Supplemental information

The seroprevalence of neutralizing antibodies

against the adeno-associated virus capsids

in Japanese hemophiliacs

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Table S1. Comparison of AAV Nab positive subjects for each serotype among generations

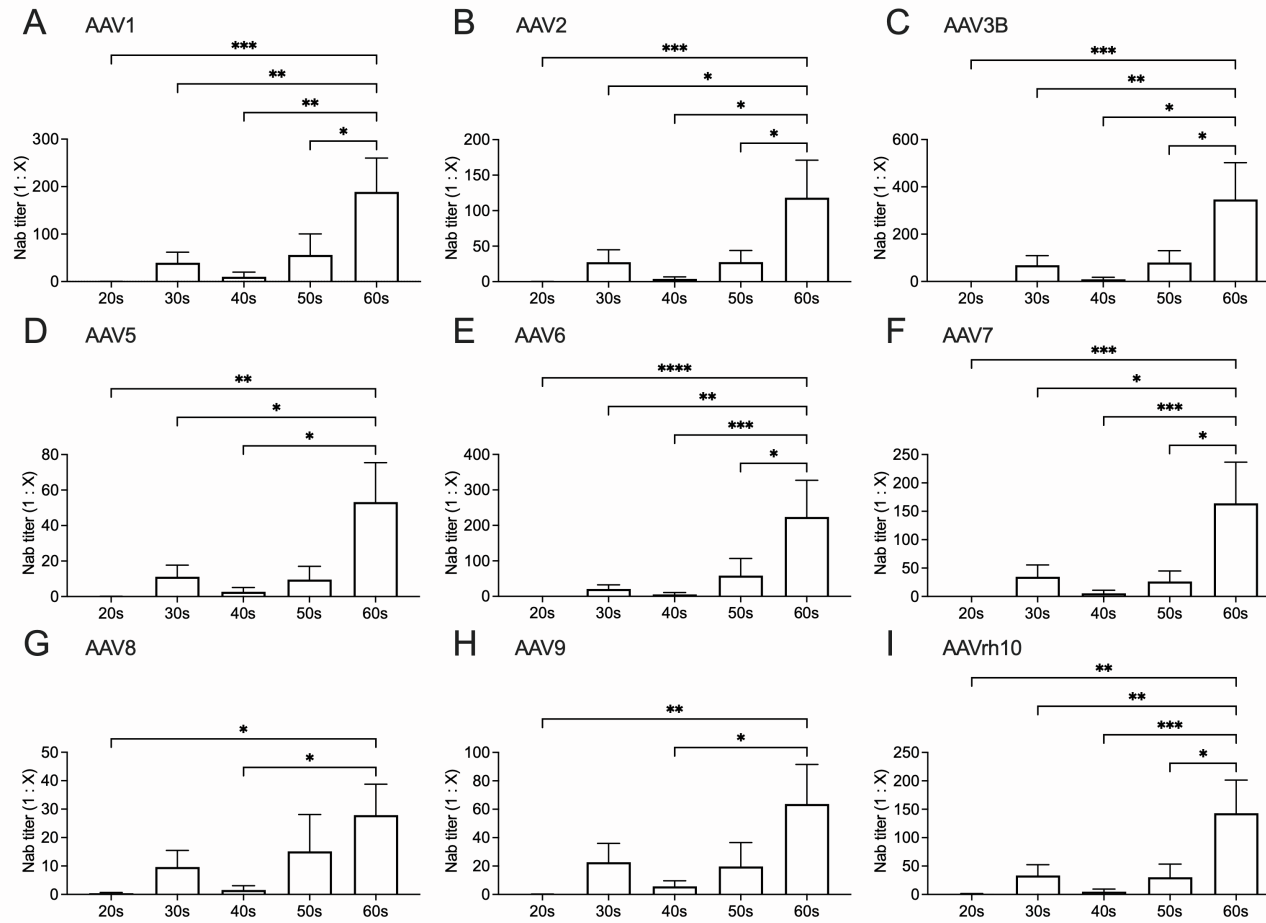| Healthy volunteers		
Serotype	Chi-square, df	*P*
AAV1	15.41, 1	<0.0001
AAV2	14.61, 1	0.0001
AAV3B	11.76, 1	0.0006
AAV5	10.19, 1	0.0014
AAV6	18.84, 1	<0.0001
AAV7	14.10, 1	0.0002
AAV8	6.556, 1	0.0105
AAV9	11.28, 1	0.0008
AAVrh10	7.922, 1	0.0049
Patients		
Serotype	Chi-square, df	*P*
AAV1	27.11, 1	<0.0001
AAV2	30.06, 1	<0.0001
AAV3B	29.36, 1	<0.0001
AAV5	24.66, 1	<0.0001
AAV6	30.47, 1	<0.0001
AAV7	30.19, 1	<0.0001
AAV8	19.75, 1	<0.0001
AAV9	28.65, 1	<0.0001
AAVrh10	24.46, 1	<0.0001

