A New Genetic Variant of MDX Mice: Study of the Phenotype

L. I. Krivov*, M. A. Stenina*, V. N. Yarygin*, A. V. Polyakov*,, V. I. Savchuk*, S. A. Obrubov*, and N. V. Komarova*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 147, No. 5, pp. 557-561, May, 2009
Original article submitted September 29, 2008

Genetic selection in a colony of mdx mice (suffering from X-chromosome-linked muscular dystrophy) resulted in generation of their new genetic variant. In this new variant, the genetic, biochemical, and histological markers of muscular dystrophy are combined with signs of oculocutaneous albinism (skin and fur depigmentation), transillumination of the iris, sharply reduced pigmentation of the retinal epithelium, and increase of the eyeball refraction). Two sensorimotor tests (negative geotaxis and wire back down hanging) detected other phenotypical characteristics of albino mdx mice carrying, in addition to the mutation in the dystrophin gene exon 23 (intrinsic of the "classical" black mdx mice), an extra mutation responsible for pigmentation disorders. Slow geotaxis, despite longer wire back down hanging capacity, was regarded as aggravation of the neurological dysfunction in albino mdx mice in comparison with black mdx mice.

Key Words: *mdx mice; genetics; behavioral phenotype; Duchenne's progressive muscular dystrophy*

The first publications about mdx mice suffering from X-chromosome-linked muscular dystrophy appeared in 1984 [2]. These mice appeared spontaneously in C57Bl/10Sn colony; they carry a point mutation in exon 23 of dystrophin gene located on X chromosome [14] and do not express full-length isoform (Dp427) of this protein in the muscle tissue and brain [5]. Muscular dystrophy in mdx mice is regarded as a homologue of Duchenne progressive muscular dystrophy (DMD) in humans. This incurable disease of the human muscular system is caused by a mutation in the dystrophin gene. It manifests in childhood by cardiac (cardiomyopathies, arrhythmias) and extramuscular (mental retardation) disorders. The similarity of mdx mouse disease and DMD is seen from histological signs of involvement of the skeletal and diaphragmatic muscles progressing with age [3], heart involvement [1], and such characteristics of the clinical phenotype as cognitive dysfunction [15] and dilatation cardiomyopathy developing by old age [11].

Genetic variants of mdx mice were described: mdx2cv, mdx3cv, mdx4cv, mdx5cv mice with mutations located in other exons of the dystrophin gene [4,7], and mdx mice with a combination of mutation in the dystrophin gene and mutations in other genes, *e.g.* mdx mice with additional genetic defects in the utrophin (protein homologous to dystrophin) gene [6] or in α_7-integrin gene [13]. One more genetic variant are transgenic mdx mice with hyperexpression of NO synthase, calpastatin, calcineurin, α_7-integrin, *etc.* in muscles. Phenotypical differences in the severity of clinical course of muscular dystrophy and lifespan of genetic variants of mdx mice were demonstrated in experimental studies.

*Russian State Medical University; **Center of Molecular Genetics; ***Medical Genetic Center, the Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** rsmu@rsmu.ru. L. I. Krivov

0007-4888/09/1475-0625

The problem of specific features of the clinical phenotype in relation to the genotype remains insufficiently studied. For mdx mice it is closely linked with the problem of the development of new technologies for DMD treatment: gene therapy and cell therapy. The authors of these technologies using mdx mice as a model of hereditary myodystrophy focus their attention on the efficiency of skeletal muscle reparation and neglect cardiac and other manifestations of DMD; in other words, mdx mice with a "preset" phenotype are needed, in which the involvement of the skeletal muscles is combined with pronounced neurological or cardiological disorders.

A special reproductive colony of mdx mice is maintained at Russian State Medical University for more than 10 years. A variant of mice with oculocutaneous albinism is distinguished in this colony. These albino mice represent a subcolony in the colony of black mdx mice.

