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Mouse model of chorea-acanthocytosis exhibits male infertility caused by impaired sperm motility as a result of ultrastructural morphological abnormalities in the mitochondrial sheath in the sperm midpiece

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ABSTRACT

Chorea-acanthocytosis (ChAc) is an autosomal recessive hereditary disease characterized by neurodegeneration in the striatum and acanthocytosis caused by loss-of-function mutations in the Vacuolar Protein Sorting 13 Homolog A (*VPS13*A) gene, which encodes chorein. We previously produced a ChAc-model mouse with a homozygous deletion of exons 60–61 in *Vps13a*, which corresponded to the human disease mutation. We found that male ChAc-model mice exhibited complete infertility as a result of severely diminished sperm motility. Immunocytochemical study revealed that chorein-like immunoreactivity is abundant only in the midpiece, mitochondria-rich region, of the sperm of wild type mice. They showed no significant differences from wild types in terms of the adenosine 5′-triphosphate (ATP) concentration of their sperm, sperm count, or sexual activity. Electron microscopy revealed abnormal ultrastructural morphology of the mitochondria in the midpiece of sperm from ChAc-model mice. These results suggest that chorein is essential in mouse sperm for the maintenance of ultrastructural mitochondrial morphology and sperm motility.

1. Introduction

In humans, loss-of-function mutations in the Vacuolar Protein Sorting 13 Homolog A (*VPS13*A) gene, which encodes a large protein named chorein, causes chorea-acanthocytosis (ChAc; MIM, 200150) [1–4], an autosomal recessive neurodegenerative disorder. Clinically, ChAc is characterized by adult-onset chorea, acanthocytosis in erythrocytes, and Huntington's disease-like neuropsychiatric symptoms. The main neuropathological feature of ChAc is the neurodegeneration of the striatum.

We produced a ChAc-model mouse with a hybrid genetic background (129/sv and C57BL/6J) by deleting exons 60–61 of *Vps13a*, which mimics the human disease mutation [5]. ChAc-model mice that were backcrossed with different inbred strains exhibited differences in symptoms [6]. While conducting these experiments, we found male infertility in ChAc-model mice carrying homozygous exon 60–61 deletions in *Vps13a* [7]. While we experienced human male ChAc patients with normal reproductive capacity, there is one case report of male infertility in a patient with neuroacanthocytosis [8]. Recently, Kevin Peikert et al. reported two male ChAc patients with infertility due to asthenoteratozoospermia and oligoasthenozoospermia, respectively in the ninth International Meeting on Neuroacanthocytosis Syndromes. The abstract of this study in the meeting is in press in Tremor and Other Hyperkinetic Movements.

In the present study, we confirmed male infertility in ChAc-model mice and performed assessment tests for sexual activity and sperm condition, together with an electron-microscopy analysis of sperm morphology, to identify the causes of male infertility in ChAc-model mice.

Abbreviations: ChAc, chorea-acanthocytosis; VPS13A, Vacuolar Protein Sorting 13 Homolog A; PBS, phosphate-buffered saline; *ATP, Adenosine 5′-triphosphate.*

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2. Materials and methods

2.1. Animals

ChAc-model mice (*Vps13a*^{tm1asan} mice) with a homozygous deletion of exons 60–61 in *Vps13a*, corresponding to a human disease mutation, were produced by gene targeting as previously described [5,9]. We primarily used an inbred strain 129S6/SvEv (wild type) (Taconic Labs, Hudson, NY, USA), and strain 129S6/SvEv-*Vps13a*^{tm1asan} (ChAc$^{Del/Del}$), which we produced previously [6]. This study was carried out in accordance with the guidelines for Animal Experimentation (MD14017) and Gene Recombination Experiments (24054) of the Graduate School of Medical and Dental Sciences, Kagoshima University, Japan. Mice were group-housed with normal light–dark cycles (lights on at 7:00 a.m., lights off at 7:00 p.m.) in a clean facility, and were given free access to food and water.

2.2. Infertility test

Each of four male ChAc$^{Del/Del}$ mice were mated with female wild-types for 19.5 ± 5.2 (15–24) weeks. Each of five female ChAc$^{Del/Del}$ mice was also mated with male wild-types for 5.4 ± 2.9 (3–9) weeks.

2.3. Odor-sniffing test

The odor sniffing test was performed as described by Kobayakawa et al. [10] with minor modifications. Mice (n = 8 wild-type; n = 7 ChAc$^{Del/Del}$) were habituated to the cage, after which a cotton swab soaked with distilled water was presented for 3 min. This was repeated three times at 1-min intervals. Then a cotton swab soaked with the indicated amount of the test odorants (first female urine and then male urine) was presented in the same way. Mouse behavior was recorded with a digital video camera (1440 × 1080 pixels) for analysis. We defined 'an investigation' as nasal contact within 1 mm of the cotton swab, and we measured the duration of each investigation.

