Virus Research 272 (2019) 197716

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Molecular characterization of novel Adeno-associated virus variants infecting human tissues

Nishtha Purohit[a], Apurv Jain[b], Vikram Mathews[c], Giridhara R. Jayandharan[a,b,c,*]

[a] Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, UP, India
[b] Centre for Stem Cell Research, Vellore, TN, India
[c] Department of Hematology, Christian Medical College, Vellore, TN, India

ARTICLE INFO

Keywords:
AAV
Genotyping
PCR
Biodistribution

ABSTRACT

Despite the many advantages with Adeno-associated virus (AAV) based vectors for gene therapy, certain barriers related to host permissivity and immune response precludes their widespread application in humans. A comprehensive study of the distribution and complexity of naturally occurring AAV in human tissues should facilitate their optimal utilization for gene therapy and tissue targeting in humans. A total of 205 samples, comprising 198 tissue samples from individuals of Indian origin and 7 different cell lines were investigated. A panel of 8 primate samples was used as controls. DNA from these samples was screened for the AAV capsid specific signature regions by a modified PCR and DNA sequencing approach. Further, we generated a single point mutation (S224A) in AAV3 vector, analogous to the mutation identified in a novel AAV3 sequence variant isolated from a peripheral blood stem cell (PBSC) sample. We further studied the infectivity of these vectors in HeLa and HS5 cells *in vitro*. Of the 205 samples analyzed, an AAV specific signature DNA sequence was detected in 92 samples (45%), including 85 out of 198 human tissues and in all the 7 human cell lines investigated. DNA sequencing analysis showed that AAV6(34%) was the most common serotype and identified predominantly in PBSCs. Interestingly, a comparative genotypic analysis in primate samples identified AAV3 specific DNA in most of the bone marrow or liver tissue analyzed (n = 7/8) suggesting species-specific differences in AAV infectivity. Further characterization of an AAV3 serotype variant isolated from the PBSCs was non-infectious *in vitro*, possibly due to altered receptor affinity. Our data outlines the genetic diversity and the distribution of AAV serotypes infecting humans and provides a basis for their further characterization to generate efficient gene delivery vectors.

1. Introduction

Adeno-associated virus (AAV) is a member of Parvovirus family that requires co-infection with a helper virus for its productive replication and gene expression. In the absence of a helper virus, AAV undergoes site-specific integration into the host genome to establish a latent cycle of infection. AAV serotype 2 is the best studied with respect to its life cycle. Molecular cloning of AAV2 aided the initial development recombinant AAV based vectors (Samulski et al., 1982). Stable transgene expression was achieved using recombinant AAV2 vectors in multiple tissues including lung, muscle, CNS, liver and retina (Conrad et al., 1996; Herzog et al., 1999) (Acland et al., 2001; During et al., 1998). Subsequently, several alternate AAV serotypes (AAV1-10), with a majority isolated from primate tissues were described (Gao et al., 2002) (Daya and Berns, 2008). Despite their significant promise in clinical settings (Manno et al., 2006; Mingozzi et al., 2007; Nathwani et al., 2011), limitations related to lower transgene expression, inactivation by neutralizing antibodies and a strong immune response against AAV vectors have considerably impacted their translational applications. These factors underscore the need to isolate newer AAV serotypes preferably from human tissues, with diverse tissue tropism, stable transduction and an ability to bypass host immune system. Furthermore, it is well recognized that AAV serotypes have an unique ability to bind specific receptors and co-receptors on the host cells (Balakrishnan

Abbreviations: AAV, adeno-associated virus; HSPG, heparin sulfate proteoglycan; CNS, central nervous system; PBSC, peripheral blood stem cells; U937, human leukemic monocyte lymphoma; HL60, human promyelocytic leukemia cells; K562, human erythromyeloblastoid leukemia cells; NB4, human acute promyelocytic leukemia cells; Huh7, human hepatocellular carcinoma cells; HEK293, human embryonic kidney cells; HeLa, human cervical carcinoma cells; HS5, human bone marrow/ stromal cell line; PCR, polymerase chain reaction; EGFP, enhanced green fluorescent protein; Vgs, vector genomes per cell; WT, wildtype

* Corresponding author at: Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208 016 U.P., India.
E-mail address: jayrao@iitk.ac.in (G.R. Jayandharan).

https://doi.org/10.1016/j.virusres.2019.197716
Received 19 June 2019; Received in revised form 12 August 2019; Accepted 12 August 2019
Available online 13 August 2019
0168-1702/

and Jayandharan, 2014; Asokan et al., 2012). For *e.g.*AAV1 and AAV5 use N-linked sialic acid, AAV2 uses heparin sulfate proteoglycan (HSPG), AAV4 utilizes O-linked sialic acid, for host cell binding (Vandenberghe et al., 2009). Despite sharing similar receptors, AAV1 transduces muscle, liver and heart efficiently, while AAV4 and AAV5 transduce the eye and central nervous system (CNS), effectively (Daya and Berns, 2008). Understanding the basis of such differences in tropism despite the virus sharing similar receptors requires a comprehensive analysis of human tissues for their natural permissivity to AAV serotypes or their variants. In addition, while the seroprevalence of AAV2 is ˜80% in humans, suggesting that humans serve as natural host for AAV infections (Chirmule et al., 1999) it is also established that different populations have varying levels of neutralizing antibodies to different AAV serotypes (Calcedo et al., 2009; Selot et al., 2014). Thus, profiling population specific AAV variants may be necessary to optimize gene delivery approaches. Such molecular genotyping strategies has led to better understanding of virus (hepatitis C Virus)- host relationship, particularly their molecular and cellular interactions (Ellwanger et al., 2017). In this report, we have profiled the distribution of naturally occurring AAV in a cohort of tissue samples from India collected from various clinical or diagnostic settings by an optimized molecular screening protocol.

