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Short Communication

Homozygous mice with mutant protein FUS[1-359] overexpression: Innovative possibilities for ALS treatment

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Abstract

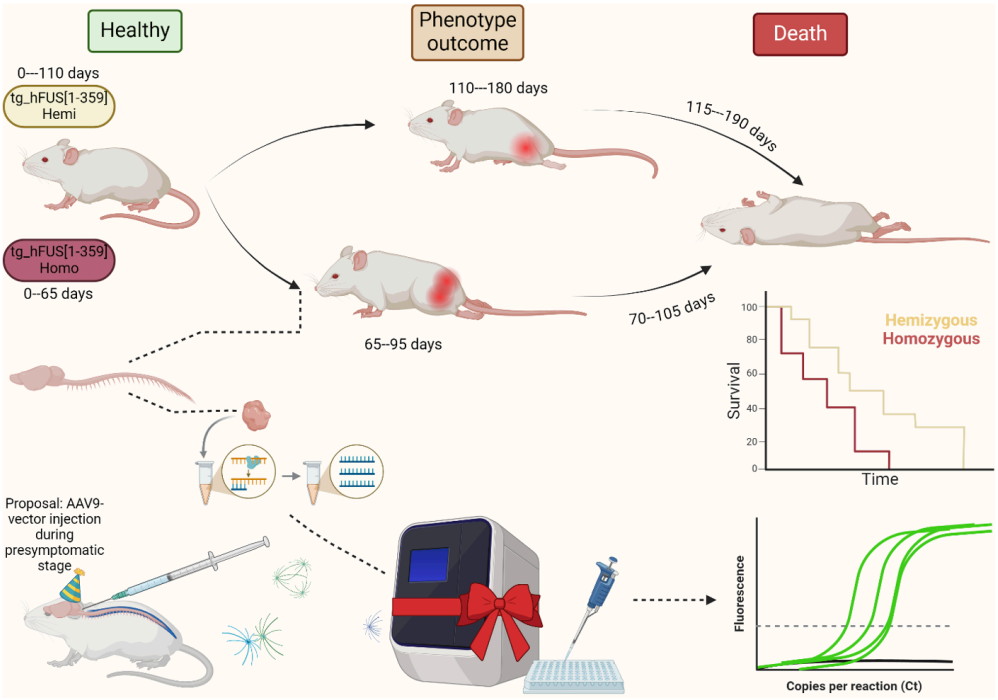
Introduction: This study investigates a mouse model with overexpression of the mutant FUS[1-359] protein, which can be used to evaluate the effectiveness of gene therapy and other pharmacological interventions for amyotrophic lateral sclerosis (ALS). The model enhances the deeper understanding of the mechanisms underlying disease progression and allows for testing new therapeutic strategies.

Materials and Methods: The study utilized tg_hFUS[1-359] animal lines with a transgenic cassette expressing the human mutant FUS[1-359] protein. Animal groups were formed by crossing hemizygous individuals, and analyses were conducted on lifespan, age of disease manifestation, as well as relative copy number and expression levels of the transgenic cassette.

Results and Discussion: The results demonstrated statistically significant differences in the age of onset of initial disease symptoms between homozygous and hemizygous mice. Differences in the copy number of the transgenic insertion were also identified, revealing that homozygous animals exhibited increased expression of the mutant FUS protein in various structures of the central nervous system, consistent with existing literature on ALS pathogenesis.

Conclusion: Mice with hyperexpression of the mutant FUS[1-359] protein represent a promising genetic model for evaluating therapeutic approaches to ALS treatment. This model exhibits clear phenotypic manifestations of the disease and can be utilized for investigating gene therapy methods.

Graphical abstract



Keywords

amyotrophic lateral sclerosis, fused in sarcoma, gene expression, gene therapy

Introduction

Developments and new approaches in the study and application of gene therapeutic drugs are increasingly resonating within the scientific and medical communities (Yazdani et al. 2018; Grandchamp 2020; Polikarpova et al. 2022; Korokin et al. 2023), especially with the emergence of publications on approaches to treating neurodegenerative diseases (Alzheimer's disease, Parkinson's disease), as well as orphan diseases (Duchenne muscular dystrophy, Miyoshi myopathy). Consequently, there is a rising necessity for more extensive phenotyping and validation of genetic models of experimental animals in preclinical studies assessing the efficacy of gene-engineered therapeutics.

