AAV-8 Is More Efficient than AAV-9 in Transducing Neonatal Dog Heart

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Abstract

Adeno-associated virus serotype-8 and 9 (AAV-8 and 9) are the leading candidate vectors to test bodywide neonatal muscle gene therapy in large mammals. We have previously shown that systemic injection of $2\text{--}2.5 \times 10^{14}$ viral genome (vg) particles/kg of AAV-9 resulted in widespread skeletal muscle gene transfer in newborn dogs. However, nominal transduction was observed in the heart. In contrast, robust expression was achieved in both skeletal muscle and heart in neonatal dogs with $7.14\text{--}9.06 \times 10^{14}$ vg particles/kg of AAV-8. To determine whether superior cardiac transduction of AAV-8 is because of the higher vector dose, we delivered 6.14×10^{14} and 9.65×10^{14} vg particles/kg of AAV-9 to newborn puppies via the jugular vein. Transduction was examined 2.5 months later. Consistent with our previous reports, we observed robust bodywide transduction in skeletal muscle. However, increased AAV dose only moderately improved heart transduction. It never reached the level achieved by AAV-8. Our results suggest that differential cardiac transduction by AAV-8 and AAV-9 is likely because of the intrinsic property of the viral capsid rather than the vector dose.

Introduction

OVER THE LAST TWO DECADES, adeno-associated virus (AAV) has become a leading vector for gene therapy. AAV is a single-stranded DNA virus discovered in mid-1960s.¹ More than 100 AAV variants are now available for gene transfer studies.^{2,3} These AAV variants are either isolated from natural resources (such as adenovirus stocks and animal tissues) or engineered in the laboratory by rational design and/or evolution. While all AAV variants have a similar icosahedral capsid, the difference in the amino acid composition has yielded distinctive biological properties that are now been capitalized for different gene therapy applications. Among these, the ability to escape from the vasculature while remaining transduction competent is particularly attractive for treating diseases like Duchenne muscular dystrophy (DMD). Muscle makes up $\sim 40\%$ of the body mass and it is distributed all over the body. An effective therapy for DMD will require an efficient delivery of a therapeutic gene to the whole body.

The breakthrough in systemic gene delivery was made about a decade ago.^{4–8} Studies from several laboratories show that a single intravenous injection of AAV-6, 8, or 9 leads to sustained whole-body gene transfer in rodents. Subsequent

studies in mouse models of DMD and other types of muscular dystrophies revealed excellent bodywide muscle transduction and disease amelioration.^{9–14} We conducted the first systemic AAV gene delivery in a large mammal in 2008.¹⁵ In the study, we delivered $1\text{--}2.5 \times 10^{14}$ viral genome (vg)/kg of AAV-9 to newborn dogs. We picked AAV-9 because this serotype worked extremely well in the rodent heart. We reasoned that the cardiac tropism would help to treat cardiomyopathy, a lethal complication of muscular dystrophy. Although robust bodywide skeletal muscle transduction was observed, surprisingly, very few cardiomyocytes were transduced.¹⁵ To search for alternative methods, we tested AAV-8 in a recently published study.¹⁶ At the dose of 1.35×10^{14} vg/kg, we did not see much cardiac gene transfer. However, when the dose was increased to $7.14\text{--}9.06 \times 10^{14}$ vg/kg, we observed widespread gene transfer throughout the entire heart. The results of the AAV-8 study suggest that the vector dose may play an important role in determining cardiac transduction efficiency in neonatal dogs.¹⁶

In the present study, we tested whether AAV-9 has a similar dose response. We delivered AAV-9 to two neonatal dogs at the dose of 6.14 and 9.65×10^{14} vg/kg. The increased vector dose resulted in moderate heart transduction but never reached that of AAV-8.

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Materials and Methods

Animals

All animal experiments were approved by the Animal Care and Use Committee of the University of Missouri and were in accordance with the National Institutes of Health guideline. Two newborn dogs, Christa and Barbara, received medium-dose (6.14×10^{14} vg/kg) and high-dose (9.65×10^{14} vg/kg) AAV-9 (see below for details), respectively. Both were female carriers for DMD. An age-matched female carrier dog (Generic) was used as the noninjected control (Table 1). Two more dogs (Artemis and Dojo) were used for comparison and they were from previously published studies.^{15,16} Artemis was a female carrier dog and Dojo was a normal male dog. Artemis received 9.06×10^{14} vg/kg of AAV-8 and was euthanized at the age of 2.5 months. Dojo received 2×10^{14} vg/kg of AAV-9 and was euthanized at the age of 6 months. All experimental dogs were generated by artificial insemination at the University of Missouri and they were on a mixed genetic background of golden retriever, Labrador retriever, beagle, and Welsh corgi. The genotyping was determined by polymerase chain reaction (PCR) as we described before.^{17,18}

