Adenylate Kinase 9 Involvement in Sperm Flagellar Movement and Ependymal Cell Cilia of Mouse Brain Ventricles

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The extreme subfertility observed in a commercial dairy bull, with only a 10% pregnancy rate despite passing routine laboratory tests of semen quality, led to the discovery of an intronic variant in the bovine adenylate kinase 9 (AK9) gene (doi: 10.1073/pnas.2305712120). This variant activates cryptic splicing, resulting in a truncated protein. While AK9 is known to be involved in nucleoside homeostasis, its specific functions remain unclear. To further explore the role of the Ak9 gene in male fertility, we utilized CRISPR/Cas9 technology to generate mice deficient in Ak9. We established four lines of mice with deletions induced in exon 21 and between exons 21 and 23, causing a frameshift and premature termination of the encoded protein. This was confirmed through western blotting and immunohistochemistry. While both male and female edited mice exhibited normal development, males with homozygous mutations were found to be infertile. Despite the normal appearances of seminiferous tubules and acrosomal caps in knockout mice, the loss of Ak9 resulted in complete immotility of flagella. While sperm parameters such as testis size, gonadosomatic index, and sperm concentration showed no differences compared to wild-type mice, Ak9 knockout resulted in sperm with a high incidence of abnormalities, reduced ATP levels, and impaired fertilization capacity. RNA-seq analysis of testis samples from Ak9 gene knockout mice (Ak9-/-) and wild-type mice revealed differential expression of genes associated with cilium- and flagellum-dependent motility, axoneme and flagellum assembly, microtubule movement, spermatid differentiation, sperm-egg recognition, energy state of the cell, and fertilization. Ultrastructure analysis of sperm cells from Ak9-/- mice via transmission electron microscopy showed severe disorganization of the (9 + 2) axonemal structure. These results indicated that the cause of infertility was the lack of sperm motility due to both a metabolic and structural deficit of the sperm flagella. Interestingly, AK9-/- animals exhibited abnormal behavior, primarily hyperactivity and impaired balance on the hanging test. Analysis of AK9 expression in different brain regions using immunofluorescence revealed high expression in the ciliated ependymal cells of the ventricles, as well as in other regions of the hypothalamus and frontal cortex. Ultrastructure analysis of the cilia of the ependymal cells from Ak9-/- mice performed by electron microscopy did not identify any disorganization of the axonemes. However, quantification of the lateral brain ventricles showed a significant increase in size in AK9-/- mice, suggesting that the mutation in AK9 may impair ventricular multiciliated neuroepithelial cell function. Our results suggest a dual function of AK9 in fertility and brain functions, mediated by its role in ciliated cells. Our findings highlight the essential metabolic role of AK9 in sperm flagellar structure and motility. Additionally, alterations in mouse behavior and lateral ventricle sizes suggest that AK9 may be involved in the composition and movement of ependymal cilia, potentially affecting cerebrospinal fluid flow.

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