AAV, adeno-associated virus; CI, confidence interval; Nab, neutralizing antibody

**Table S2.** Comparison of AAV Nab titer between patients and healthy volunteers according to generations

AAV1	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−66.2 (−284.6 to 152.2)	0.9419
	30s	37.61 (−195.5 to 270.8)	0.9965
	40s	−41.62 (−269.2 to 186.0)	0.9937
	50s	−46.61 (−276.8 to 183.6)	0.9899
	60s–	−72.94 (−310.8 to 164.9)	0.9391
AAV2	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−38.49 (−223.9 to 147.0)	0.9887
	30s	23.84 (−174.2 to 221.8)	0.9991
	40s	−31.66 (−224.9 to 161.6)	0.9962
	50s	−82.82 (−278.3 to 112.7)	0.7987
	60s–	−82.93 (−284.9 to 119.0)	0.8188
AAV3B	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−80.23 (−379.8 to 219.4)	0.9653
	30s	61.41 (−258.4 to 381.3)	0.9921
	40s	−82.01 (−394.3 to 230.2)	0.968
	50s	−55.21 (−371.0 to 260.6)	0.9949
	60s–	−197.6 (−523.9 to 128.7)	0.4677
AAV5	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−14.45 (−62.75 to 33.84)	0.9449
	30s	10.15 (−41.41 to 61.72)	0.9911
	40s	−4.685 (−55.02 to 45.65)	0.9998
	50s	−8.222 (−59.13 to 42.69)	0.9965
	60s–	−11.42 (−64.02 to 41.18)	0.9861
AAV6	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−67.99 (−319.2 to 183.2)	0.9637
	30s	16.95 (−251.3 to 285.2)	>0.9999
	40s	−67.36 (−329.2 to 194.5)	0.9707
	50s	−76.1 (−340.9 to 188.7)	0.9533
	60s–	−45.16 (−318.8 to 228.4)	0.9961
AAV7	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−65.18 (−277.3 to 146.9)	0.9386
	30s	32.48 (−194.0 to 259.0)	0.998
	40s	−9.235 (−230.3 to 211.9)	>0.9999

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	50s	−82 (−305.6 to 141.6),	0.8784
	60s–	−72.88 (−303.9 to 158.1)	0.9318
AAV8	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−28.95 (−171.1 to 113.2)	0.9896
	30s	7.659 (−144.1 to 159.4)	>0.9999
	40s	−16.36 (−164.5 to 131.8)	0.9994
	50s	−50.32 (−200.1 to 99.47)	0.9126
	60s–	−161.4 (−316.1 to −6.587)	0.0366
AAV9	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−28.37 (−160.9 to 104.1)	0.987
	30s	20.91 (−120.5 to 162.4)	0.9977
	40s	−13.85 (−151.9 to 124.2)	0.9996
	50s	−19.51 (−159.2 to 120.1)	0.9982
	60s–	−82.07 (−226.4 to 62.22)	0.5365
AAVrh10	Generation	Predicted mean difference (95% CI)	*P*
	10–20s	−62.11(−270.9 to 146.7)	0.9462
	30s	30.99 (−191.9 to 253.9)	0.9983
	40s	−51.97 (−269.6 to 165.7)	0.9788
	50s	−55.04 (−275.1 to 165.1)	0.9741
	60s–	−112.7 (−340.0 to 114.7)	0.6751