AAV preparation

In this study, recombinant AAV-9 stock was generated using the exactly same triple-plasmid transfection protocol as we did in our published neonatal dog systemic gene delivery studies.^{15,16,19,20} The three plasmids were (1) a *cis*-plasmid containing the Rous sarcoma virus (RSV) promoter, the heat-resistant *human placental alkaline phosphatase* (AP) reporter gene, and the simian virus 40 polyadenylation signal; (2) an AAV capsid/replication protein helper plasmid (a generous gift from Dr. James Wilson at the University of Pennsylvania, Philadelphia, PA)²¹; and (3) an adenoviral helper plasmid (Agilent Technologies, Clara, CA). The experimental vector (AAV.RSV.AP) was purified through three rounds of isopycnic CsCl ultracentrifugation followed by three changes of HEPES buffer at 4°C for 48 hr. The endotoxin level (as determined by the limulus amoebocyte lysate assay) was within the acceptable level recommended by the Food and Drug Administration. Minor technical differences can greatly influence AAV titer determination. To this end, we have taken several precautions. Specifically, we have used the same set of primers, same quantitative PCR (qPCR) kit, same plasmid standard, same machine, and same PCR condition for viral titer determination. For each

titer determination, we run triplicated qPCRs. The average of three reactions was designated as the vector titer. Further, we have confirmed the biological activity of different batches of the vectors (the batch used in the current study versus the batches used in our previous studies) in C57/BL10 mice by local and systemic delivery.^{15,16} There was no apparent difference in transduction efficiency when the same dose, but different batches, of vectors were injected in mice. The PCR primers used in the titer determination amplify a fragment in the RSV promoter. The forward primer is 5'-GGTTGTACGCGGTTAGGAGT. The reverse primer is 5'-GGCATGTTGCTAACTCATCG.

Gene delivery and dog necropsy

AAV-9 from the same batch was delivered to 2-day-old conscious puppies by a single bolus injection through the jugular vein as we described before (Table 1).^{15,16,20} Full-body necropsy was performed at the age of 2.5 months. A total of 53 skeletal muscles were collected from the head, neck, shoulder, thorax, back, abdomen, forelimb, and hind limb. These muscles included extra-ocular muscle, geniohyoid, digastricus, masseter, temporalis (Tem), tongue (Ton), sternocephalicus (Ste), sternohyoideus, cleidocervicalis, trapezius, deltoideus (Del), supraspinatus, infraspinatus, teres major, teres minor, serratus dorsalis cranialis, serratus ventralis thoracis, latissimus dorsi, superficial pectoralis (SP), deep pectoralis, inter-costal muscle, abdominal rectus (AR), external abdominal oblique, internal abdominal oblique, transversus abdominis, extensor carpi ulnaris (ECU), extensor carpi radialis (ECR), extensor digitorum lateralis, extensor digitorum communis, flexor carpi ulnaris (FCU), flexor carpi radialis (FCR), flexor digitorum superficialis, flexor digitorum deep, pronator teres, pronator quadratus, biceps brachii (BB), triceps brachii (TB), brachialis, cranial tibialis (CT), extensor digitorum longus (EDL), vastus lateralis, vastus intermedius, semitendinosus, semimembranosus, cranial sartorius (CS), rectus femoris (RF), biceps femoris (BF), gluteus, adductor, pectineus, gracilis, gastrocnemius (Gas), and levator ani muscle. The diaphragm (DPH) samples were obtained from three different locations: the sternal (DPH-S), costal (DPH-C), and lumbar (DPH-L) part of the diaphragm. AP expression in 17 represented muscles was examined to evaluate bodywide gene transfer. These include BF, CT, CS, Del, DPH, ECU, ECR, EDL, FCR, FCU, Gas, SP, Ste, BB, TB, Tem, and Ton. Heart tissues were collected from 10 locations, including the