2. Materials and methods

2.1. Samples

The study was approved by Institutional Review board and Institutional ethics Committee (CMC, Vellore, IIT-Kanpur), Institutional animal ethics committee (National Institute of Reproductive Research and Health, Mumbai). Human samples including donor derived peripheral blood stem cells (PBSC, n = 88), bone marrow aspirates from acute promyelocytic leukemia cells (APL, n = 57) and formalin-fixed surgical excision tissues such as uterus (n = 7), gall bladder (n = 7), appendix (n = 7), prostrate (n = 5), ovary (n = 4), lymph node (n = 4), liver (n = 3), colon (n = 3), ileum (n = 2), endometrium (n = 2), spleen (n = 2), muscle (n = 2), jejenum (n = 1), urinary bladder (n = 1), lung (n = 1), tonsil (n = 1) and kidney (n = 1) were collected. In addition, seven cell lines of human origin, namely, *U937 (*human leukemic monocyte lymphoma), *HL60* (human promyelocytic leukemia), *K562 (*human erythromyeloblastoid leukemia), *NB4* (human acute promyelocytic leukemia), *Huh7* (human hepatocellular carcinoma), *HEK293* (human embryonic kidney), *HeLa* (human cervical carcinoma) were included in the screening. Control samples including, primate liver (n = 4) and bone marrrow samples (n = 4) were harvested from marmosets.

2.2. DNA isolation and PCR

Genomic DNA was isolated from the human cell lines by standard phenol – chloroform extraction method. DNA from fresh or formalin-fixed and paraffin-embedded tissues was isolated by Gentra Puregene Tissue kit (QIAgen, Maryland, USA) or FFPE Kit (QIAgen). PCR for capsid specific signature regions within AAV genome was performed using primers [19 s/18as (SER1 F/R), Av1Ns/Av2CaS (SER4 F/R), UNI-C /POLY-A] as described previously (Gao et al., 2002; Schmidt et al., 2008) (Table 1). In addition, two other flanking signature regions in the AAV genome were amplified by primers SER2F/R and SER3 F/R (Table 1, Fig. 1). Briefly, polymerase chain reaction (PCR) was performed with ˜100 ng of DNA in a 25 µl reaction volume containing 10 pmol of each primer in a 1X concentration of a ready reaction mix (Hotstart PCR Mastermix, QIAgen or NEB PCR mastermix, New England Biolabs, Ipswich, MA, USA). The cycling conditions for SER1-3 F/R primers included an initial denaturation at 95° C for 5–15 min, with 30 cycles of PCR amplification performed, with denaturation at 95° C for 30 s, annealing at 60° C for 30 s and extension at 72° C for 30 s. The final extension was at 72° C for 5 min. For the primers SER4F/R and UNI-C /POLY-A, which generates larger amplicons, the extension time was suitably increased to 1 min/Kb of the PCR amplicon size. To check the quality of DNA isolated from formalin fixed tissues, a human coagulation factor IX gene segment (exon h) was amplified as described previously (Jayandharan et al., 2003).

2.3. DNA sequencing

Samples displaying positive amplification for the AAV signature/ entire capsid regions were further sequenced by the Big Dye® Terminator v3.1 Cycle Sequencing Kit (Life technologies, Applied Biosystems, Warrington, UK) on an ABI 3130 genetic analyzer (*PE* Applied Biosystems, Foster City, CA). The nomenclature of novel nucleotide/amino acid substitutions in different AAV serotypes is according to their VP1 capsid sequence and their NCBI accession numbers are provided in Supplementary data file 1.

2.4. Comparative sequence analysis

The polynucleotide sequence obtained was compared for local alignment search by BLASTn analysis. Further, multiple sequence alignment of the isolate DNA sequence to known AAV serotypes were also performed (http://www.ebi.ac.uk/Tools/msa/).

2.5. Recombinant vector generation

A single point mutation, S224A identified from one of the tissue sample (HSC-17) was modeled on the parental AAV3 capsid encoding R2C3 plasmid (Stratagene, Agilent technologies). The following primers were used for generating this mutation: P1: 5'-CCAATTTCCTGAGGCA TTACCCACTCCATCGGC-3', P2: 5'-GCCGATGGAGTGGGTAATGCCTCA GGAAATTGG-3'. Self-complementary AAV3 wild type (scAAV3-CBa-EGFP-WT) containing an enhanced green fluorescent protein (EGFP) gene or their mutant forms (HSC17-AAV3) was generated and quantified as previously described (Gabriel et al., 2013; Kube et al., 1997; Sen et al., 2013).

2.6. AAV transduction assays

To study the transduction potential of the AAV3 variant, we used HeLa or a human bone marrow/ stromal cell line (HS5). These cells were seeded at a density of ˜8×10^4 cells/well in a 24 well plate in complete Iscove's Modified Dulbecco's Medium (IMDM) (Gibco, Life Technologies, Carlsbad, USA) with 10% Fetal Bovine Serum (Gibco) at 37 °C with 5% CO_2, supplemented with 1.2 g/L of sodium bicarbonate and 1% (by volume) of 100X stock solution of antibiotics (10,000 U penicillin + 10,000 mg streptomycin). For vector transduction, cells were mock (PBS) infected or infected with 5×10^3 vgs/cell of either wildtype (WT)-AAV3 or the mutant HSC17-AAV3 vectors for 2 h in the presence of incomplete IMDM (without FBS). Cells treated with phosphate buffered saline (PBS) were considered as the mock group. After two hours, cells were washed in 1XPBS and replaced with complete IMDM. Two days later, GFP positive cells were detected and quantified by flow-cytometric analysis (FACS Calibur, BD, USA).

3. Results and discussion

The strategy initially employed to isolate newer AAV serotypes involved the rescue of latent AAVs from potential sources by super-infection with helper viruses such as adenovirus (Blacklow et al., 1967; Rutledge et al., 1998). Alternatively, latent AAV genomes can be detected by PCR amplification of integrated AAV DNA using degenerate primers designed against conserved regions in capsid which flank sequences variable yet unique to specific serotypes termed as 'signature regions' (255bp) (Gao et al., 2002). Further, the complete AAV capsid

Table 1
Primers designed and used for detecting the presence of AAV genome in the present study.

