Published in final edited form as:
Muscle Nerve. 2010 August ; 42(2): 268–270. doi:10.1002/mus.21700.

Sequencing protocols to genotype *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice

Glen B. Banks[1], **Ariana C. Combs**[1], and **Jeffrey S. Chamberlain**[*,1,2,3]

[1]Department of Neurology, University of Washington, Seattle, Washington 98195, USA

[2]Department of Medicine, University of Washington, Seattle, Washington 98195, USA

[3]Department of Biochemistry, University of Washington, Seattle, Washington 98195, USA

Abstract

Currently available PCR genotyping methods for point mutations in the mouse dystrophin gene can lead to false positives resulting in wasted time and money breeding or treating the wrong mice. Here we describe a simple and accurate method for sequencing the point mutations in *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice. This method clearly distinguishes between wild-type, heterozygous and mutant transcripts thereby saving time and money by avoiding false positives.

Introduction

Duchenne muscular dystrophy is caused by X-linked recessive mutations in the dystrophin gene. Our understanding of the pathogenesis of DMD and development of potential treatments has benefited enormously from the multiple *mdx* mouse models. Here we describe simple genotyping protocols for the most commonly used *mdx*, *mdx*^{4cv} and *mdx*^{5cv} strains. The original and naturally occurring mutation in *mdx* mice is a G to A transition that leads to a premature stop codon in exon 23 [1,2]. Four additonal *mdx* strains (2cv to 5cv) were generated with N-ethylnitrosourea chemical mutagenesis [3]. The *mdx*^{4cv} mouse has a C- to T- transition in exon 53, creating a nonsense ochre codon [4]. The *mdx*^{5cv} allele has an A to T transition in exon 10, creating a new splice donor site that generates a premature stop codon in RNA transcripts [4]. Each mutation leads to a loss of dystrophin protein expression in striated muscles. The mice vary in the number of revertant muscle fibers in which some muscle fibers express partially functional truncated dystrophins [5]. Both *mdx*^{4cv} and *mdx*^{5cv} have the fewest revertant fibers and each strain varies in which isoforms of dystrophin are expressed in non-muscle tissues [4,6]. Various methods have been published to genotype the different *mdx* mice ([7] and references within), but we and others find these methods difficult to apply on a routine basis as they can lead to false positives that waste large amounts of time and money breeding or treating the wrong mice. Here, we describe methods to genotype the *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice by sequence analyses to avoid false positives.

Materials and Methods

Mouse strains

The strains used in this study were *mdx* (C57BL/10ScSn-*mdx*/J), *mdx*^{4cv} (B6Ros.Cg-*Dmd*$^{mdx\text{-}4Cv}$/J), and *mdx*^{5cv} (B6Ros.Cg-*Dmd*$^{mdx\text{-}5Cv}$/J). The mice were originally obtained from the Jackson Labs (Bar Harbor, ME), but have been maintained *via* sibling matings at the University of Washington for more than 5 generations. All procedures were carried out in accordance with approved protocols by the Institutional Animal Care and Use Committee (IACUC) of the University of Washington.

*Corresponding Author: Professor Jeffrey S Chamberlain, Fax: 206 616 8272 Telephone: 206 616 6645, jsc5@uw.edu.

Preparation of DNA

Approximately 1 cm of tail was clipped using a clean razor and the mice appropriately marked. The tails were digested with proteinase K in a 56°C water bath overnight and the DNA was isolated and dissolved in 100 μl of Tris-EDTA (TE) buffer using the spin column protocol from the Qiagen DNEasy blood and tissue extraction kit (Valencia, CA), as per manufacturers instructions.

Polymerase chain reaction

We prefer to utilize a proofreading Taq in a pre-made mix from Invitrogen called Accuprime (Carlsbad, CA), although other PCR reagents may suffice. We added 30–150 ng of genomic DNA and 20 pM of each primer to the Accuprime mix. The primers were *mdx* F1: AACTCATCAAATATGCGTGTTAGT *mdx* R1:5′ CTCAATCTCTTCAAATTCTG. *mdx*^{4cv} F1 TCAAGAACAGCTGCAGAACAGGAGA. *mdx*^{4cv} R1 GGATTGCATCTACTGTGTGAGGACC. *mdx*^{5cv} F1 ATTTGGAAGCTCCCAGAGAC. *mdx*^{5cv} R1 TGCTTTAGCTTCAGAAGTCA. The PCR conditions for each of the *mdx* strains is 94°C for 5′, followed by 35 cycles of 94°C for 30″, 60°C for 30″ and 72°C for 30″ followed by an extension of 72°C for 3′.

Isolation and sequencing of the amplified DNA

We isolated the amplified DNA using the Qiaquick PCR purification kit (Qiagen, Valencia, CA) into 50 μl of filter-sterilized water. The DNA concentration was quantified using a spectrophotometer (NanoDrop 1000; Thermo Scientific; Miami, OK). 5 μl of the cleaned DNA was run on a 1.25% agarose gel to verify the correct PCR product was amplified (179 bp for *mdx*, 157 bp for *mdx*^{4cv} and 221 bp for *mdx*^{5cv}) and no product was found in the negative control that had 1 μl TE instead of DNA.