TABLE 1. SUMMARY OF EXPERIMENTAL PROTOCOL AND DOGS

	Christa	Barbara	Artemis ^a	Dojo ^a	Generic
Gender	Female	Female	Female	Male	Female
Genotype	Carrier	Carrier	Carrier	Normal	Carrier
Age at the time of injection (day)	2	2	2	2	N/A
BW at the time of injection (g)	380	380	412	520	N/A
AAV serotype	AAV-9	AAV-9	AAV-8	AAV-9	N/A
Injection volume (ml/kg BW)	9.21	14.47	14.56	8	N/A
AAV dosage (vg particle/kg BW)	6.14×10^{14}	9.65×10^{14}	9.06×10^{14}	2×10^{14}	N/A
BW at 2.5 months of age (kg)	5.40	5.44	6.02	—	5.8
Age at necropsy (month)	2.5	2.5	2.5	6	2.5

AAV, adeno-associated virus; BW, body weight; N/A, not applicable; —, not available.

^aDogs used in previous published experiment.

right and left atria (RA and LA), the cranial and caudal wall of the right ventricle (RVcr and RVca), interventricular septum (IS), anterior and posterior papillary muscles (PMa and PMp), and the apex, anterior, and posterior wall of the left ventricle (LVap, LVa, and LVp). We also collected the liver, pancreas, spleen, kidney, lung, and axillary lymph nodes.

Blood chemistry

Blood was collected immediately before euthanasia. Laboratory biochemical test was performed at the UMC Vet Med Diagnostic Lab (Columbia, MO).

AP expression

Histochemical staining was carried out on 8 μ m cryosections as we described before.^{15,16,22,23} Enzymatic activity was measured using the Stem TAG Alkaline Phosphatase Activity Assay Kit (Cell Biolabs, San Diego, CA) according to manufacturer's instruction. Before histochemical staining and enzymatic assay, endogenous heat-sensitive AP was inactivated at 65°C for 45 min to 1 hr.

T-cell infiltration

Eight-micrometer cryosections were cut from the extensor carpi ulnaris muscle and left ventricle. CD4⁺ and CD8⁺ cells were revealed using the canine-specific anti-CD4 (1:1000) and anti-CD8 (1:200) antibodies, respectively (AbD Serotec, Raleigh, NC). The quantity of CD4⁺ or CD8⁺ cells was determined from counting five random 10 $\times$ fields.

AAV genome copy

Genomic DNA was extracted from liquid-nitrogen-frozen samples. Average DNA concentration was determined from three independent measurements using the NanoDrop ND-1000 spectrophotometer (260/280 ratio was 1.8–2.0). The viral ge-

nome copy in tissue samples was determined by quantitative PCR using the Fast SYBR Green Master Mix kit (Applied Biosystems, Foster City, CA) in an ABI 7900 HT qPCR machine. An amount of 100 ng genomic DNA was used in each qPCR. The same primers used for AAV copy number quantification were used for AAV titer determination. The qPCR efficiency from all experiments was among 90–93%. R^2 was >0.99.

Results

High-dose intravenous AAV-9 delivery did not cause adverse reaction in newborn dogs

Administration of medium-dose (Christa, 6.14×10^{14} vg/kg) and high-dose (Barbara, 9.65×10^{14} vg/kg) AAV-9 was well tolerated. Both puppies showed similar activity, behavior, food/water intake, and body weight gain as uninjected littermates. Except for the expected elevation of serum alkaline phosphatase (because of expression from AAV.RS-V.AP), the blood panel was unremarkable (Table 2).¹⁶ Histological examination of major internal organs revealed a similar morphology as that of the noninjected control (Fig. 1).

Saturated expression was observed in skeletal muscle throughout the body

In our previous study, we demonstrated bodywide skeletal muscle transduction after intravenous injection of 2×10^{14} vg/kg of AAV-9 in newborn puppies.¹⁵ However, gene transfer in many muscles (such as RF, CS, and AR) remains suboptimal (50–80%) (Fig. 2A).¹⁵ A 3-fold increase in the vector dose (to 6.14×10^{14} vg/kg) resulted in complete (~100%) transduction of every limb muscle in Christa (Fig. 2A and Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/hgtb). Boosting the dose further to 9.65×10^{14} vg/kg in Barbara (~5-fold higher than the dose used in Dojo) increased the vector genome