Primer Name	Primer	Amplicon Size	Reference
SER 1F	19 s and 18as	255 bp	Gao et al. (2002)
SER 1R			
SER 2F	Fwd-5 'GGACCCTTCAACGGACTCGACA-3'	534 bp	Present study
SER 2R	Rev-5 'ATCGCAATGCCAATTTCCTGAG-3'		
SER 3 F	Fwd-5'GGTAATGCCTCAGGAAATTGGCATT-3'	533 bp	
SER 3R	Rev-5' GGAAAGTACTCCAGGCAGTAAAA-3'		
SER 4F	Av1Ns and Av2Cas	3 Kb	Gao et al. (2002)
SER 4R			
UNI-C	UNI-C and POLY-A	3 Kb	Schmidt et al. (2008)
POLY-A			

gene segment (3Kb) can be isolated from target tissues with a similar PCR based strategy (Schmidt et al., 2008). Such molecular screening protocols have facilitated the isolation of several novel AAV variants and expanded the repertoire of vectors that are available for gene delivery. Apart from humans (Gao et al., 2004) and primates (Gao et al., 2002), novel serotypes of AAV have also been genotyped and characterized from murine (Lochrie et al., 2006), caprine (Arbetman et al., 2005), snake (Farkas et al., 2004) and porcine sources (Bello et al., 2009). To further complement this screening strategy, we have designed two additional primers (SER2F/R and SER3F/R) that spanned extended signature regions within the AAV capsid (Fig. 1). The limit of detection for both these primers was estimated to be 1 ng of purified AAV cap DNA diluted in a background of ~1 µg human genomic DNA (~0.001%, data not shown).

With this approach, we screened 205 samples of human origin comprising 198 tissue samples and 7 human cell lines. The type and distribution of the tissue samples screened are illustrated in Fig. 2. Genomic DNA from these samples were first amplified by pre-defined AAV signature region primer sets. Amplicons in the positive samples were then sequenced bi-directionally with their respective PCR primers. The sequence outputs were analysed for similarity alignment using BLASTn tool to ascertain the similarity of the DNA sequence to the known AAV genomes. With this screening protocol, an AAV specific signature DNA sequence was detected in 92 samples (45%), including 85 out of 198 human tissues and in all the cell lines investigated (Table 2). Out of 7 cell lines screened, a PCR positivity was seen in all cell lines (100%) with SER2F/R primers, in 4 out of 7 cell lines (57%) with SER1F/R primers while the degenerate primers UNI-C/POLYA amplified an AAV specific full-length capsid from the NB4 cell line (Table 3). Interestingly, AAV6 genome was found to be the most frequently identified in HL60, HEK293, U937 and K562 cells lines, followed by the detection of AAV1, 3 and 7 signature genomes in other cell lines. One novel isolate from NB4 cell line where the entire capsid DNA sequence (~3 kb) was isolated is a variant of AAV1, that included two silent mutations (ACG > ACA; T265T and AGC > AGT; S268S) and one missense mutation (AAT > AAA; N304K) (Table 3). The detection of AAV specific genomes in these cell lines could represent either a natural infection and its further evolution as in the case of the NB4 isolate or their cross contamination *via* supplements added in cell culture such as animal serum. However, further studies are needed to confirm the presence of intact AAV particles, if any, in these cells by either immunofluorescent staining or *in situ* hybridization assays. Since AAV infection does not lead to a cytopathic effect, macroscopic changes in cells are not visible. It must be therefore noted that commonly cultured cell lines in laboratory can potentially harbor such novel AAV variants, and these could further impact the routine *in vitro* testing of AAV transduction characteristics in such cell lines.

Of the remaining 198 human tissue samples studied (Table 2), 145 samples were from either donor of cytokine primed PBSCs or acute promyelocytic leukemia (APL) patient derived bone marrow aspirate samples. Our genotyping data from this cohort showed that ~43% (63/145) of these samples were positive for AAV6 DNA while a small fraction (1.3%, n = 2/145) were positive for AAV3 specific signature DNA. These data mirror previous studies that have reported about 70% prevalence of AAV in human cytokine primed PBSC (Smith et al., 2014). In contrast, a low incidence of AAV viremia has also been reported in consecutive recipients of hematopoietic stem cell transplantation (2.8%) (Heugel et al., 2011). Such differences in the pattern of incidence of AAV viremia could be possibly due to the distinct study populations investigated, and due to differences in samples tested (blood plasma *vs.* PBSC) utilized for assaying AAV genome. Nonetheless, it is well established that AAV6 is the most optimal vector to transduce human HSCs (Song et al., 2013) and stable long-term integration of AAV is observed only in the most primitive, quiescent CD34(+)CD38(-) subsets, but not progenitor cells (Paz et al., 2007). Our findings further confirm that AAV6 by virtue of its common prevalence is probably the most suitable vector system for targeting human HSCs. Interestingly, a comparative genotypic analysis in primate

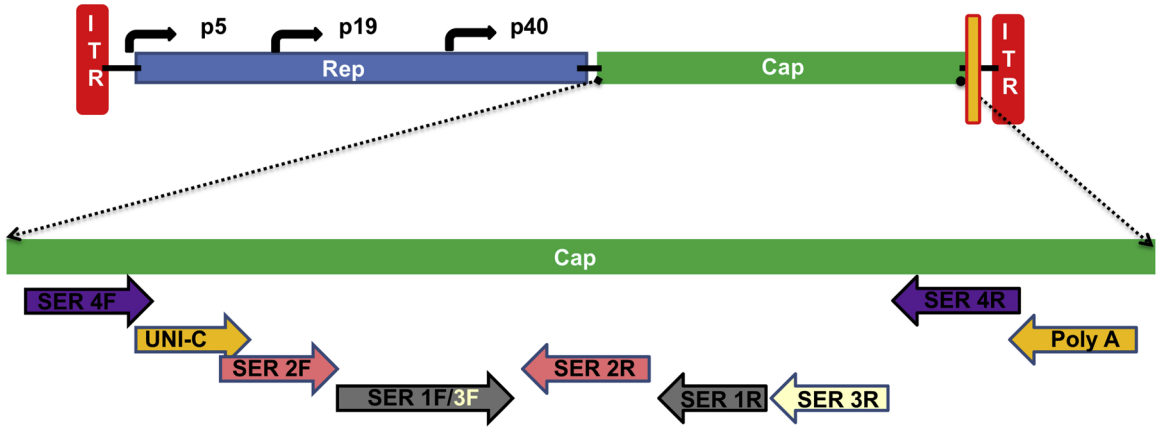

Fig. 1. Schematic representation of PCR amplification strategy of AAV genome.