Approximately 100 ng of the PCR product was required for sequencing (Biochemistry sequencing facility, University of Washington, WA). The facility utilizes the Applied Biosystems 3730XL sequencer (Applied Biosystems, USA). We used the forward primers for sequencing *mdx* and *mdx*^{5cv} products and the reverse primer for sequencing the *mdx*^{4cv} products.

Results

The chromatogram clearly showed a single peak for wild-type, *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice, and two peaks at the appropriate nucleotide for heterozygous mice (example of *mdx* in Fig. 1). Only the chromatogram was used to analyze the sequence, because the nucleotide in the letter file for heterozygous mice usually reads as a wild-type or N (unable to decipher) (Fig. 1). As expected, heterozygous mice were only found in the DNA samples from female mice. We successfully retrieved the sequence data from 92% of samples (232 readable sequences from 253 mice) on the first attempt from *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice. All but one of the sequences showed the mouse genotype on the second attempt (20 readable sequences from 21 mice). The second attempt for sequencing the amplified DNA utilized the same PCR product as the first. The single failure resulted from problems extracting the genomic DNA from the tail sample. Thus, the genotype of only one mouse was not positively identified out of 253 samples, and we have not had a single false positive in over a year of subsequent analyses of the genotyped mice.

Discussion

In the present study we developed a sequencing method for genotyping the point mutations in *mdx*, *mdx*^{4cv} and *mdx*^{5cv} mice. We chose to sequence the PCR amplified DNA

encompassing the point mutations, which gave a high level of success. Previous methods developed by our lab and others for genotyping *mdx* mice using PCR alone are typically faster and cheaper than sequencing the DNA as described in this study ([7] and references within). However, despite years of methodological optimization we find that PCR alone can lead to false positives and difficulty in obtaining reproducible results, which can be a constant source of frustration when trying to breed various *mdx* mice to different strains or examining mice that have been treated with expensive reagents. The methods described here did not lead to any false positives thereby saving time and money for housing, breeding, treating and examining the mice.

Acknowledgments

We would like to thank the staff at the Biochemistry sequencing facility, University of Washington. GBB was supported by the CJ Martin postdoctoral fellowship and an MDA development grant. This work was also supported by NIH grants AR044533 and PO1N5046788 to JSC.

References

1. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse. Proc Natl Acad Sci U S A. 1984; 81:1189–1192. [PubMed: 6583703]
2. Sicinski P, Geng Y, Ryder-Cook AS, Barnard EA, Darlison MG, et al. The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. Science. 1989; 244:1578–1580. [PubMed: 2662404]
3. Chapman VM, Miller DR, Armstrong D, Caskey CT. Recovery of induced mutations for X chromosome-linked muscular dystrophy in mice. Proc Natl Acad Sci U S A. 1989; 86:1292–1296. [PubMed: 2919177]
4. Im WB, Phelps SF, Copen EH, Adams EG, Slightom JL, et al. Differential expression of dystrophin isoforms in strains of mdx mice with different mutations. Hum Mol Genet. 1996; 5:1149–1153. [PubMed: 8842734]
5. Danko I, Chapman V, Wolff JA. The frequency of revertants in mdx mouse genetic models for Duchenne muscular dystrophy. Pediatr Res. 1992; 32:128–131. [PubMed: 1635838]
6. Haenggi T, Fritschy JM. Role of dystrophin and utrophin for assembly and function of the dystrophin glycoprotein complex in non-muscle tissue. Cell Mol Life Sci. 2006; 63:1614–1631. [PubMed: 16710609]
7. Trebbin AL, Hoey AJ. A novel and simple method for genotyping the mdx mouse using high-resolution melt polymerase chain reaction. Muscle Nerve. 2009; 39:603–608. [PubMed: 19347923]

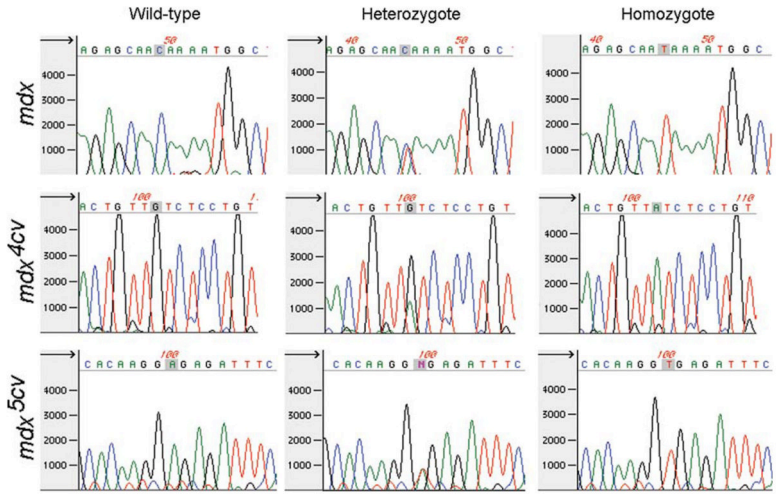

Figure 1.

Representative chromatograms from wildtype, heterozygous, and homozygous mice for the point mutation in *mdx*, *mdx*^{4cv}, and *mdx*^{5cv} mice (shaded). Note that the heterozygous mouse samples have two peaks, but the nucleotide sequence reads as a wildtype nucleotide or "N," necessitating manual analysis of chromatograms for genotyping these mice.