Global seroprevalence of pre-existing immunity against AAV serotypes in people with hemophilia A

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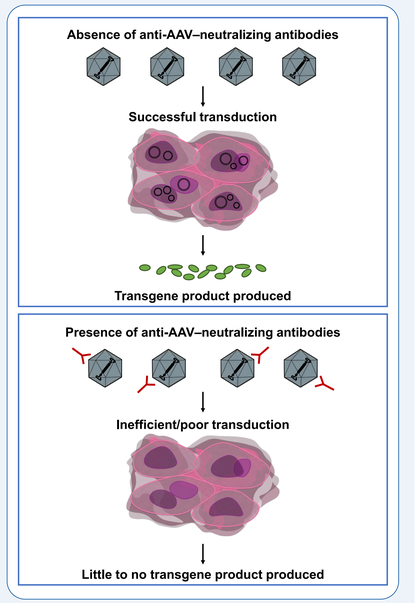
Introduction



Methods

Study design

- BMN 270-901 was a prospective, noninterventional study evaluating the seroprevalence of antibodies and neutralizing factors against adeno-associated virus (AAV) serotypes
- Conducted in 9 countries: Brazil, France, Germany, Italy, Japan, Russia, South Africa, the UK, and the USA
- On day 1, participants visited the clinic for biospecimen collection (plasma and serum)
  - At 3 or 6 months, additional biospecimens were collected from approximately 20% of participants in each country

Participants

- Eligibility criteria
  - Age ≥12 years
  - Hemophilia A, with residual factor VIII (FVIII) levels ≤2 IU/dL
  - Previous treatment with FVIII, either prophylaxis or on-demand
  - With or without FVIII inhibitors

Assessments

- Antibodies against AAV5 were measured with a validated bridging total antibody electrochemiluminescent (ECL) assay on the Meso Scale Discovery platform (limit of detection [LoD] = 33 ng/mL)
  - Antibodies against the AAV2, AAV6, AAV8, and AAVrh10 serotypes were measured using qualified research-use-only bridging ECL assays with similar design (LoD of 60, 15, 70, 42 ng/mL, respectively)
- Titers of confirmed positive samples were measured by serial dilution
- Assay validation and sample testing were performed at ARUP Laboratories (Salt Lake City, Utah)

Results

Participants

- Participant baseline demographics and clinical characteristics are shown in **Table 1**

Table 1. Participant demographics and baseline characteristics

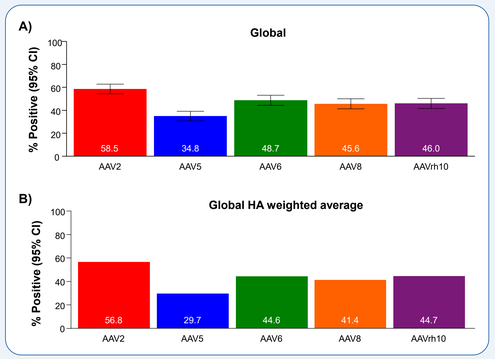
Parameter	Overall population (N = 546)
Age at enrollment, mean (SD), years	36.0 (14.9)
Age at enrollment, n (%)	
12 to <18 years	68 (12.5)
18 to ≤30 years	147 (26.9)
>30 to ≤40 years	127 (23.3)
>40 to ≤50 years	103 (18.9)
>50 to ≤60 years	67 (12.3)
>60 years	34 (6.2)
Sex, male, n (%)	542 (99.3)
Race, n (%)	
Asian	91 (16.7)
Black or African American	66 (12.1)
White	293 (53.7)
Native Hawaiian or other Pacific Islander	1 (0.2)
Not provided due to patient privacy rules	95 (17.4)
Time since hemophilia diagnosis, mean (SD), years	31.4 (14.7)
History of exposure to hepatitis B, n (%)	103 (18.9)
History of exposure to hepatitis C, n (%)	282 (51.6)
Type of FVIII treatment, n (%)	
On demand	109 (20.0)
Prophylaxis	437 (80.0)
Country of enrollment	
Brazil	26
France	87
Germany	90
Italy	20
Japan	84
Russia	91
South Africa	60
UK	17
USA	71

FVIII, factor VIII; SD, standard deviation

Overall AAV seropositivity

- Global seropositivity for all serotypes is shown in **Figure 1**

Figure 1. Global seropositivity for all serotypes based on A) observed data and B) factoring in prevalence of hemophilia A in countries assayed

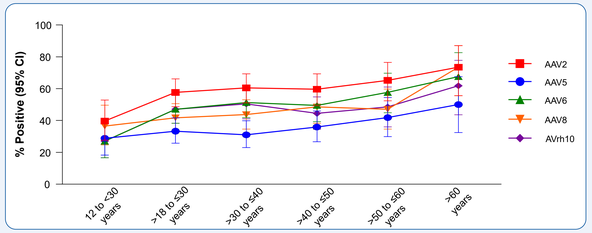


Global HA weighted average was calculated by multiplying the percentage of participants who tested positive in each country by the number of people with HA in that country, per 2018 WFH survey, divided by the total number of people with HA in all countries in this study, per WFH.
AAV, adeno-associated virus; CI, confidence interval; HA, hemophilia A; WFH, World Federation of Hemophilia.