TABLE 2. BLOOD EXAMINATION RESULTS

	Age (2.5 months)			Age (1.5–3.5 months) (n = 20)	
	Christa	Barbara	Artemis	Uninjected control, mean $\pm$ SEM	Uninjected control, range
Calcium (mg/dl)	11.8	11.8	10.8	11.2 $\pm$ 0.1	10.5–11.9
Chloride (mEq/liter)	109	113^a	109	106.6 $\pm$ 0.6	99–111
Phosphorus (mg/dl)	9.4	9.2	9.2	8.5 $\pm$ 0.2	7.0–10.1
Potassium (mEq/liter)	7	5.1	5	5.8 $\pm$ 0.1	5.2–7.1
Sodium (mEq/liter)	142	145	148	141.5 $\pm$ 0.8	133–148
Albumin (g/dl)	2.5	2.6	2.3	2.7 $\pm$ 0.1	2.1–3.6
Alkaline phosphatase (ALP) (U/liter)	209	310	239	147.9 $\pm$ 5.4	111–200
Alanine aminotransferase (ALT) (U/liter)	70	52	42	25.7 $\pm$ 4.4	9–97
ALP/ALT ratio	2.9	5.9	5.7	8.1 $\pm$ 1.1	1.3–22
Anion gap (mEq/liter)	20	17	25	22.2 $\pm$ 0.5	19–28
Cholesterol (mg/dl)	236	194	186	254.2 $\pm$ 14.7	173–427
Creatinine (mg/dl)	0.5	0.4	0.5	0.4 $\pm$ 0.0	0.2–0.7
Gamma-glutamyl transpeptidase (GGT) (U/liter)	<3	2	<3	0.9 $\pm$ 0.4	0–6
Globulin (g/dl)	2.2	2.2	1.9	2.2 $\pm$ 0.1	1.7–2.7
Glucose (mg/dl)	197	110	88	105.3 $\pm$ 2.4	89–126
Total bilirubin (mg/dl)	0.2	0.2	0.3	0.2 $\pm$ 0.0	0.1–0.4
Total CO ₂ (mEq/liter)	20	20	19	18.6 $\pm$ 0.9	7–24
Total protein (g/dl)	4.7	4.8	4.2	4.9 $\pm$ 0.1	4.1–6.3
Urea nitrogen (mg/dl)	10	14	19	11.8 $\pm$ 1.4	4–22

^aBold font indicates the value is not within the range of uninjected control.

FIG. 1. High-dose AAV-9 delivery in neonatal dogs does not alter histology of major internal organs. Representative photomicrographs of HE-stained tissue sections from dogs that were injected with medium- and high-dose AAV-9 (Christa and Barbara), high-dose AAV-8 (Artemis), and non-injected dog (Generic). No abnormality was observed in the liver, pancreas, spleen, kidney, and lung. AAV, adeno-associated virus; N/A, not applicable. Color images available online at www.liebertpub.com/hgtb

copy number in most muscles but it did not yield dramatically much higher AP activity in muscle lysate, suggesting a saturation effect in skeletal muscle (Fig. 2C and D, and Supplementary Fig. S1D). No AP positive cells were observed in uninjected control (Supplementary Fig. S2).

Increased AAV-9 dosage yielded only moderate myocardial transduction

The main goal of this study is to determine whether the vector dose is the primary limiting factor for AAV-9 transduction in the newborn dog heart. In our published studies ($1\text{--}2.5 \times 10^{14}$ vg/kg), hardly any expression was detected in the heart.¹⁵ In Christa (6.14×10^{14} vg/kg), AP-positive cells became readily detectable by histochemical staining in the interventricular septum, left ventricle, and papillary muscle (Fig. 3A). A dose-dependent increase in AP expression was observed in almost every region of the heart in Barbara (9.65×10^{14} vg/kg). Widespread AP-positive cells were seen in the left atrium, interventricular septum, papillary muscle, and left ventricle (Fig. 3A). However, it never reached the level seen in Artemis, a puppy injected with 9.06×10^{14} vg/kg of AAV-8 (Fig. 3A and B). Interestingly, on histochemical staining, the right heart (RA and RV) was efficiently transduced by AAV-8 but barely transduced by AAV-9 (Fig. 3A).

