Young mice administered adult doses of AAV5-hFVIII-SQ achieve therapeutic factor VIII expression into adulthood

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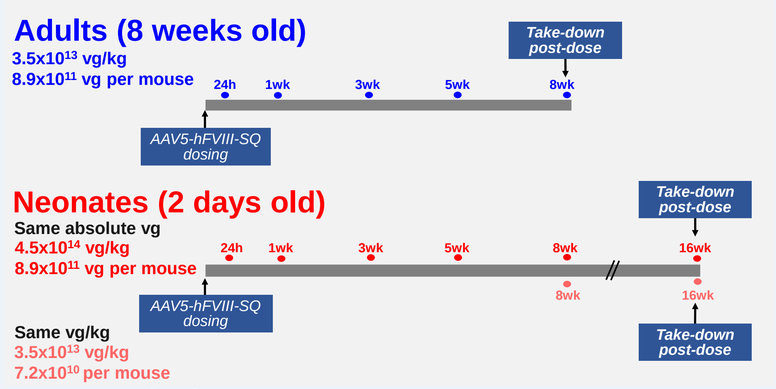
Introduction

- Haemophilia A (HA) is an X-linked genetic bleeding disorder caused by a deficiency in coagulation factor VIII (FVIII). Individuals with severe HA (FVIII <1 IU/dL) experience spontaneous internal bleeding into joints or muscles[1,2]
- Hemarthrosis typically develops before 2 years of age in children with severe HA and, if untreated, leads to recurrent bleeds with musculoskeletal deformity and loss of mobility[3,4]
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) led to prolonged endogenous FVIII production and reduced bleeding in adults with severe HA. However, paediatric clinical feasibility and outcomes are unknown[5]
- Previous preclinical research in neonatal mice and non-human primates with adeno-associated virus (AAV) vectors indicates robust liver cell proliferation early in life can prevent stable transgene expression[6–8]
- Here we examined the effect of vector dose on transgene production and persistence in neonatal vs adult mice using a mouse model of HA

Methods

- RAG2$^{-/-}$ x FVIII$^{-/-}$ double-knockout (DKO) mice were used in the study
- The adult cohort (5 groups of 10 eight-week-old mice) were dosed with AAV5-hFVIII-SQ by intravenous (IV) injection via tail vein at a dose of 3.5x10^{13} vg/kg (ie, an absolute dose of 8.9x10^{11} vg/mouse; **Figure 1**)
  - Adult mice were then euthanised at 24 hours or 1, 3, 5, and 8 weeks after dosing
- The neonatal cohort (8 groups of 10 two-day-old mice) were dosed with AAV5-hFVIII-SQ by IV injection via temporal vein
  - Six groups received the same absolute dose as adults: 8.9x10^{11} vg/mouse, equivalent to 4.5x10^{14} vg/kg
    - Mice were then euthanised at 24 hours or 1, 3, 5, 8, and 16 weeks after dosing
  - Two groups received the same dose as adults based on body weight: 3.5x10^{13} vg/kg, equivalent to 7.2x10^{10} vg/mouse
    - Mice were euthanised at 8 and 16 weeks after dosing

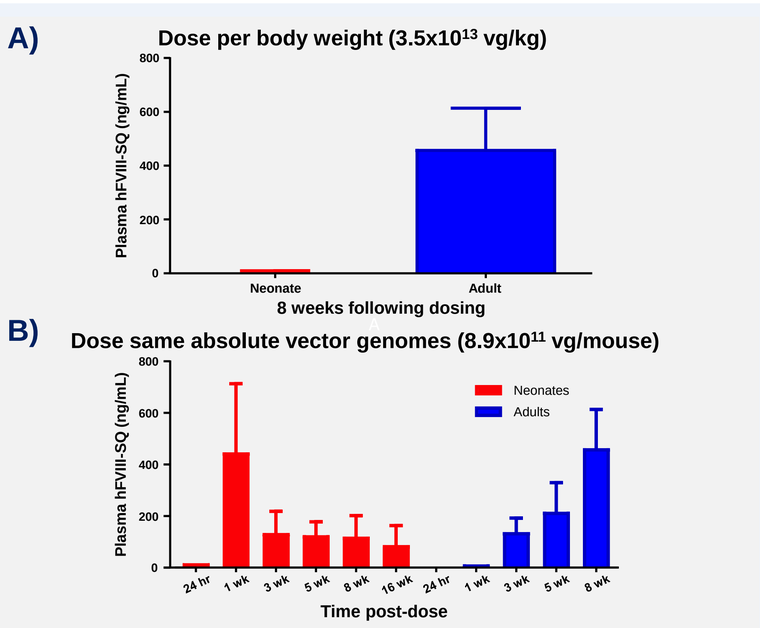
Figure 1. Adult vs neonate study design



vg, vector genome; wk, week.