The synthesis of tyrosinase (the key enzyme of melanin pigment synthesis) in these mice is encoded by c-albino locus on chromosome 7 [9]. Studies of the interactions of albinism gene with nonallele genes demonstrated the impact of the mutation in this gene for behavioral characteristics of mice [8]. We failed to find reports about the phenotype of mdx mice with mutation in the dystrophin gene combined with albinism.

The aim of this study was to describe the clinical phenotype of albino mdx mice with X-chromosome-linked muscular dystrophy in comparison with black mdx mice.

MATERIALS AND METHODS

The study was carried out on 392 mice (256 males and 136 females): 186 animals with mutation in the dystrophin gene and 206 carriers of normal dystrophin gene (control).

The mutation in dystrophin gene exon 23 was detected by PCR with DNA isolated from the tip of mouse tail. Analysis of DNA was carried out at Center of Molecular Genetics.

Creatine phosphokinase (CPC) concentration in the blood was measured on a Reflotron IY biochemical express analyzer (F. Hoffmann La Roche Ltd.). Whole blood for the analysis was collected from the caudal vein of conscious mice.

The degenerative process in the skeletal muscles was evaluated by examining cryostat sections (8 μ) of skeletal muscles supravitally stained with cresyl fast violet. The sizes of fibers were evaluated and the fibers differing sharply by size and with central nuclei were detected.

In the negative geotaxis test, the mice were placed on a metal wire netting (8×8 mm cells) and it was positioned vertically so that the mouse was hanging at the net with its head down. In our modification of the standard test [12] we recorded the time needed for 180° rotation of the animal to have its head up (negative geotaxis) during the 1st, 2nd, and 3rd attempts.

In the other test, the wire netting with the mouse was turned so that the animal was hanging with its back down. The time during which the animal could hang on the wire netting was recorded, irrespective of its behavior on the net (active movements or just clutching with all four paws).

RESULTS

The core of our colony of mdx mice was a small branch of 2-3-month-old black mice bred at Laboratory of Experimental Biological Models of the Russian Academy of Medical Sciences (Head of Department of Genetics: A. M. Malashenko). Five genotypes of animals were distinguished by DNA diagnosis of mutations in the dystrophin gene exon 23: *a*) homozygous females with dystrophin gene mutations in both X chromosomes (mdx/mdx): *b*) heterozygous female carriers of the mutant gene in one X chromosome (mdx/x); *c*) mutant hemizygous males carrying this gene in the only X chromosome (mdx/y); *d*) females with normal dystrophin gene (x/x); and *e*) males with normal dystrophin gene (x/y).

In accordance with the protocol for reproduction of mdx mice used by Jackson Laboratory (the leading world center for studies and breeding of mice for research purposes), we used homozygous females and mutant males for reproduction. Mice with phenotypical manifestations of oculocutaneous albinism appeared in the very first litters. These animals presented with skin and fur depigmentation, transillumination (transparency) of the iris, sharply reduced pigmentation of the retinal epithelium, and high refraction of the eyeball. Several albino animals eliminated from the reproductive colony of black mdx mice formed the core of the reproductive subcolony of albino mice. Mutation in the dystrophin gene exon 23 was detected in these animals by DNA diagnosis (in other words, their appurtenance to mdx mice was confirmed).

Mice with normal dystrophin gene were bred specially; the absence of mutation in the dystrophin gene exon 23 was verified by DNA diagnosis. We think that male and female mice with normal dystrophin gene from our colony having a genetic background similar to that of mdx mice are preferable as controls for mutant mdx animals in studies of