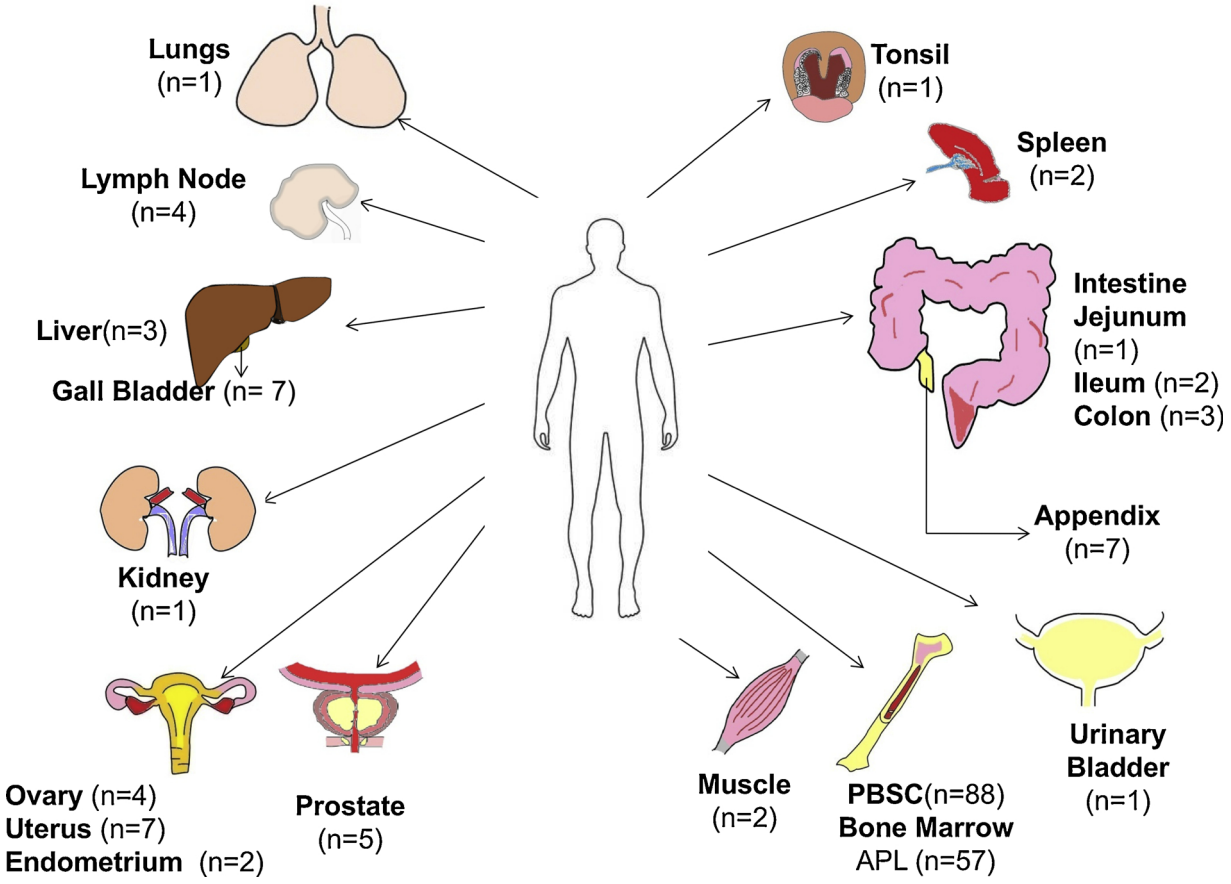

Fig. 2. Schematic representation of various human tissues screened for AAV specific DNA sequence in this study.

Table 2
Incidence and bio-distribution of AAV in various human tissues screened in this study.

Sample	No. of Samples	No. of PCR positive samples	AAV genotype
Cell lines	7	7	AAV1, AAV3, AAV6, AAV7
Mobilized PBSC	88	41	AAV6
Bone marrow (APL)	57	22	AAV6, AAV3
Uterus	7	2	AAV2 Hu.45 variant
Gall bladder	7	3	AAV2 Hu.45 variant
Kidney	1	1	AAV2 Hu.45 variant
Lung	1	0	–
Lymph node	4	3	AAV6, AAV2 Hu.45 variant
Spleen	2	2	AAV1, AAV2 Hu.45 variant
Ovary	4	2	AAV2 Hu.45 variant
Colon	3	1	AAV1
Prostate	5	2	AAV2 Hu.45 variant
Urinary Bladder	1	0	–
Appendix	7	1	AAV2 Hu.45 variant
Ileum	2	0	–
Muscle	2	2	AAV2 R58E, AAV2 Hu.45 variant
Liver	3	2	AAV6
Jejunum	1	1	AAV2 Hu.45 variant
Tonsil	1	0	–
Endometrium	2	0	–
Total	**205**	**92**	

Table 3
Detection of AAV genome sequence in cell lines of human origin.