AAV, adeno-associated virus; CI, confidence interval; Nab, neutralizing antibody

Figure S1. Comparison of the AAV Nab titer among age generations in healthy volunteers. The Nab titer to each AAV serotype (A: AAV1, B: AAV2, C: AAV3B, D: AAV5, E: AAV6, F: AAV7, G: AAV8, H: AAV9, I: AAVth10) in each age generation in healthy volunteers. Values are mean ± SEM (n = 20). The value comparison between the groups is analyzed by the Kruskal–Wallis test with post hoc multiple comparisons. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. AAV, adeno-associated virus; Nabs, neutralizing antibodies.
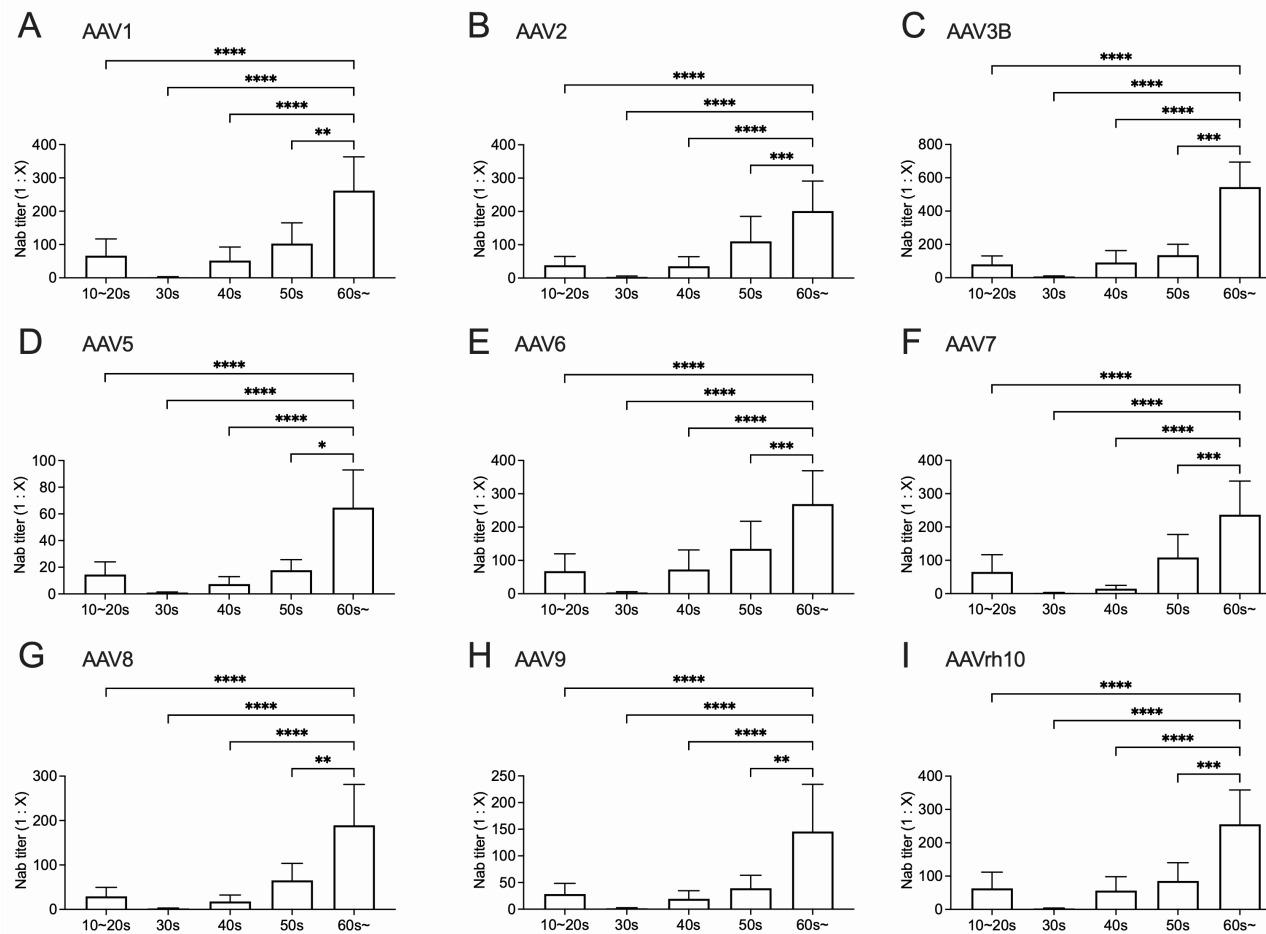
Figure S2. Comparison of the AAV Nab titer among age generations in hemophilia patients. The Nab titer to each AAV serotype (A: AAV1, B: AAV2, C: AAV3B, D: AAV5, E: AAV6, F: AAV7, G: AAV8, H: AAV9, I: AAVth10) in each age generation in hemophiliacs. Values are mean ± SEM (10−20s, n = 59; 