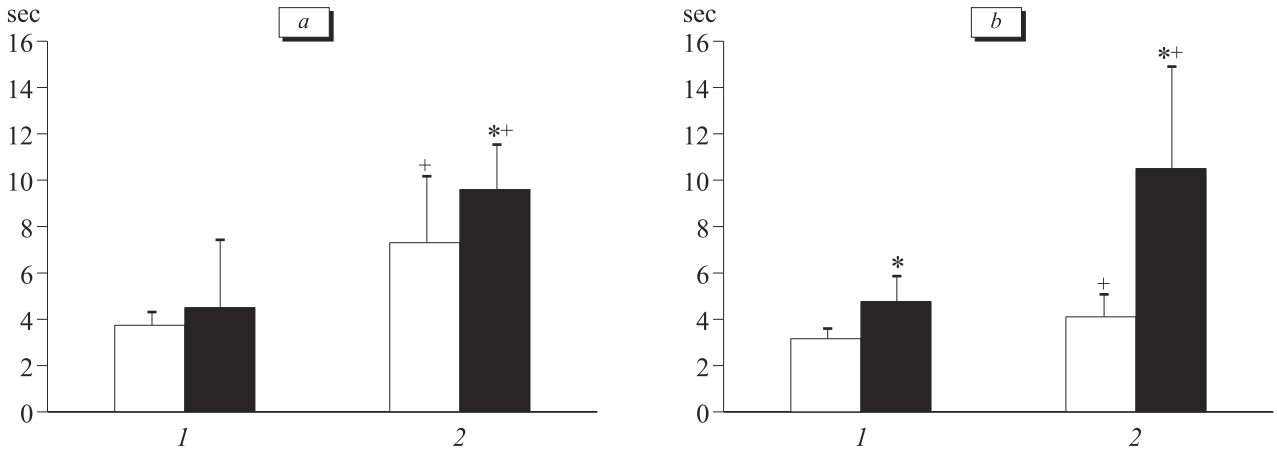

Fig. 1. Activities of black and albino mice with normal (*1*) and mutant (*2*) genotypes in the negative geotaxis test. Ordinate: mean duration of turning. *a*) males: *b*) females. Light bars: black mice; dark bars: albino mice. $p<0.05$ compared to: *black mice of the same gender and genotype; +normal animals of the same gender and color.

mdx mouse phenotype in comparison with inbred C57Bl/10Sn strain.

Increased serum CPC activity is attributed to increased membrane permeability in dying myofibril; high CPC values are regarded as a laboratory marker of progressive muscular dystrophy in DMD patients and mdx mice. CPC activity in adult control albino male and female mice and in black mdx controls varied from 50 to 500 U. Similarly as in black mdx mice, high CPC activity in albino animals was a phenotypical manifestation of mutation in the dystrophin gene. In mutant males, this parameter varied from 3000 to 15,000 U, in mutant homozygous females from 5000 to 20,000 U.

The histology of the skeletal muscles of mutant albino mdx males corresponded to the picture of the degenerative process described for black mutants [1]. Our method for supravital staining of cryostat sections revealed variations of muscle fibers by size with fibers of large diameter or fibers with centrally located nuclei.

Body weight served as the indicator of physical development of mice. Analysis of body weight was carried out in 8 samples of adult animals from our colony. The samples were represented by black and albino mutant or normal by dystrophin gene males and homozygous females.

Statistically significant differences in body weights were detected for black and albino males without mutation in the dystrophin gene. The number of large mice weighing more than 35 g was higher in the population of albino males than among black mice (Table 1), the mean body weight of the respective females being significantly higher in comparison with black females.

Differences in body weights of albino and black variants of mdx mutants were statistically negligible. The absence of significant differences can be explained by accumulation of large animals in albino and pigmented populations with mutation in the dystrophin gene. Body weights of mutant mice of any age can be higher than the weights of ani-

TABLE 1. Body Weights of Adult Black and Albino Mice of Different Genotypes

Genotype/phenotype			Percentage of large animals	Mean weight, g
Males	mdx/y	black (*n*=64)	30	38.1±0.9
		albino (*n*=66)	40	38.3±1.2
	x/y	black (*n*=65)	3	33.9±0.6*
		albino (*n*=61)	2	34.6±0.6*
Females	mdx/mdx	black (*n*=20)	45	34.5±1.4
		albino (*n*=36)	50	34.6±1.0
	x/x	black (*n*=37)	14	32.6±0.7*
		albino (*n*=43)	40	34.1±0.7*+

Note. Males weighing more than 40 g and females heavier than 35 g were considered large. $p<0.05$ compared to: *mutants of the same gender and color, +normal black females.