AAV copy number quantification revealed an interesting pattern. In the right ventricle and interventricular septum, we detected more vector genome in AAV-8-injected Artemis, consistent with high transduction. But in the right atrium, similar number of the AAV genome copy was found despite a substantially much more robust expression in the AAV-8-injected puppy (Fig. 3). Most surprisingly, in the left atrium, papillary muscle, and left ventricle, AAV copy number in Barbara (9.65×10^{14} vg/kg of AAV-9) was 3–5-fold higher than that in Artemis (9.06×10^{14} vg/kg of AAV-8). However, the high copy number did not result in high expression, suggesting that a significant portion of the AAV-9 genome may have been trapped in a yet-to-be-defined dead compartment and/or not converted to the transcription-competent form in cardiomyocytes and cannot express the transgene.

Transduction of internal organs by systemic high-dose AAV-9 injection in newborn puppies

AAV transduction in the liver, pancreas, spleen, lung, and kidney was examined (Fig. 4). Similar to the results of high-dose AAV-8,¹⁶ very few AP-positive cells were detected in the liver, but substantial AP expression was found in the pancreas, spleen, lung, and kidney (Fig. 4).

FIG. 2. Intravenous injection of high-dose AAV-9 results in improved skeletal muscle transduction in neonatal dogs. **(A)** Representative photomicrographs of alkaline phosphatase (AP) histochemical staining from muscles that were injected with low (Dojo, 2×10^{14} vg/kg) and medium (Christa, 6.14×10^{14} vg/kg) doses of the AAV-9 AP reporter vector. AR, abdominal rectus; CS, cranial sartorius; RF, rectus femoris. **(B)** Representative photomicrographs of skeletal muscle AP histochemical staining from AAV-9-injected dogs Christa and Barbara as well as AAV-8-injected dog Artemis. DPH, diaphragm; ECU, extensor carpi ulnaris; SP, superficial pectoralis; TB, triceps brachii. **(C)** Quantitative examination of AP activity in muscle lysate. **(D)** Comparison of the AAV vector genome copy number in different skeletal muscles. Color images available online at www.liebertpub.com/hgtb

Systemic AAV-9 in newborn dogs did not induce T-cell infiltration in transduced muscles

To determine whether high-dose systemic AAV injection induced the cellular immune response in muscle, we examined the infiltration of CD4^+ and CD8^+ T-cell. Only occasional residential CD4^+ and CD8^+ T-cells were detected. There was no apparent difference in AAV-injected and noninjected dog muscles (Supplementary Fig. S3).

High-dose systemic AAV injection in newborn dogs resulted in minimum transduction in draining lymph nodes

AP expression was evaluated in the axillary lymph nodes from injected and noninjected dogs. Nominal AP expression was detected in the lymph node of AAV-9-injected puppies (Christa and Barbara) (Supplementary Fig. S4). Slightly more AP expression was seen in the puppy injected with AAV-8 (Artemis). Interestingly, there was strong AP expression in the capsule of the lymph node harvested from this puppy (Artemis) (Supplementary Fig. S4).

Discussion

In this study, we tested the hypothesis that high-dose AAV-9 can efficiently transduce the newborn dog heart. We found a positive correlation between the vector dose and transgene expression in the heart. However, cardiac transduction of AAV-9 never reached that of AAV-8.

Studies performed in rodents suggest that AAV-9 may represent a cardiac tropic serotype (reviewed in refs.^{24,25}). Surprisingly, the heart was barely transduced when we delivered AAV-9 to newborn puppies at a dose comparable to that used in rodent studies.¹⁵ To identify a serotype that can be used for dog heart gene transfer, we screened several additional AAV serotypes, including Y445F/Y731F AAV-1, Y445F AAV-6, AAV-8, and Y731F AAV-9.^{15,16,20} Robust myocardial gene transfer was observed with AAV-8 at doses that were 3–4-fold higher than the highest dose (2.5×10^{14} vg/kg) we used in the original AAV-9 study.¹⁵

The difference in the vector dose and/or the viral serotype may have contributed to the superior heart transduction by AAV-8. To dissect the underlying mechanisms, we delivered high-dose AAV-9 to two newborn puppies (Christa and Barbara). To evaluate the potential dose response, we used two doses (6.14×10^{14} and 9.65×10^{14} vg/kg). Bodywide gene transfer in these two puppies was examined at the same time point as we did for Artemis, a dog used in the AAV-8 study.¹⁶ As expected from our previous publications,^{15,16,20} vigorous skeletal muscle transduction was observed, while liver is poorly transduced (Figs. 2 and 4, and Supplementary Fig. S1). However, unlike what we have seen with medium- to high-dose AAV-8,¹⁶ heart transduction by AAV-9 only reached moderate levels at these doses (Fig. 3). For example, puppy Barbara received 9.65×10^{14} vg/kg of AAV-9. This is higher than the AAV-8 dose administered to puppy Artemis (9.06×10^{14} vg/kg). Yet, the number of AP-positive cells seen in the heart of