2.4. Male sexual behavior test

The male sexual behavior test was performed as described by Haga et al. [11], with minor modifications. Each of the male mice (n = 6 wild-type; n = 6 ChAc$^{Del/Del}$) was individually housed in a test cage 24 h before the test. After 24 h, the mice were paired with a sexually naive female wild-type mouse and their behavior was video-recorded (1440 × 1080 pixels) for 1 h during the dark phase under a 50-lux light. The total number of male mounting behaviors was scored.

2.5. Sperm collection, count, and motility

Epididymides from wild-type and ChAc$^{Del/Del}$ mice were dissected, and then the cauda regions were separated. Immediately, multiple incisions were made in the tissue, and sperm from each tissue were gently scraped out or allowed to swim into 1 ml of warmed saline. The sperm solution was loaded into the hemocytometer chamber. After assessing samples for 10–15 min, at least 200 sperm were counted per replicate (n = 3 wild-type; n = 3 ChAc$^{Del/Del}$). Then the concentration of sperm per region was calculated. Microscopic observation video recordings were made of three randomly selected regions (n = 10 wild-type; n = 10 ChAc$^{Del/Del}$). Under light-microscope observation, the number of sperm displaying abnormal morphology, classified according to World Health Organization (WHO) guidelines [12], was also blindly calculated. The motility of all sperm within a defined area of the field was assessed according to WHO categories of sperm movement [12]. First, the grid section being scored for progressive motility (PR) cells was scanned. Next, non-progressive motility (NP) sperm, and finally immotile (IM) sperm, were counted in the same grid section. The total number of sperm that were classified as either NP or IM was calculated as the "motility-failure rate".

2.6. Immunocytochemical study

To prepare the discontinuous Percoll (Sigma) density gradient, Percoll solution was diluted with phosphate-buffered saline (PBS) to make 20–80% Percoll solutions at graduated intervals of 20%. The epididymides from wild-type mice were dissected, then the cauda regions were separated. Immediately, multiple incisions were made in the tissue, which was placed into 0.3 ml of PBS and incubated for 1 h. After removing the epididymis, the 0.3-ml sperm suspension was placed at the top of the Percoll density-gradient column and centrifuged at 400 × g for 20 min. The bottom fraction was washed twice with 1 ml PBS. The supernatant was removed and 375 μl of 400 nM MitoTracker Green FM (Invitrogen) with PBS was added. Sperm stained with MitoTracker Green FM were washed three times with PBS. Subsequently, sperm were fixed for 15 min in 0.1 M phosphate buffer containing 4% (w/v) paraformaldehyde. The fixed sperm were washed three times with PBS–Glycine (0.01 M glycine in PBS, pH 7.4), then permeabilized with PBS containing 0.1% (w/v) Triton X-100 for 5 min. Sperm were then washed three times with PBS–Glycine and blocked for 1 h at room temperature with 10% (w/v) non-fat dried milk in PBS containing 0.1% (w/v) tween 20 and 6% (w/v) glycine (PBS-T). Sperm were incubated with rabbit polyclonal antibody against chorein (NBP1-85641; Novus Biologicals, Littleton, CO, USA) overnight at 4 °C. After washing with PBS-T, the sperm were incubated with secondary antibodies (Alexa Fluor 555 Goat Anti-Rabbit IgG (H + L), Invitrogen) for 1 h at room temperature. Coverslips were washed, mounted with Vectashield medium containing DAPI (Vector Laboratories, Burlingame, CA, USA), and viewed with a BZ-X 710 fluorescence microscope system (Keyence) using a sectioning technique.

2.7. Measurement of sperm adenosine 5′-triphosphate (ATP) concentration

The ATP concentration was measured using an ATP bioluminescent assay kit (Sigma-Aldrich, St. Louis, USA) according to the manufacturer's instructions.

2.8. Electron-microscopy analysis

Sperm were fixed with equal amounts of 4% paraformaldehyde (PFA) and 4% glutaraldehyde (GA) in 0.1 M cacodylate buffer pH 7.4 at incubation temperature, then refrigerated to lower their temperature to 4 °C. Thereafter, they were fixed with 2% GA in 0.1 M cacodylate buffer pH 7.4 at 4 °C overnight. After fixation, the samples were washed three times with 0.1 M cacodylate buffer for 20 min each, and were post-fixed with 2% osmium tetroxide (OsO4) in 0.1 M cacodylate buffer at 4 °C for 2 h. The samples were dehydrated in graded ethanol solutions (50%,70%, 90%, 100%). The schedule was as follows: 50% and 70% for 20 min each at 4 °C, 90% for 20 min at room temperature, and four changes of 100% for 20 min each at room temperature. The samples were infiltrated with propylene oxide (PO) twice for 20 min each, and then put into a 70:30 mixture of PO and resin (Quetol-812; Nisshin EM Co., Tokyo, Japan) for l h. The cap of the tube was then left open and the PO was volatilized overnight. The samples were transferred to fresh 100% resin, and were polymerized at 60 °C for 48 h. The

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