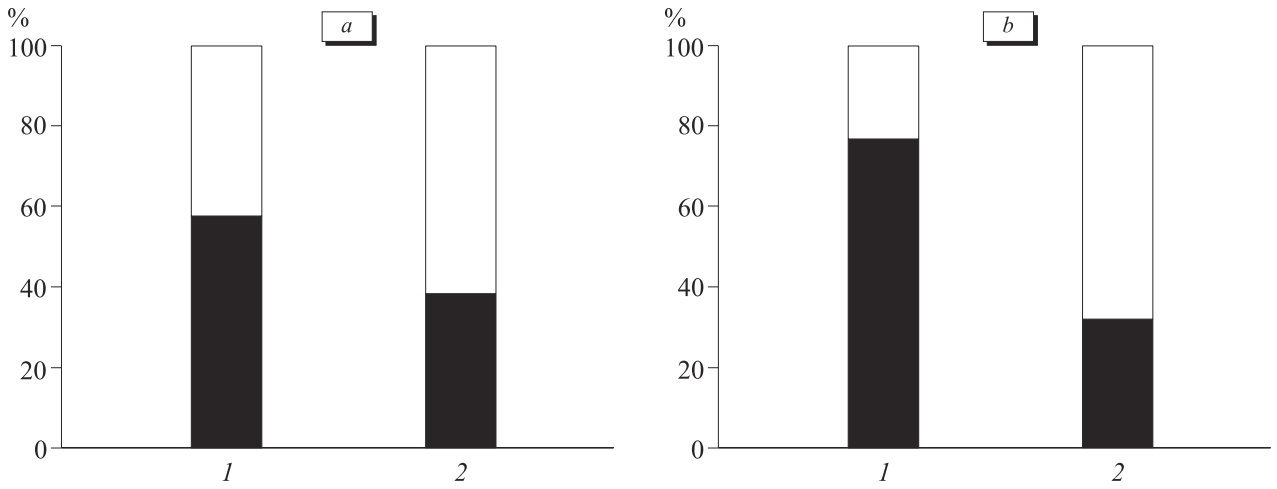

Fig. 2. Proportion of "slow" (light section of the diagram) and "rapid" (dark section) turns in mutant black (*1*) and albino (*2*) males (*a*) and females (*b*).

mals of parental strain (C57Bl/10Sn), which was previously demonstrated for 6-7-month-old mutant and normal black females [10].

Mice with body weights at which there was no clear-cut relationship between the results of tests and animal body weight (30-35 g for females and 35-40 g for males) were studied in the above tests.

The overwhelming majority of mice (normal and with mutant dystrophin gene) finding themselves hanging at a vertical wire netting with their heads down changed their position to have their heads up (realized negative geotaxis). Just few animals from the group of mutants started to move down (positive geotaxis).

Mice with myodystrophy changed their position slower than normal mice with intact skeletal muscles irrespective of color and gender (Fig. 1).

Comparison of black and albino mdx mice by the mean parameters demonstrated their clear-cut phenotypical differences (Fig. 1). Albino mutants turned significantly slower than black ones.

The relationship between the rate of turning and the pattern of animal movements is worthy to note. Controls rapidly turned by 180° around their axis. Mice with slow geotaxis moved along a greater or lesser arch making stops during which they turned their heads looking around. We classified the turns made during more than 5 sec as "slow" and found that the percent of "slow" turns was higher in albino mdx mice in comparison with black ones for the total number of attempts made by mice of this genotype (Fig. 2).

Albino mdx mice hanged longer on the wire with their backs down than black ones (Fig. 3).