Results

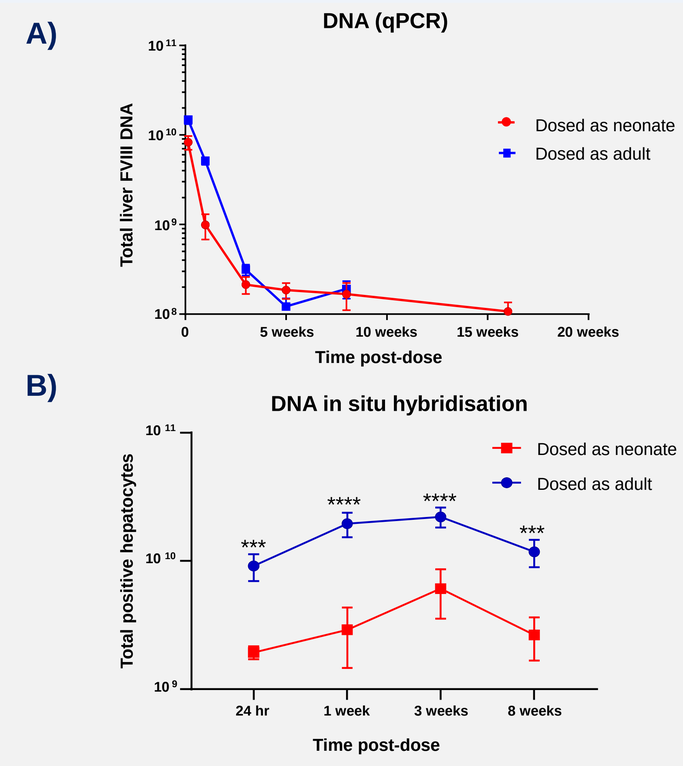
Figure 2. Neonatal mice require same total vector genomes as adults to achieve therapeutic levels of hFVIII-SQ in plasma



Data are shown as mean ± standard deviation. Red indicates mice dosed as neonates and blue indicates mice dosed as adults. hFVIII-SQ, human factor VIII SQ variant; vg, vector genome.

- Neonates and adults were administered AAV5-hFVIII-SQ and plasma hFVIII-SQ levels were measured
- When the dose was administered based on body weight, neonatal mice did not achieve meaningful plasma hFVIII-SQ protein levels (**Figure 2A**)
- When the dose was administered at the same absolute vector genomes (adult dose), neonates maintained therapeutic levels of hFVIII-SQ expression into adulthood (**Figure 2B**)
  - Neonatal mice initially exhibited high hFVIII-SQ plasma levels followed by a partial decline potentially due to age-related body mass and blood volume increases

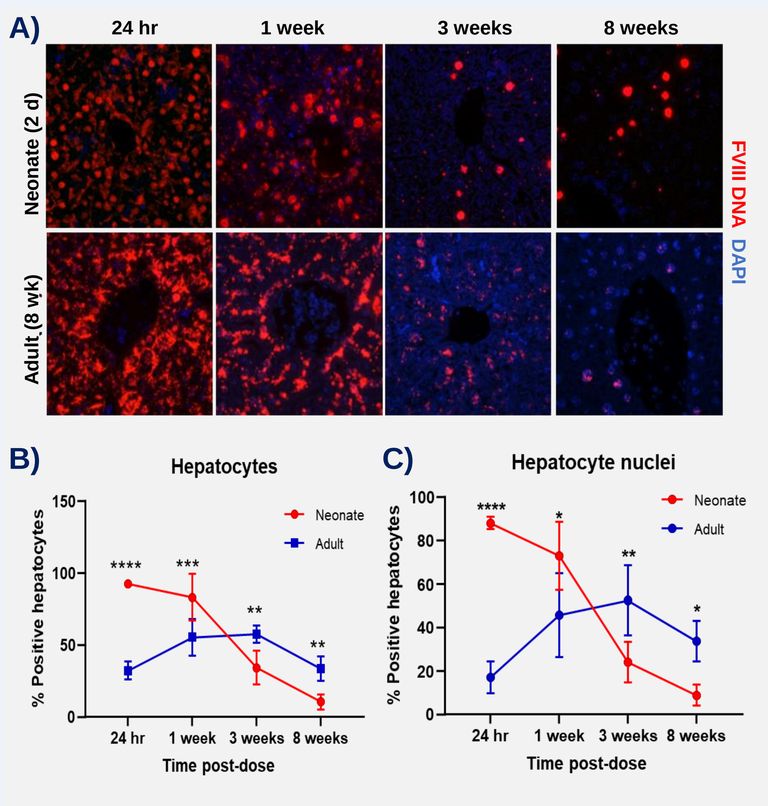
Figure 3. Neonatal mice are capable of taking up the same amount of vector genomes as adult mice



****P <0.0001, ***P <0.001; **P <0.01 comparing neonates vs adults at each timepoint with a one-way ANOVA and a Tukey's multiple comparison test.
Data are mean ± SD; Red indicates mice dosed as neonates and blue indicates mice dosed as adults.
ANOVA, analysis of variance; FVIII, factor VIII; qPCR, quantitative real-time PCR; SD, standard deviation;

