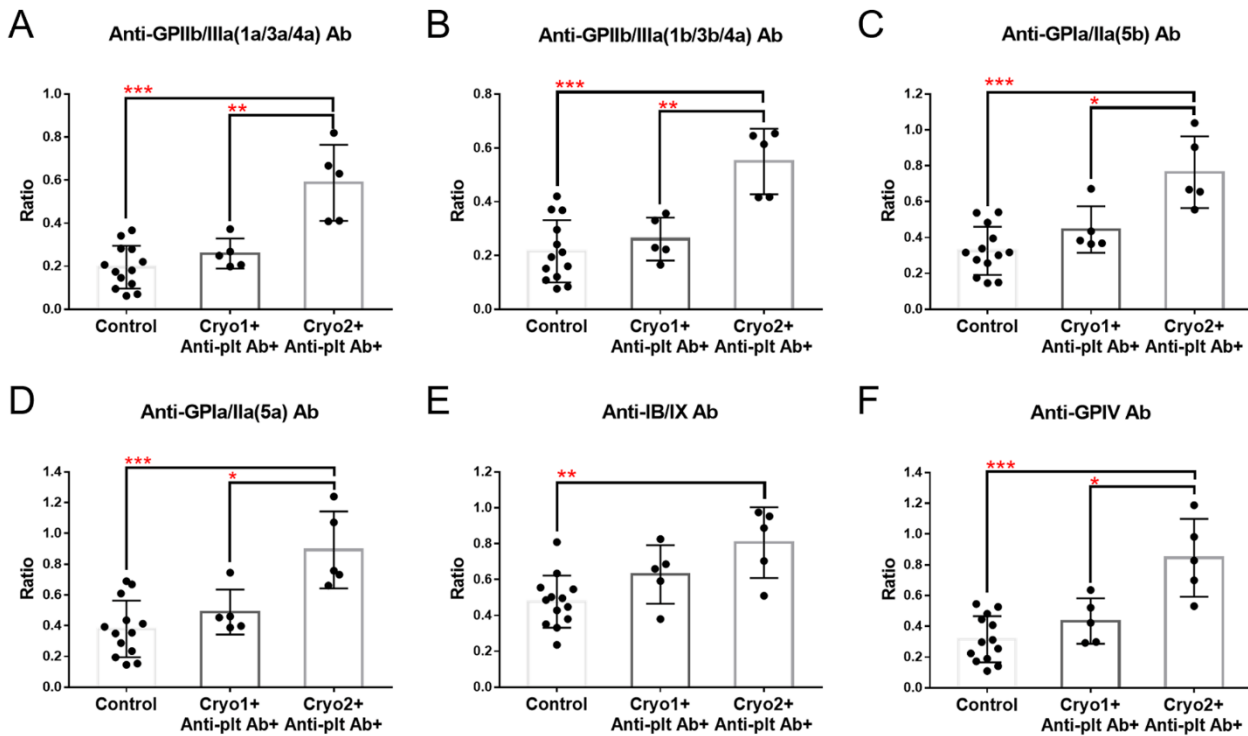
**Supplementary Figure 1. Detection results of antiplatelet antibodies and cryoglobulins.** A. Antiplatelet antibody profiles. Anti-GPIIb/IIIa antibodies were the most common antiplatelet antibody. Compared with HCV controls, HCV-ITP patients had higher positive rates of anti-Ib/IX antibodies, anti-HLA Class I antibodies, and all antiplatelet antibodies ( $p = 0.012$ ,  $0.040$ , and  $0.002$ , respectively). B. Antiplatelet antibody complexity. More than three types of antiplatelet antibodies were detected in the primary and secondary ITP patients, but they were not detected in the HCV control. C. Cryoglobulin profile. Cryoglobulin IgM was the most frequently detected immunoglobulin, whereas cryoglobulin IgA was the least detected immunoglobulin in ITP patients. The positive detection rates of cryoglobulin IgA, IgM and total cryoglobulin were significantly higher in HCV-ITP patients than in HCV control ( $p = 0.018$ ,  $0.049$ , and  $0.023$ , respectively). D. Cryoglobulin complexity. All three types of cryoglobulins were most frequently detected in HCV-ITP patients. \* denotes a significant difference.