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of motor neurons, leading to muscle atrophy and paralysis. One of the key molecular targets associated with this disease is the FUS gene, mutations which result in protein aggregation and disruption of cellular functions (Funikov et al. 2018). The hyperexpression of the mutant FUS[1-359] protein may serve as an important marker for studying the pathogenesis of ALS (Rezvykh et al. 2024).

This study presents a description of a homozygous mouse model with hyperexpression of the mutant human FUS[1-359] protein, which can be utilized in preclinical research to evaluate the efficacy of gene therapy and other pharmacological interventions. The application of this model provides an opportunity to investigate disease mechanisms and test new therapeutic strategies aimed at slowing the progression of ALS (Probert et al. 2022, de Munter et al. 2020).

The aim of the study is to describe the homozygous state of the mouse model with hyperexpression of the mutant FUS[1-359] protein and to assess the prospects for testing gene therapy and other pharmacological interventions in amyotrophic lateral sclerosis (ALS).

Materials and Methods

Animals

The study investigated a line of FUS[1-359] mice with a transgenic cassette integrated into chromosome 11, consisting of cDNA from the mutant variant of the human FUS gene, expressing a truncated FUS protein with a high level of cytoplasm aggregation (Shelkovnikova et al. 2013). Groups of animals were obtained by crossing mutant hemizygous animals with synchronized cohorts of hemizygous male (Hemi M, n=12), hemizygous female (Hemi F, n=12), homozygous male (Homo M, n=12), and homozygous female (Homo F, n=12) mice. The study was conducted on mice aged 70 days – this is the threshold age for homozygous animals, at which paralysis begins to develop within 1-6 days.

Analysis of lifespan and disease onset

Data on lifespan and the age of initial phenotypic disease manifestations in homozygous and hemizygous animals were collected over five generations of mice within one year. The total number of animals included in the analysis was: Hemi M – 62, Homo M – 37, Hemi F – 61,and Homo F – 46.

Analysis of transgene cassette copy number

Real-time PCR analysis was conducted using the qPCRmix-HS SYBR kit (Evrogen, Russia) and involved comparing the target FUS[1-359] fragment with four housekeeping genes of the known copy number in the mouse genome. Each sample was analyzed in triplicate, and average values were used in calculations. The purified DNA content in the reaction mix was 40 ng.

Analysis of transgene cassette expression in CNS structures

Brain and spinal cord tissues were isolated on ice immediately after decapitation of animals under injectable anesthesia. All tissues were placed in tubes containing lysis buffer from the RNeasy Mini Kit (250) (Qiagen, Germany) and homogenized ultrasonically on ice. RNA extraction followed the kit instructions. Purified RNA solutions were analyzed spectrophotometrically using the NanoDrop OneC instrument (Thermo Scientific, USA) and diluted to an equal concentration of 20 ng/μL.

Reverse transcription was performed using the MMLV RT reagent kit (Evrogen, Russia) following the manufacturer's instructions. The synthesized cDNA was used as a template in real-time PCR reactions with the 5X qPCRmix-HS SYBR reagent kit (Evrogen, Russia). Specific primers targeting the truncated human FUS cDNA were used to determine transgene cassette expression levels, along with primers specific to the reference mouse Gapdh gene. Relative normalized quantification of transgene cassette expression was conducted using the $2^{-\Delta\Delta CT}$ method, where $\Delta\Delta Ct = Ct(sample) - \Delta Ct(standard)$.

Statistical analysis

Statistical processing was performed using GraphPad Prism Software 8.0 (GraphPad Software Inc., USA). Normality of data distribution was assessed using the Shapiro–Wilk test. Two-way ANOVA was applied to evaluate differences between multiple sample groups. Post hoc analysis employed unpaired Student's t-test or the Mann–Whitney test. Statistical differences in survival distributions were assessed using the log-rank Mantel–Cox test. Results were considered significant at $p \leq 0.05$.