Cell Line	1F1R (255bp)	2F2R (534bp)	3F3R (533 bp)	4F4R (3 kb)	UNI-C /POLY-A (3 kb)	AAV DNA homology
Huh7 – human hepatocellular carcinoma cell line	+	+	–	–	–	AAV 3
HeLa- human cervical carcinoma cell line	+	+	–	–	–	AAV 7
HL60 - human promyelocytic leukemia cells	–	+	–	–	–	AAV 6
HEK293- Human embryonic kidney cell line	–	+	–	–	–	AAV 6
U937 - human leukemic monocyte lymphoma cell line	+	+	–	–	–	AAV 6
K562 - Human erythromyeloblastoid leukemia cell line	–	+	–	–	–	AAV 6
NB4 - Human acute promyelocytic leukemia cell line	+	+	+	–	+	AAV1 variant (T265T, S268S, N304K)

A positive amplification in PCR is denoted by (+) while no amplification in PCR is denoted by (-) sign.

Table 4
List of novel variants isolated from human tissues in the present study.

Tissue/ cells	Sample ID	Parental AAV Serotype	Homology	NCBI Reference	DNA sequence variation Vs. reference	Gene Bank Accession No. of novel variant
NB4 cell line	NB4	AAV1	97%	AF063497.1	3%	KY711389
PBSC	HSC17	AAV3	99%	AF028705.1	1%	KY711390
Lymph Node	5H	AAV6	94%	EU368909.1	6%	KX708593
Spleen	6H	AAV2	95%	AF043303.1	5%	KX788853
Colon	9H	AAV1	96%	AF063497.1	4%	KX788854
Prostate	10H	AAV Hu.45	98%	AY530608.1	2%	KX788855
Kidney	11H	AAV Hu.45	98%	AY530608.1	2%	KX788856
Thigh Muscle	14H	AAV2R58E hu.45	98%	AY530608.1	2%	KX788857
Uterus	36H	AAV Hu.45	99%	AY530608.1	1%	KX932075
Appendix	37H	AAV Hu.45	99%	AY530608.1	1%	KX932076
Spleen	43H	AAV1	93%	AF063497.1	7%	KX932082
Ovary	44H	AAV Hu.45	97%	AY530608.1	3%	KX932083

samples identified AAV3 specific DNA in most of the bone marrow or liver tissue (87.5%, n = 7/8) analyzed suggesting species-specific differences in AAV serotype infectivity.

Furthermore, 53 formalin fixed biopsy specimens from multiple tissues were also screened, of which 22 samples showed PCR positivity to the AAV signature region. Table 2 shows the distribution of AAV serotypes in these tissue samples. AAV2 or AAV2 hu.45 variant was frequently present, with its signature DNA identified in 17 out of 53 samples analyzed including uterus, prostrate, kidney, ovaries, gall bladder, lymph nodes, spleen, appendix, muscle and the jejunum. In addition, the presence of AAV6 specific DNA sequence was confirmed in the liver (n = 2) and in the lymph node tissue (n = 1), while AAV1 was detected in two tissue samples. These partial genome sequences which are possibly non-coding also had a significantly higher number of acquired mutations (1-7% variation) when compared to parental reference sequence (Table 4). Our observations are in agreement with previous studies, where AAV2 or its variants were predominantly identified from multiple tissues screened from children (Chen et al., 2005). The increased incidence of mutations within the AAV genomes isolated in our study here suggests that genomic plasticity within the conventional AAV serotypes is possibly crucial for their persistence and dissemination in humans as has been observed earlier (Chen et al., 2005; Gao et al., 2004).

To further study the effect of these genomic alterations in AAV, we selected a partially conserved AAV3 variant with a S224A substitution isolated from PBSCs (sample HSC17, Fig. 3). To study the effect of this mutation on vector transduction, we generated a S224A mutant on the AAV3 capsid. Both the WT-AAV3 and HSC17-AAV3 vectors were packaged with EGFP as the transgene in self-complementary forms. Subsequently, the transduction efficiency of these vectors was evaluated *in vitro* at a MOI of 5×10^3 vgs/cell. As can be seen in Fig. 4, WT-AAV3 infected cells showed ˜35% GFP expression in a highly permissive cell line such as HeLa (Fig. 4A, B) whereas in a non-permissive human bone marrow/ stromal cell line (HS5) we observed only ˜5% transgene expression (Fig. 4C, D). Interestingly, this HSC-17-AAV3 variant completely abrogated the transgene expression in both HeLa as well as HS5,

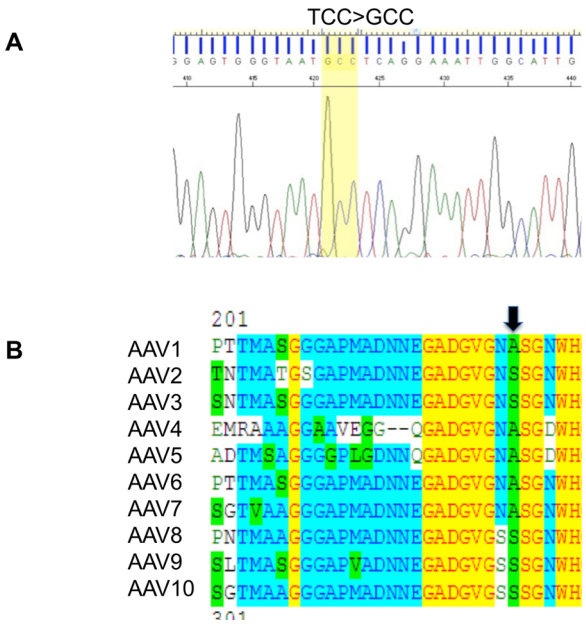

Fig. 3. (a) Sequencing electropherogram indicating the mutation S224A identified in a Peripheral blood stem cell isolate. (b) Multiple sequence alignment shows that the mutated amino acid (marked) is partially conserved among the common AAV serotypes.

a cell line similar to its host cell of origin (PBSCs). It must be noted that hematopoietic stem cells are known to carry endogenous AAVs of clade F and some of these AAV variants have also been demonstrated to improve targeting, transduction and engraftment of the multi-potential HSCs in *vivo* (Smith et al., 2014). While the basis of non- infectious nature of HSC-17-AAV3 is not clear, it is possible that this mutation (S224A) could have evolved *de novo* during multiple rounds of viral replication in the PBSC so as to improve the viral persistence *in vivo*. It