Hence, genetic selection in the colony of mdx mice resulted in derivation of a new variant of these animas. Combination of mutations in these animals

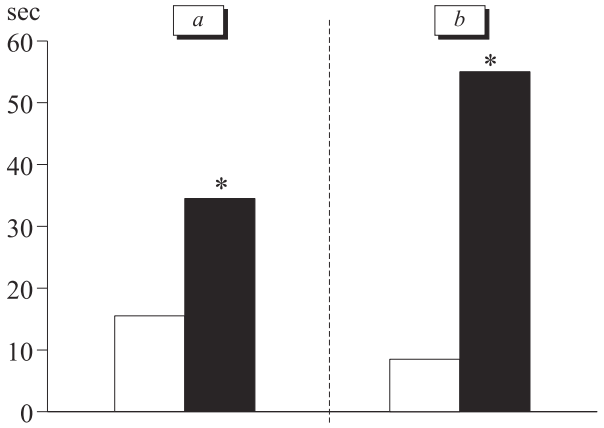

Fig. 3. Activity of black (light bars) and albino (dark bars) males (*a*) and females (*b*) during clutching at the net with one's back down. Ordinate: mean duration of clutching. *$p<0.01$ compared to black mice.

(in the dystrophin gene and locus) led to serious phenotypical changes manifesting in a more severe neurological dysfunction in comparison with black mdx mice (slow geotaxis in combination with longer clutching at the net with one's back down). Further detailed analysis of the clinical phenotype with emphasis on the neurological and cardiovascular status of albino mdx mice is the task of our next study.

REFERENCES

1. L. R. Bridges, *J. Neurol. Sci.*, **72**, Nos. 2-3, 147-157 (1986).
2. G. Bulfield, W. G. Siller, P. A. Wight, and K. J. Moore, *Proc. Natl. Acad. Sci. USA*, **81**, No. 4, 1189-1191 (1984).
3. J. S. Chamberlain, J. Metzger, M. Reyes, *et al.*, *FASEB J.*, **21**, No. 9, 2195-2204 (2007).
4. V. M. Chapman, D. R. Miller, D. Armstrong, and C. T. Caskey, *Proc. Natl. Acad. Sci. USA*, **86**, No. 4, 1292-1296 (1989).
5. K. Culligan, L. Glover, P. Dowling, and K. Ohlendieck, *BMC Cell Biol.*, **2**, No. 2, 2 (2001).

6. R. M. Grady, H. Teng, M. C. Nichol, *et al.*, *Cell*, **90**, No. 4, 729-738 (1997).
7. W. B. Im, S. F. Phelps, E. H. Copen, *et al.*, *Hum. Mol. Genet.*, **5**, No. 8, 1149-1153 (1996).
8. R. J. Katz and R. L. Doyle, *Behav. Genet.*, **11**, No. 2, 167-172 (1981).
9. B. S. Kwon, A. K. Haq, S. H. Pomerantz, and R. Halaban, *Proc. Natl. Acad. Sci. USA*, **84**, No. 21, 7473-7477 (1987).
10. C. Olichon-Berthe, N. Gautier, E. Van Obberghen, and Y. Le Marchand-Brustel, *Biochem. J.*, **291**, Pt. 1, 257-261 (1993).
11. J. G. Quinlan, H. S. Hahn, B. L. Wong, *et al.*, *Neuromuscul. Disord.*, **14**, Nos. 8-9, 491-496 (2004).
12. J. A. Rafael, Y. Nitta, J. Peters, and K. E. Davies, *Mamm. Genome*, **11**, No. 9, 725-728 (2000).
13. J. E. Rooney, J. V. Welser, M. A. Dechert, *et al.*, *J. Cell. Sci.*, **119**, Pt. 11, 2185-2195 (2006).
14. P. Sicinski, Y. Geng, A. S. Ryder-Cook, *et al.*, *Science*, **244**, No. 4912, 1578-1580 (1989).
15. C. Vaillend, A. Rendon, R. Misslin, and A. Ungerer, *Behav. Genet.*, **25**, No. 6, 569-579 (1995).