Results and Discussion

Survival curve results

After the observation period, statistically significant differences in median age of disease onset were identified between groups: Hemi M – 133.5 days and Homo M – 86.5 days; Hemi F – 134.5 days, and Homo F – 85 days (Figure 1).

The median age at death for the animals was as follows: Hemi M – 136.5 days and Homo M – 89.5 days; Hemi F – 132 days and Homo F – 90.5 days (Figure 2).

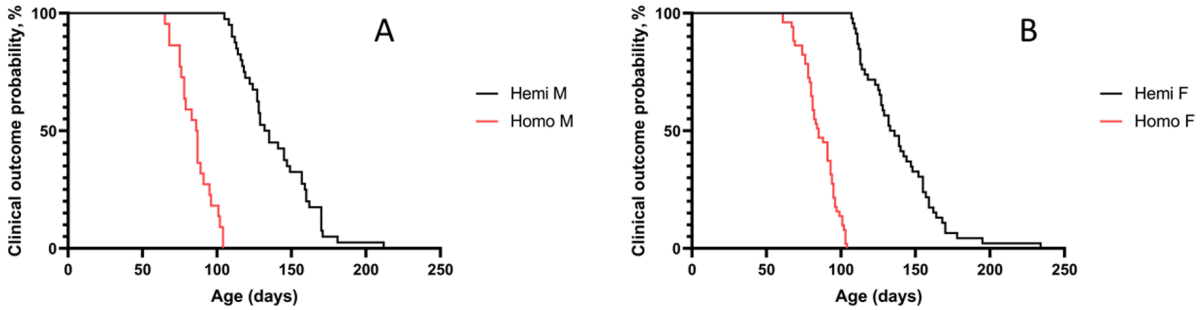


Figure 1. Kaplan-Meier plot of the probability of developing pathological condition with age in animals from the Hemi M and Homo M groups (**A**), and Hemi F and Homo F groups (**B**).

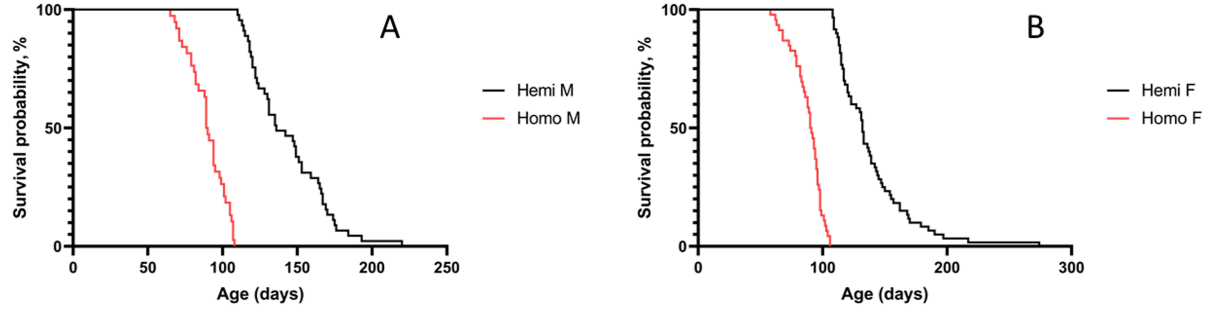


Figure 2. Kaplan-Meier plot of the probability of death with age in animals from the Hemi M and Homo M groups (**A**), and Hemi F and Homo F groups (**B**).

The progression of loss of locomotor function in the animals, from the onset of the disease due to denervation of muscle fibers to death, is 5-7 days for homozygous animals and 6-9 days for hemizygous animals. Additionally, observations revealed that in 87% of animals of both sexes and genotypes, paresis began in the hind limbs (59% on the left, 41% on the right), while in 13%, the innervation of the forelimbs was primarily affected, leading to an inability to use them for movement.