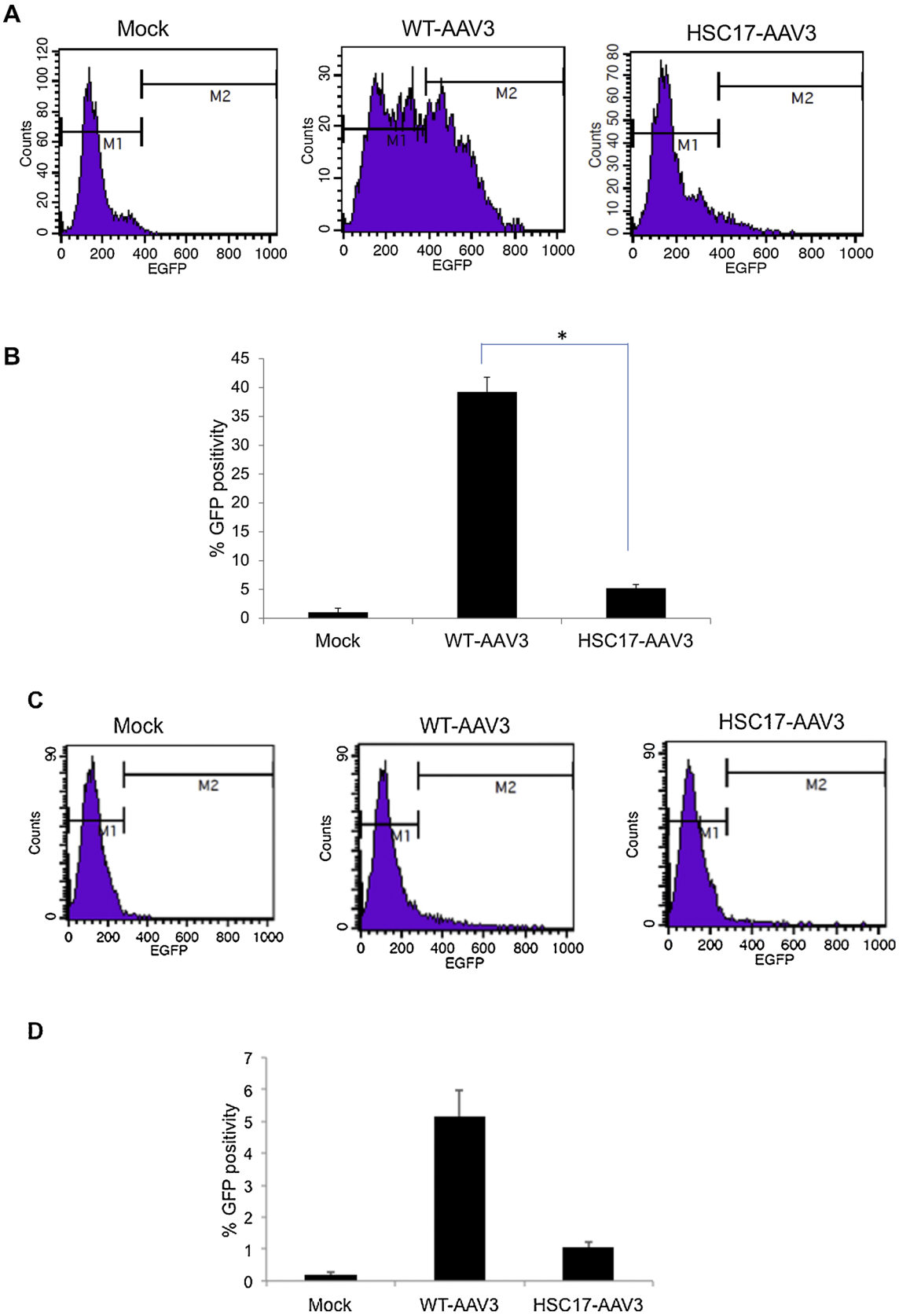

Fig. 4. Transduction efficiency of wildtype (WT) and mutant HSC17-AAV3 vectors *in vitro*. HeLa or HS5 cells were either mock-infected or infected with 5×10^3 vgs/cell of WT-AAV3 and mutant HSC17-AAV3 vectors. Forty-eight hours post-transduction, cells were analyzed for EGFP expression by flow cytometry. Representative histogram showing % EGFP positive cells in HeLa (a) or HS5 (c) cells are shown. The quantitative data from mean of triplicate analysis and confirmed with two independent experiments in HeLa (b) and HS5 (d) cells are represented. *$P < 0.05$ by analysis of variance statistical test.

is also feasible that this S224A mutation introduced into the AAV3 vector altered its receptor binding characteristics *in vitro,* which requires further detailed studies for its characterization.

Our study has certain limitations. The PCR based screening approach despite being very effective in detecting small regions of capsid sequence could identify only one full length capsid sequence in NB4 cells. This could be either due to the presence of only truncated endogenous viral gene sequences or a limitation of the PCR based technique to detect low copies of viral DNA. Further the cross-contamination of multiple cell lines with laboratory based recombinant AAV serotypes could not be completely ruled out, although the serotypes identified were varied and no full length AAV genome was detected except in NB4 cells with missense substitutions. This phenomenon thus requires further detailed analysis of various cell types and cell lines from multiple sources.

4. Conclusions

Our studies have confirmed the diversity of AAV genetic variants in human tissues and further detailed characterization of the other novel variants reported here is likely to shed light on their utility as a gene delivery vector.

Author contributions

NP and AJ performed the experiments and analyzed the data. GRJ supervised the study and wrote the paper. VM provided clinical samples and analyzed the data. All authors reviewed the manuscript.

Funding

GRJ is supported by an endowment from Joy-Gill Chair (IIT-Kanpur) and research grants from Department of Science of Technology, Government of India (Swarnajayanti Fellowship 2011; Nanomission grant SR/NM/NS-1084/2016) and an Initiation grant (2014-256) from IIT-Kanpur.