30s, n = 38; 40s, n = 44; 50s, n = 41; 60s−, n = 34;). The value comparison between the groups is analyzed by the Kruskal–Wallis test with posthoc multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. AAV, adeno-associated virus; Nabs, neutralizing antibodies.
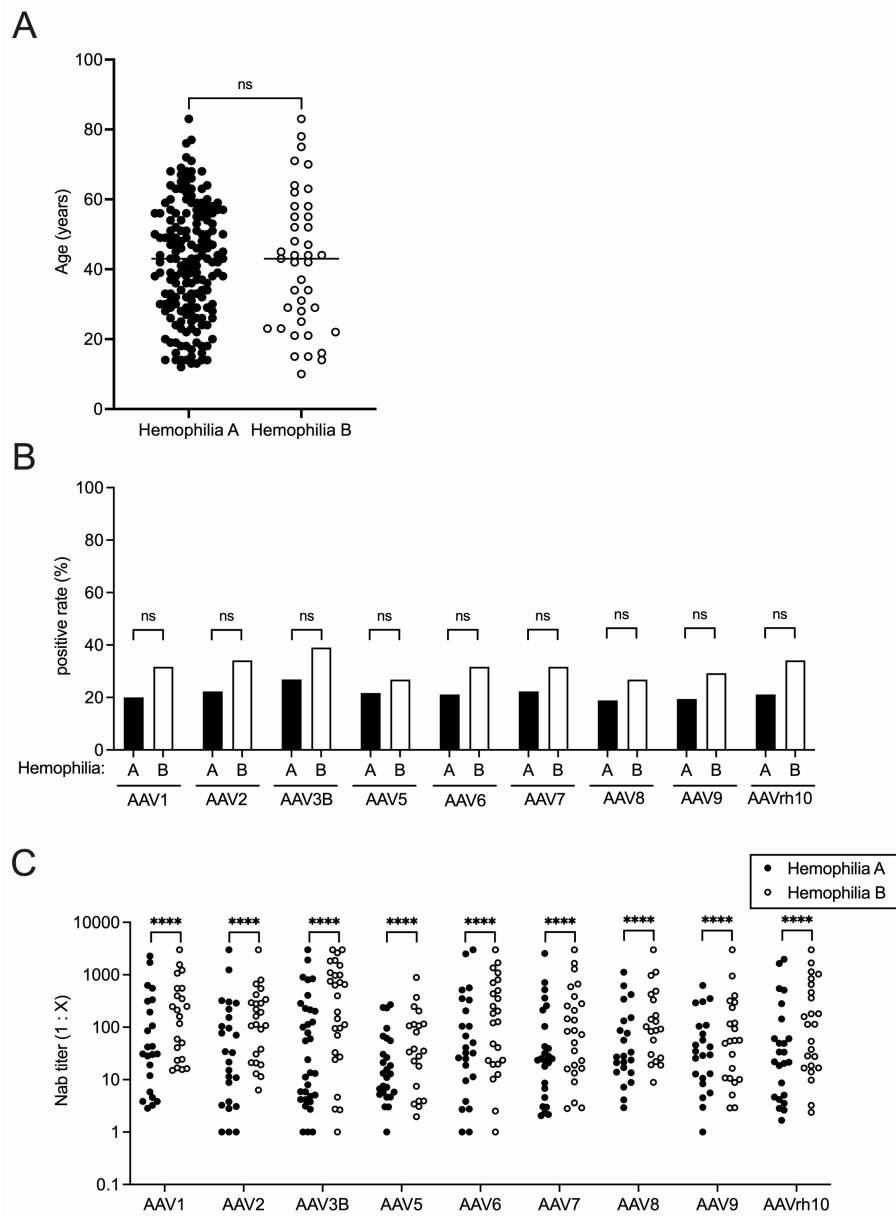
Figure S3. Comparison of the seroprevalence and titer of AAV Nabs between hemophilia A and hemophilia B. (A) Comparison of the ages between the groups. The statistical difference was analyzed by the Mann–Whitney U test. Bar, median. (B) The seroprevalence of AAV Nabs. The between-group statistical significance was analyzed by the Fisher's extract test. (C) The Nab titer against AAV. The value comparison between the groups is analyzed by the Mann–Whitney U test. ****$P < 0.0001$. AAV, adeno-associated virus; Nabs, neutralizing antibodies; n.s., not significant.

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