Supplementary Figure 2

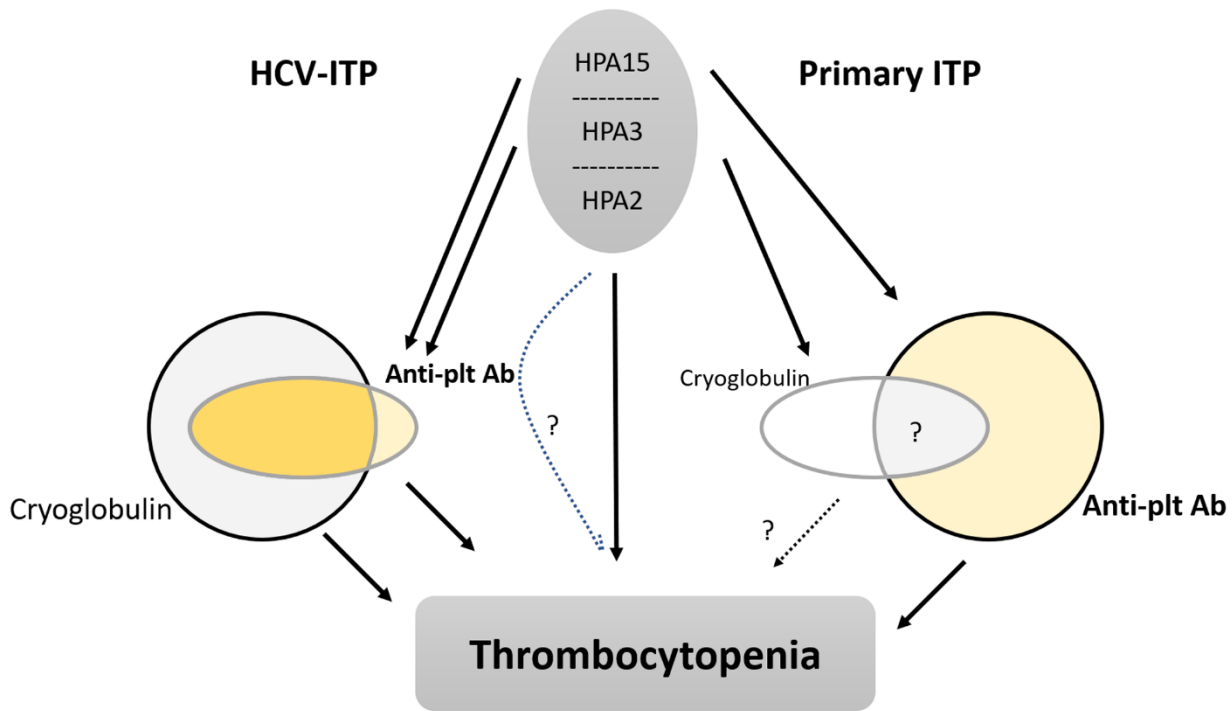


**Supplementary Figure 2. Correlations of HPA2 with platelet level.** A. In the whole ITP cohort, patients with HPA2ab had a significantly higher percentage of severe thrombocytopenia (level 5) than those with HPA2aa (p=0.023). B. The ITP patients with HPA2ab had a significantly lower mean platelet level than those with HPA2aa (31.36 x10<sup>9</sup>/L vs. 49.93 x10<sup>9</sup>/L, p=0.037). \* denotes a significant difference.

Supplementary Figure 3



**Supplementary Figure 3. Detection of antiplatelet antibodies in cryoglobulins.** We selected 5 ITP patients with cryoglobulin (2+: IgG 2+/IgA 1+ or 2+/IgM2+) and positive anti-GPIIb/IIIa antibodies, 5 with cryoglobulin (1+: IgG 1+/IgA 0 or 1+/IgM 1+) and positive anti-GPIIb/IIIa antibodies, and 13 controls including 4 with cryoglobulin (2+: IgG 2+/IgM 2+) and negative anti-GPIIb/IIIa antibody, 5 with negative cryoglobulin and positive anti-GPIIb/IIIa antibodies, and 4 with both negative cryoglobulin and anti-GPIIb/IIIa antibody. The ITP patients with positive anti-GPIIb/IIIa antibodies always had multiple antiplatelet antibodies simultaneously. A and B, the patients with cryoglobulin (2+)/anti-GPIIb/IIIa antibody (+) had significantly higher detection ratios of anti-GPIIb/IIIa antibodies than the controls and the ITP patients with cryoglobulin (1+)/anti-GPIIb/IIIa antibody (+). C, D, E, and F. The anti-GPIa/IIa, GPIb/IX, and IV antibodies were detected with a higher ratio in the patients with cryoglobulin (2+)/anti-GPIIb/IIIa antibody (+) than the controls. The data suggests that cryoglobulins shared the characteristics of antiplatelet antibodies in the ITP patients with strongly positive cryoglobulins and antiplatelet antibodies. \*, \*\*, and \*\*\* denote a significant difference of  $p < 0.05$ ,  $< 0.01$ , and  $< 0.001$ , respectively.



**Supplementary Figure 4. Summary of the associations among HPA alleles, antiplatelet antibodies, and cryoglobulins in primary ITP and HCV-ITP patients.** According to the results of this study, we found that HPA2b was associated with thrombocytopenia. However, it was not clear whether the production of HPA2b-associated anti-GPIb/IX antibodies or the specificity of these antibodies was responsible for clinical thrombocytopenia. HPA15b promoted the complexity of antiplatelet antibody profiles in ITP patients. On the other hand, HPA3b was associated with anti-GPIIb/IIIa antibody production in the HCV-ITP patients, but with cryoglobulin IgG/A/M in the primary ITP patients. The correlations between antiplatelet antibodies and cryoglobulins were different in primary ITP and HCV-ITP patients. In HCV-ITP patients, the positive rates of antiplatelet antibodies were lower than cryoglobulins. Most of the HCV-ITP patients with positive antiplatelet antibodies had positive cryoglobulins. Clinically, the cryoglobulins, similar to antiplatelet antibodies, were correlated with thrombocytopenia. In laboratory, the cryoglobulins exhibited characteristics of antiplatelet antibodies. HCV-ITP may be considered as one of cryoglobulinemia-related complications. However, the associations between antiplatelet antibodies and cryoglobulins were not as strong in the primary ITP patients as in the HCV-ITP patients. The correlation between cryoglobulin and clinical thrombocytopenia was not obvious in the primary ITP patients. This implies that the pathophysiology of antiplatelet antibody formation is different between HCV-ITP patients and primary ITP patients.