Ethical approval

The study was approved by Institutional Review board and Institutional ethics Committee (CMC, Vellore, IIT-Kanpur), Institutional animal ethics committee (National Institute of Reproductive Research and Health, Mumbai).

Declaration of Competing Interest

The authors declare that there are no conflicts of interests.

Acknowledgements

We thank N Gabriel for generation of primers and initial standardization of PCR assays; G Saravanan, D Sen, [CMC and CSCR, Vellore] and D Verma [VIT university, Vellore] for their experimental and technical assistance; Dr D Bhartiya and Dr D Manjramkar [NIRRH, Mumbai] for providing primate tissue and Dr P Saraswat [Saraswat Pathology and Kanpur medical college, Kanpur] and clinical colleagues at Departments of Hematology and Pathology, CMC, Vellore for providing human tissue samples.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.197716.

References

Acland, G.M., Aguirre, G.D., Ray, J., Zhang, Q., Aleman, T.S., Cideciyan, A.V., Pearce-Kelling, S.E., Anand, V., Zeng, Y., Maguire, A.M., Jacobson, S.G., Hauswirth, W.W., Bennett, J., 2001. Gene therapy restores vision in a canine model of childhood blindness. Nat. Genet. 28 (1), 92–95.

Arbetman, A.E., Lochrie, M., Zhou, S., Wellman, J., Scallan, C., Doroudchi, M.M., Randlev, B., Patarroyo-White, S., Liu, T., Smith, P., Lehmkuhl, H., Hobbs, L.A., Pierce, G.F., Colosi, P., 2005. Novel caprine adeno-associated virus (AAV) capsid (AAV-Go.1) is closely related to the primate AAV-5 and has unique tropism and neutralization properties. J. Virol. 79 (24), 15238–15245.

Asokan, A., Schaffer, D.V., Samulski, R.J., 2012. The AAV vector toolkit: poised at the clinical crossroads. Mol. Ther. 20 (4), 699–708.

Balakrishnan, B., Jayandharan, G.R., 2014. Basic biology of adeno-associated virus (AAV) vectors used in gene therapy. Curr. Gene Ther. 14 (2), 86–100.

Bello, A., Tran, K., Chand, A., Doria, M., Allocca, M., Hildinger, M., Beniac, D., Kranendonk, C., Auricchio, A., Kobinger, G.P., 2009. Isolation and evaluation of novel adeno-associated virus sequences from porcine tissues. Gene Ther. 16 (11), 1320–1328.

Blacklow, N.R., Hoggan, M.D., Rowe, W.P., 1967. Isolation of adenovirus-associated viruses from man. Proc. Natl. Acad. Sci. U. S. A. 58 (4), 1410–1415.

Calcedo, R., Vandenberghe, L.H., Gao, G., Lin, J., Wilson, J.M., 2009. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J. Infect. Dis. 199 (3), 381–390.

Chen, C.L., Jensen, R.L., Schnepp, B.C., Connell, M.J., Shell, R., Sferra, T.J., Bartlett, J.S., Clark, K.R., Johnson, P.R., 2005. Molecular characterization of adeno-associated viruses infecting children. J. Virol. 79 (23), 14781–14792.

Chirmule, N., Propert, K., Magosin, S., Qian, Y., Qian, R., Wilson, J., 1999. Immune responses to adenovirus and adeno-associated virus in humans. Gene Ther. 6 (9), 1574–1583.

Conrad, C.K., Allen, S.S., Afione, S.A., Reynolds, T.C., Beck, S.E., Fee-Maki, M., Barrazza-Ortiz, X., Adams, R., Askin, F.B., Carter, B.J., Guggino, W.B., Flotte, T.R., 1996. Safety of single-dose administration of an adeno-associated virus (AAV)-CFTR vector in the primate lung. Gene Ther. 3 (8), 658–668.

Daya, S., Berns, K.I., 2008. Gene therapy using adeno-associated virus vectors. Clin. Microbiol. Rev. 21 (4), 583–593.

During, M.J., Samulski, R.J., Elsworth, J.D., Kaplitt, M.G., Leone, P., Xiao, X., Li, J., Freese, A., Taylor, J.R., Roth, R.H., Sladek Jr., J.R., O'Malley, K.L., Redmond Jr., D.E., 1998. In vivo expression of therapeutic human genes for dopamine production in the caudates of MPTP-treated monkeys using an AAV vector. Gene Ther. 5 (6), 820–827.

Ellwanger, J.H., Kaminski, V.L., Valverde-Villegas, J.M., Simon, D., Lunge, V.R., Chies, J.A.B., 2017. Immunogenetic studies of the hepatitis C virus infection in an era of pan-genotype antiviral therapies – effective treatment is coming. Infect. Genet. Evol. 66 (12), 376–391.

Farkas, S.L., Zadori, Z., Benko, M., Essbauer, S., Harrach, B., Tijssen, P., 2004. A parvovirus isolated from royal python (Python regius) is a member of the genus Dependovirus. J. Gen. Virol. 85 (Pt 3), 555–561.

Gabriel, N., Hareendran, S., Sen, D., Gadkari, R.A., Sudha, G., Selot, R., Hussain, M., Dhaksnamoorthy, R., Samuel, R., Srinivasan, N., Srivastava, A., Jayandharan, G.R., 2013. Bioengineering of AAV2 capsid at specific serine, threonine, or lysine residues improves its transduction efficiency in vitro and in vivo. Hum. Gene Ther. Methods 24 (2), 80–93.

Gao, G., Vandenberghe, L.H., Alvira, M.R., Lu, Y., Calcedo, R., Zhou, X., Wilson, J.M., 2004. Clades of Adeno-associated viruses are widely disseminated in human tissues. J. Virol. 78 (12), 6381–6388.

Gao, G.P., Alvira, M.R., Wang, L., Calcedo, R., Johnston, J., Wilson, J.M., 2002. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc. Natl. Acad. Sci. U. S. A. 99 (18), 11854–11859.