FIG. 3. AAV-9 is less efficient than AAV-8 in transducing neonatal dog heart. **(A)** Representative lower magnification microphotographs of AP staining in different parts of the heart, including right atrium (RA), left atrium (LA), right ventricular cranial wall (RVcr), right ventricular caudal wall (RVca), interventricular septum (IS), anterior papillary muscle (PMa), posterior papillary muscle (PMp), left ventricular anterior wall (LVa), left ventricular posterior wall (LVp), and left ventricular apex (LVap). **(B)** Quantitative examination of AP activity in muscle lysate from different parts of the heart. **(C)** AAV genome copy quantification in different parts of the heart. Color images available online at www.liebertpub.com/hgtb

Barbara was substantially lower than that of Artemis (Fig. 3). While additional studies are needed to corroborate these findings (we only have a small sample size), our results suggest that some yet unidentified properties of the AAV-8 capsid may have contributed to the efficient myocardial transduction in newborn dogs by AAV-8. The crystal structure of AAV-8 capsid and AAV-9 capsid was resolved recently by the Agbandje-McKenna laboratory at the resolution of 2.6 and 2.8 angstrom, respectively.^{26,27} Major differences between two serotypes are located in three surface variable regions. Future site-directed mutagenesis in these regions will help to pinpoint structural features that are responsible for the superior myocardial transduction by AAV-8 in newborn dogs.²⁸

It is currently unclear why AAV-9 can effectively transduce rodent heart but not newborn dog heart. One apparent explanation would be species differences. Such species-specific changes have been shown to at least partially contribute to the phenotypic differences among dystrophin-deficient mice, dogs, and human patients.²⁹ Our findings raise a critical concern on the future translation of canine study results to DMD patients. It is our belief that animal studies may inform the design of the clinical trial rather than predict the outcome of a clinical trial.

Besides skeletal muscle and the heart, we also examined the transduction profile in major internal organs (Fig. 4). Moderate AP expression was observed in pancreas, spleen,

FIG. 4. Internal organ transduction following high-dose systemic AAV-9 injection in neonatal dogs. **(A)** Representative photomicrographs of AP histochemical staining from the liver, pancreas, spleen, kidney, and lung. **(B)** Quantitative examination of AP activity in tissue lysate from the liver, pancreas, spleen, kidney, and lung. **(C)** AAV genome copy quantification in the liver, pancreas, spleen, kidney, and lung. Color images available online at www.liebertpub.com/hgtb

kidney, and lung. However, very few AP-positive cells were detected in the liver. The AP activity assay further confirmed low-level liver transduction. This is in contrary to the results of AAV genome copy quantification. Substantial amount of the vector genome was detected in the liver. We speculate that this may relate to the promoter shutdown or other yet unrecognized mechanisms that may have limited the formation of the transcriptionally competent vector genome.^{30–32}

The safety of systemic gene delivery has always been a concern. The dosage administrated to puppy Barbara (9.65×10^{14} vg/kg) is so far the highest AAV dose ever been given to a living animal. Consistent with our previous studies (from 1×10^{14} to 9.06×10^{14} vg/kg), we did not see any severe side effect in Barbara. There was no growth retardation (Table 1). Her liver and kidney function was normal (Table 2). Collectively, our data (from a total of 14 dogs) suggest that systemic AAV injection in neonatal dogs is relatively safe.^{15,16,20}

In summary, our results suggest that AAV-9 is not a cardiotropic virus in newborn dogs. Further, our results provide additional safety data supporting neonatal gene therapy in large mammals. Highly sensitive and reproducible neonatal screening methods have developed for DMD and many other muscular dystrophies. Neonatal gene therapy will allow treatment before the onset of clinical symptoms. Recently, a clinical trial was approved to treat neonatal spinal muscular atrophy patients with systemic injection (clinical trial # NCT02122952). More neonatal gene therapy studies are expected to follow in the coming years. A detailed characterization of the transduction profile of different AAV serotypes in newborn large animals will provide necessary information to help guide these trials.

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Author Disclosure Statement

D.D. is a member of the scientific advisory board for Solid GT, a subsidiary of Solid Ventures. For all other authors, no competing financial interests exist.

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