AAV seropositivity by age

- Seropositivity increased with age across all AAV serotypes (**Figure 2**)

Figure 2. AAV serotype positivity by age group

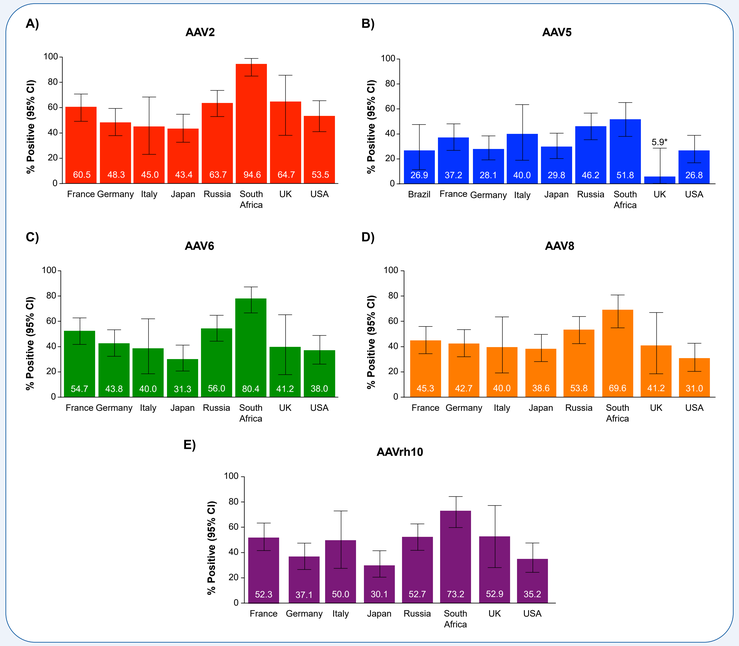


Data are for the global population on day 1.
AAV, adeno-associated virus; CI, confidence interval.

Geographic variability

- Considerable geographic variability was observed in the prevalence of pre-existing antibodies against each serotype (**Figure 3**)

Figure 3. AAV serotype positivity by country for A) AAV2, B) AAV5, C) AAV6, D) AAV8, and E) AAVrh10

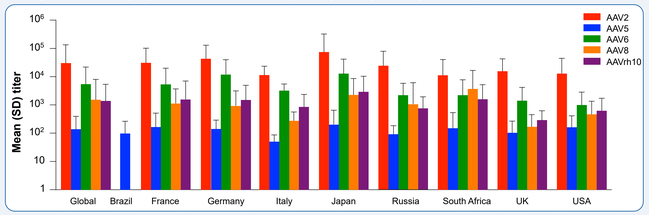


*In a larger sample (n = 100) of HA participants, 21% were AAV5 TAb-positive (Stanford S, et al. *Res Prac Thromb and Haemost.* 2019;3 [2]:261–67.)
Samples from Brazil were only tested using the validated AAV5 assay, not RUO assays.
AAV, adeno-associated virus; CI, confidence interval; HA, hemophilia A; RUO, research use only; TAb, total antibody.

Antibody titers

- Antibody titers differed across AAV serotypes and across geographic regions (**Figure 4**)
- In comparison with AAV5, global mean antibody titers were higher for all other serotypes by 1–2 orders of magnitude

Figure 4. Mean (SD) titer for all AAV serotypes globally and by country

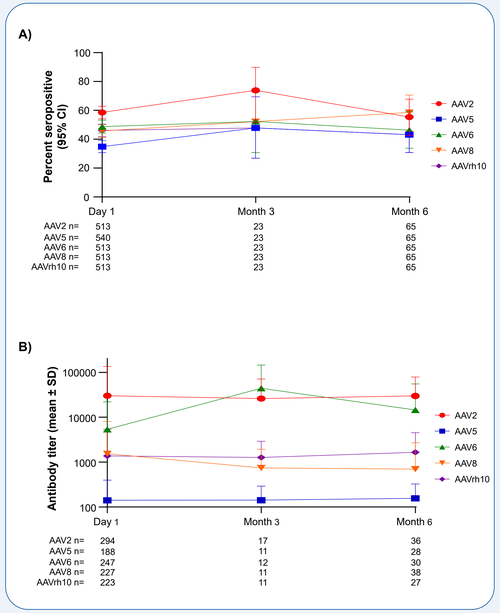


The detection threshold for the titer assay was 20, corresponding to the minimum required dilution. Samples from Brazil were only tested using the validated AAV5 assay, not RUO assays. Titer was only evaluated in seropositive positive participants. AAV, adeno-associated virus; RUO, research use only; SD, standard deviation.

Stability of seropositivity

- Serostatus was generally stable for all serotypes over the 6-month sampling period (**Figure 5A**)
- Antibody titer was also generally stable for all serotypes (**Figure 5B**)

Figure 5. For all serotypes over 6 months, A) percentage of positive participants and B) mean antibody titers in positive participants



Data are for participants in the global population. Titer was only evaluated in seropositive positive participants.
AAV, adeno-associated virus; CI, confidence interval; SD, standard deviation.