Herzog, R.W., Yang, E.Y., Couto, L.B., Hagstrom, J.N., Elwell, D., Fields, P.A., Burton, M., Bellinger, D.A., Read, M.S., Brinkhous, K.M., Podsakoff, G.M., Nichols, T.C., Kurtzman, G.J., High, K.A., 1999. Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. Nat. Med. 5 (1), 56–63.

Heugel, J., Boeckh, M., Huang, M.L., Dierks, B., Hackman, R., Fredricks, D., Kuypers, J., Corey, L., 2011. Detection of adeno-associated virus viremia in hematopoietic cell transplant recipients. J. Infect. Dis. 204 (11), 1746–1749.

Jayandharan, G., Shaji, R.V., Chandy, M., Srivastava, A., 2003. Identification of factor IX gene defects using a multiplex PCR and CSGE strategy-a first report. J. Thromb. Haemost. 1 (9), 2051–2054.

Kube, D.M., Ponnazhagan, S., Srivastava, A., 1997. Encapsidation of adeno-associated virus type 2 Rep proteins in wild-type and recombinant progeny virions: rep-mediated growth inhibition of primary human cells. J. Virol. 71 (10), 7361–7371.

Lochrie, M.A., Tatsuno, G.P., Arbetman, A.E., Jones, K., Pater, C., Smith, P.H., McDonnell, J.W., Zhou, S.Z., Kachi, S., Kachi, M., Campochiaro, P.A., Pierce, G.F., Colosi, P., 2006. Adeno-associated virus (AAV) capsid genes isolated from rat and mouse liver genomic DNA define two new AAV species distantly related to AAV-5. Virology 353 (1), 68–82.

Manno, C.S., Pierce, G.F., Arruda, V.R., Glader, B., Ragni, M., Rasko, J.J., Ozelo, M.C., Hoots, K., Blatt, P., Konkle, B., Dake, M., Kaye, R., Razavi, M., Zajko, A., Zehnder, J., Rustagi, P.K., Nakai, H., Chew, A., Leonard, D., Wright, J.F., Lessard, R.R., Sommer, J.M., Tigges, M., Sabatino, D., Luk, A., Jiang, H., Mingozzi, F., Couto, L., Ertl, H.C., High, K.A., Kay, M.A., 2006. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat. Med. 12 (3), 342–347.

Mingozzi, F., Maus, M.V., Hui, D.J., Sabatino, D.E., Murphy, S.L., Rasko, J.E., Ragni, M.V.,

Manno, C.S., Sommer, J., Jiang, H., Pierce, G.F., Ertl, H.C., High, K.A., 2007. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nat. Med. 13 (4), 419–422.

Nathwani, A.C., Tuddenham, E.G., Rangarajan, S., Rosales, C., McIntosh, J., Linch, D.C., Chowdary, P., Riddell, A., Pie, A.J., Harrington, C., O'Beirne, J., Smith, K., Pasi, J., Glader, B., Rustagi, P., Ng, C.Y., Kay, M.A., Zhou, J., Spence, Y., Morton, C.L., Allay, J., Coleman, J., Sleep, S., Cunningham, J.M., Srivastava, D., Basner-Tschakarjan, E., Mingozzi, F., High, K.A., Gray, J.T., Reiss, U.M., Nienhuis, A.W., Davidoff, A.M., 2011. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N. Engl. J. Med. 365 (25), 2357–2365.

Paz, H., Wong, C.A., Li, W., Santat, L., Wong, K.K., Chatterjee, S., 2007. Quiescent subpopulations of human CD34-positive hematopoietic stem cells are preferred targets for stable recombinant adeno-associated virus type 2 transduction. Hum. Gene Ther. 18 (7), 614–626.

Rutledge, E.A., Halbert, C.L., Russell, D.W., 1998. Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. J. Virol. 72 (1), 309–319.

Samulski, R.J., Berns, K.I., Tan, M., Muzyczka, N., 1982. Cloning of adeno-associated virus into pBR322: rescue of intact virus from the recombinant plasmid in human cells. Proc. Natl. Acad. Sci. U. S. A. 79 (6), 2077–2081.

Schmidt, M., Voutetakis, A., Afione, S., Zheng, C., Mandikian, D., Chiorini, J.A., 2008. Adeno-associated virus type 12 (AAV12): a novel AAV serotype with sialic acid- and heparan sulfate proteoglycan-independent transduction activity. J. Virol. 82 (3), 1399–1406.

Selot, R.S., Hareendran, S., Jayandharan, G.R., 2014. Developing immunologically inert adeno-associated virus (AAV) vectors for gene therapy: possibilities and limitations. Curr. Pharm. Biotechnol. 14 (12), 1072–1082.

Sen, D., Balakrishnan, B., Gabriel, N., Agrawal, P., Roshini, V., Samuel, R., Srivastava, A., Jayandharan, G.R., 2013. Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy. Sci. Rep. 3, 1832.

Smith, L.J., Ul-Hasan, T., Carvaines, S.K., Van Vliet, K., Yang, E., Wong Jr., K.K., Agbandje-McKenna, M., Chatterjee, S., 2014. Gene transfer properties and structural modeling of human stem cell-derived AAV. Mol. Ther. 22 (9), 1625–1634.

Song, L., Kauss, M.A., Kopin, E., Chandra, M., Ul-Hasan, T., Miller, E., Jayandharan, G.R., Rivers, A.E., Aslanidi, G.V., Ling, C., Li, B., Ma, W., Li, X., Andino, L.M., Zhong, L., Tarantal, A.F., Yoder, M.C., Wong Jr., K.K., Tan, M., Chatterjee, S., Srivastava, A., 2013. Optimizing the transduction efficiency of capsid-modified AAV6 serotype vectors in primary human hematopoietic stem cells in vitro and in a xenograft mouse model in vivo. Cytotherapy 15 (8), 986–998.

Vandenberghe, L.H., Wilson, J.M., Gao, G., 2009. Tailoring the AAV vector capsid for gene therapy. Gene Ther. 16 (3), 311–319.