- Neonates and adults were administered AAV5-hFVIII-SQ, and AAV5-hFVIII-SQ vector genomes in the liver were detected by qPCR or in situ hybridisation
- The majority of total vector genomes in the liver disappeared over the first few weeks following dosing (**Figure 3A**)
  - Despite liver growth in the neonatal cohort, the slope of decline in vector genomes over time is similar between young and adult animals
- The total number of hepatocytes that stained positive for hFVIII-SQ DNA remained stable in neonatal mice, suggesting hFVIII-SQ genomes are not lost due to mitosis (**Figure 3B**)

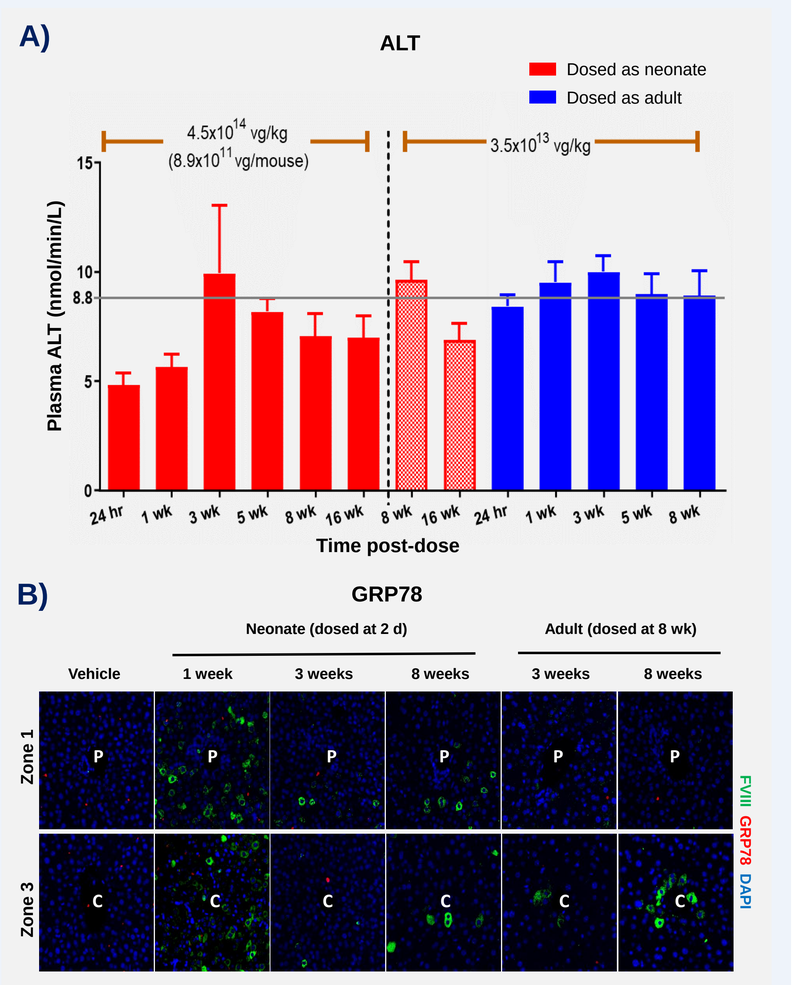
Figure 4. Kinetics of vector genome trafficking differ in neonatal mice



****P <0.0001, ***P <0.001; **P <0.01; *P <0.05 comparing neonates vs adults at each timepoint with a one-way ANOVA and a Tukey's multiple comparison test.
Data are shown as mean ± standard deviation in red for mice dosed as neonates and blue for mice dosed as adults.
ANOVA, analysis of variance; DAPI, 4′,6-diamidino-2-phenylindole; FVIII, factor VIII.

- At 24 hours after dosing, more hepatocytes in neonates stained positive for hFVIII-SQ DNA when compared with the adult cohort (**Figure 4A, 4B**)
- When looking specifically at the nucleus, hFVIII-SQ vector genomes were present in the majority of hepatocyte nuclei in mice dosed as neonates at 24 hours compared to at 3–8 weeks in adult mice (**Figure 4C**)
- This difference may explain the more rapid onset of expression of hFVIII-SQ protein observed in younger mice

Figure 5. AAV5-hFVIII-SQ does not induce hepatoxicity



Data are shown as mean ± standard deviation in red for mice dosed as neonates, blue for mice dosed as adults, and pink for neonates administered at the lower dose. The grey line indicates the mean ALT in animals dosed with vehicle.
ALT, alanine aminotransferase; C, central vein; DAPI, 4′,6-diamidino-2-phenylindole; d, day; FVIII, factor VIII; GRP78, 78 kDa glucose-regulated protein; P, portal vein; vg, vector genome; wk, week.

- There was no significant increase in alanine aminotransferase detected in either adult or neonatal cohorts (**Figure 5A**)
- There was no increase in GRP78 expression, a marker of endoplasmic reticulum (ER) stress, in hepatocytes expressing hFVIII-SQ, in either the adult or neonatal cohort (**Figure 5B**)

Conclusions

- Results demonstrate the capacity for AAV5-hFVIII-SQ transduction and rate of DNA decline is similar between neonates and adult mice when administered the same total quantity of vector
- There were no features of hepatotoxicity or ER stress that were observed
- These data suggest that young mice require the same total vector genomes as adult mice to sustain hFVIII-SQ plasma levels into adulthood

References

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Disclosures

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