CONCLUSIONS

- As AAV-mediated gene therapies for hemophilia A continue along the development pathway, pre-existing immunity against AAV serotypes may become an important eligibility criterion for enrollment in clinical trials, and it may limit who is eligible to receive the treatments once they are available
- The results of this study support the value of AAV5 as a gene therapy vector. AAV5 not only has lower prevalence of pre-existing immunity compared to other serotypes, but appears to have a mean titer orders of magnitude lower than AAV2 or AAV8

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Disclosures

RK has received consulting payments including advisory boards; has participated as a clinical trial investigator for Bayer, Biotest, BioMarin Pharmaceutical Inc., Novo Nordisk Octapharma, Pfizer, Roche/Cugai, Sanofi, Takeda/Shire, and SOBI; and has received speaker honoraria and travel support from Bayer , Biotest, BioMarin Pharmaceutical Inc., CSL Behring, Daiichi Sankyo, LEO, Novo Nordisk, Octapharma, Pfizer, Roche/Cugai, Sanofi, Takeda/Shire, and SOBI. **GH** is a former employee of and may hold stock in BioMarin Pharmaceutical Inc. **TA** has participated as a clinical trial investigator for Roche, Octapharma, Novo Nordisk, Generium, Biomarine, Catalist, and Bayesaisez and has received speaker honoraria from Roche, Octapharma, SOBI, Novo Nordisk, Generium, Biomarine, Catalist, Bayesaisez, and Takeda. **TS** has received consulting fees from Chugai, Takeda, and CSL Behring; has received research grants from Bayer; has participated as a clinical trial investigator for Chugai, BioMarin Pharmaceutical Inc., CSL Behring, and Novo Nordisk; and has received speaker honoraria for Chugai, BioMarin Pharmaceutical Inc., Bayer, Takeda, Baxalta, CSL Behring, Japan Blood Product Organization, Welfen, KM Biologics, Sekisui Medical, and Novo Nordisk. **BH** has received consulting payments from Novo Nordisk, Takeda and Pfizer; research grants from Bayer; has participated as a clinical trial investigator for BioMarin Pharmaceutical Inc., Pfizer and Takeda; has received speaker honoraria from Takeda; and has received travel support from Novo Nordisk and Takeda. **MS** has received consulting payments from Sanofi, Novo Nordisk Pharma Ltd., Chugai Pharmaceutical Co., Ltd.; has received research grant from CSL Behring, Sanofi K.K., Chugai Pharmaceutical Co., Ltd., Bayer, Novo Nordisk Pharma Ltd., BioMarin Pharmaceutical Inc., KM Biologics, Takeda, Pfizer, Sekisui Medical, Novo Nordisk Pharma Ltd., Daiichi Sankyo Co Ltd., Chugai Pharmaceutical Co., Ltd., and Teijin Pharma Ltd; has patents from Chugai Pharmaceutical Co., Ltd.; has received speaker honoraria from Chugai Pharmaceutical Co., Ltd., CSL Behring, Sanofi, Bayer, Novo Nordisk Pharma Ltd., Takeda, and Pfizer. **MCO** has received consulting payments from Bayer, BioMarin Pharmaceutical Inc., Novo Nordisk, Pfizer, Roche, Sanofi, and Takeda; has received research grants from Pfizer, Roche, and Takeda; has participated as a clinical trial investigator for BioMarin Pharmaceutical Inc., Novo Nordisk, Pfizer, Roche, Sanofi, and Takeda; has received speaker honoraria for Bayer, BioMarin Pharmaceutical Inc., Novo Nordisk, Roche, and Takeda; has received travel support from Novo Nordisk, Roche, and Takeda; and participated in grant review for Grifols. **JM** has received consulting payments from Baxalta, CSL Behring, Catalyst Biosciences, Freeline, LFB, Novo Nordisk, and Spark; has received research grants and participated as a clinical trial investigator for BioMarin Pharmaceutical Inc., CSL, NOVO Nordisk, Pfizer, SOBI, Roche, Novartis, Sanofi and Unique; and has received speaker honoraria and travel support from Novo Nordisk, Pfizer, Sanofi, SOBI, Shire, Roche, Takeda, ISTH and **WFH. DQ** has received consulting payments from Roche/Genentech, Novo Nordisk, Catalyst, BioMarin Pharmaceutical Inc., Bayer, Catalyst, Kedrion, and Sanofi; has participated as a clinical trial investigator for BioMarin Pharmaceutical Inc., Bioverativ/Sanofi, Roche/Genentech, Shire/Takeda, and uniQure; and has received speaker honoraria and travel support from Roche/Genentech, Novo Nordisk, Takeda, Bioverativ/Sanofi, and BioMarin Pharmaceutical Inc. **ML** and **WYW** are employees and stockholders of BioMarin Pharmaceutical Inc. **TP, PS, JO, S-MC, FP, RK, ADL, MC,** and **BP-P** report nothing to disclose.

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