Results of relative copy number determination of the transgene cassette

The calculation of the relative copy number of the transgene cassette was performed using a logarithmic regression model according to the formula:

$$F = b \times Ln(x) + a \quad (1)$$

where y — response variable (Ct); x — predictor variable (copy number of the fragment); a, b — regression coefficients.

As can be seen from the data (Figure 3), the comparison of the analysis results from male animals of the presented genotypes shows a twofold difference in the number of transgene cassette copies. This corresponds to the results of animal genotyping using semi-quantitative real-time PCR, as well as to the phenotypic manifestation of the disease.

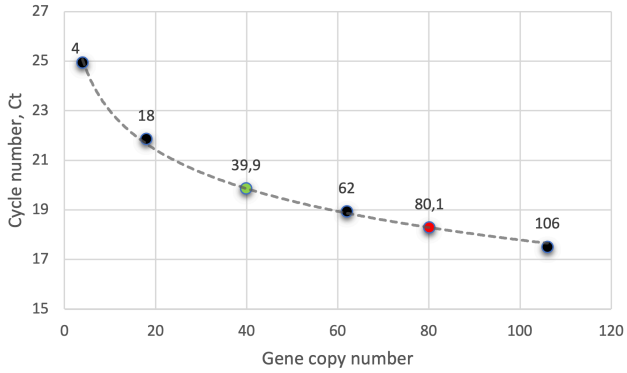


Figure 3. Logarithmic regression plot for counting the copy number of the FUS[1-359] transgene cassette; the value 39.9 corresponds to hemizygosity, and 80.1 corresponds to homozygosity; y = -2.26Ln(x) + 28.197, $R^2 = 0.9979$.

Results of transgene cassette expression level determination

The analysis of transgene cassette FUS[1-359] expression revealed statistically significant differences in the relative expression level, normalized to the expression of FUS[1-359] in the kidney (standard sample with equal transgene cassette expression across different animals within the same group). Specifically, homozygous animals of both sexes exhibited increased production of the transcript corresponding to the cDNA of the mutant human FUS protein in the sensorimotor cortex, striatum, cerebellum, brainstem, and spinal cord. These findings are consistent with literature data on the formation of cytoplasmic FUS protein aggregates in the spinal and brain regions (Shelkovnikova et al. 2013) (Figure 4).

Notably, in the hemizygous animal groups of both sexes, individual specimens exhibit delayed onset of primary disease symptoms (paresis of distal limbs), surpassing the 180-day threshold. This phenomenon was not observed in prior studies (Shelkovnikova et al. 2013; Crivello et al. 2019; Probert et al. 2022). However, no statistically significant differences in transgene cassette copy number or expression levels were identified between these individuals and other hemizygous animals (data not shown).

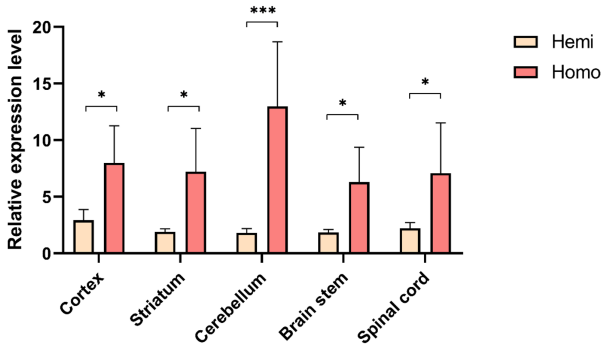


Figure 4. Relative normalized expression levels of the FUS[1-359] transgene cassette in brain structures and spinal cord of homozygous and hemizygous mice, normalized to the standard sample (* — p≤0.05; *** — p≤0.001).

Conclusion

The study highlights the significance of mice with overexpression of the mutant FUS[1-359] protein as an effective genetic model for evaluating therapeutic approaches to amyotrophic lateral sclerosis. These animals demonstrate clear phenotypic manifestations of the disease, making them a rapid and convenient model for pharmacological assessment of drug therapy efficacy, as well as gene therapy. Future research should focus on uncovering the molecular mechanisms underlying the disease and optimizing therapeutic strategies using this model. This will not only deepen our understanding of ALS, but also contribute to the development of more targeted and effective treatments for patients.

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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