Published in final edited form as:

Biol Chem. 2009 January ; 390(1): 67–73. doi:10.1515/BC.2009.011.

Differential Functions of the Apoer2 Intracellular Domain in Selenium Uptake and Cell Signaling

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Abstract

Apolipoprotein E Receptor 2 (Apoer2) is a multifunctional transport and signaling receptor that regulates the uptake of selenium into the mouse brain and testis through endocytosis of selenoprotein P (Sepp1). Mice deficient in Apoer2 or Sepp1 are infertile, with kinked and hypomotile spermatozoa. They also develop severe neurological defects on a low selenium diet, due to a profound impairment of selenium uptake. Little is known about the function of Apoer2 in the testis beyond its role as a Sepp1 receptor. By contrast, in the brain, Apoer2 is an essential component of the Reelin signaling pathway, which is required for proper neuronal organization and synapse function. Using knockin mice, we have functionally dissociated the signaling motifs in the Apoer2 cytoplasmic domain from Sepp1 uptake. Selenium concentration of brain and testis was normal in the knockin mutants, in contrast to Apoer2 knockouts. Thus, the neurological defects in the signaling impaired knockin mice are not caused by a selenium uptake defect, but instead are a direct consequence of a disruption of the Reelin signal. Reduced sperm motility was observed in some of the knockin mice, indicating a novel signaling role for Apoer2 in sperm development and function that is independent of selenium uptake.

Keywords

Disabled-1; LRP8; Male fertility; Reelin; Sperm motility; Vldlr

Introduction

Apolipoprotein E receptor 2 (Apoer2), a member of the low-density-lipoprotein (LDL) receptor family, undergoes complex splicing and proteolytic processing. Apoer2 is predominantly expressed in the brain where it regulates neuronal migration, cortical lamination, and synapse function. One of the major signaling cascades responsible for controlling these processes in the brain involves the large extracellular signaling protein Reelin, which is a ligand for Apoer2 (Herz and Chen 2006). Reelin binding to Apoer2 and the very low-density-lipoprotein receptor (Vldlr), another member of the LDL receptor family, results in receptor clustering at the plasma membrane. This clustering initiates the transphosphorylation of the adaptor protein Disabled-1 (Dab1) by Src family kinases (SFKs). Dab1 interacts with an NPxY tetraamino acid motif in

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the cytoplasmic intracellular domains (ICDs) of both receptors. Phosphorylation leads to a further increase in SFK activity, thereby activating multiple cellular signaling branches that control neuronal migration and synapse function.

Apoer2 is not only involved in the transmission of the Reelin signal but has also been implicated in regulating selenium uptake in the brain and testis. Selenium is a micronutrient that is crucial for brain function and sperm development. It is transported to target tissues by incorporation into proteins such as Selenoprotein P (Sepp1), a high capacity carrier of selenium, followed by endocytosis through Apoer2 (Burk and Hill 2005; Olson et al. 2007).

Burk and colleagues have recently reported a remarkable phenotypic similarity between Apoer2 and Sepp1 deficient animals (Trommsdorff et al. 1999; Olson et al. 2005; Burk et al. 2007; Valentine et al. 2008). Apoer2 and Sepp1 deficiency both greatly impair selenium uptake in the testis by as much as 81%, resulting in male hypofertility and structural sperm defects (Andersen et al. 2003; Hill et al. 2003; Olson et al. 2005). In addition to revealing a critical role for selenium in the testis, these findings have also shown that selenium delivery through Sepp1 and Apoer2 is the primary route of selenium supply to this tissue. In an analogous manner, selenium deficiency in the brain, caused by lack of Sepp1 or Apoer2 in combination with a low selenium diet, leads to irreversible neurological (Burk et al. 2007) and hippocampal dysfunction (Peters et al. 2006) with progressive neurodegeneration throughout the brain (Valentine et al. 2008).

We have previously shown that mutations or deletions of certain functional sequence motifs within the Apoer2 ICD, generated through a knockin approach, can also lead to hippocampal dysfunction, defects in synaptic plasticity, accelerated neurodegeneration, and reduced neuronal survival after injury (Beffert et al. 2005; Beffert et al. 2006b; Beffert et al. 2006a). These sequences include the NPxY motif and a 59 amino acid sequence encoded by an alternatively spliced exon, which provides binding sites for adaptor and scaffolding proteins that regulate the activation of JNK signaling and NMDA receptors.

The phenotypic similarity between Apoer2 and Sepp1 deficient mice raised the possibility that the neurological defects of the Apoer2 knockin mice might be caused in part by a Sepp1 uptake defect, resulting in neuronal selenium deficiency, rather than a direct effect of Apoer2 on neuronal JNK or NMDA receptor signaling. Distinguishing between these possibilities is of the utmost importance for proper interpretation of the physiological functions that have been attributed to Apoer2, in particular its critical roles in regulating synaptic transmission and neuronal survival. The purpose of the current study was to disentangle these confounding possibilities. To achieve this, we made use of the series of Apoer2 knockin mice we had generated (Beffert et al. 2005; Beffert et al. 2006b; Beffert et al. 2006a). Here, we investigated the ability of these mutant animals to mediate selenium uptake into brain and testis. We also measured a series of sperm motility parameters to reveal potential roles of the cytoplasmic signaling and docking motifs in sperm maturation and function. Our results unequivocally show that selenium uptake into the brain is not impaired in the knockin mutants, thereby effectively ruling out a compounding effect of selenium deficiency on the profound synaptic signaling defects that occur in the Apoer2 ICD mutants. Intriguingly, our findings also show that the signaling competent functional motifs in the cytoplasmic domain of Apoer2 play a previously unknown role in sperm development which is independent of selenium uptake.

Results

We have previously generated and used a series of Apoer2 ICD mutant mouse lines to molecularly define intracellular components of the Reelin signaling pathway. In this study we utilize four of these mutant lines to investigate the involvement of the Apoer2 ICD in selenium

uptake both in the brain and testis (Figure 1). The Apoer2[*ex19*] and Apoer2[*Δex19*] mice constitutively express a splice variant of Apoer2 that contains or lacks exon 19, respectively. Exon 19 encodes a proline rich 59 amino acid sequence within the intracellular domain of the receptor that contains binding sites for the adaptor proteins postsynaptic density protein of 95kDa (PSD95) and JNK interacting proteins (JIPs). This exon is essential in the regulation of synaptic plasticity by the neuronal signaling protein Reelin. Exon 19 functionally couples Apoer2 to *N*-methyl *D*-aspartate receptors (NMDAR) probably through the adaptor protein PSD95, resulting in the activation of the ionotropic receptors and regulation of NMDAR dependent synaptic plasticity (Beffert et al. 2005).

Another Apoer2 ICD mutant line, Apoer2[*Stop*], contains the deletion of the entire ICD five amino acids carboxyterminal of the NPxY motif. This deletion includes part of exon 18 and all of exons 19 and 20. These mice, along with the Apoer2[*Δex19*] mice, show an age-dependent loss of corticospinal neurons signifying the importance of exon 19 as a regulator of neurodegeneration, likely through the interaction with JIPs, adaptor proteins involved in the regulation of the JNK signaling pathway (Beffert et al. 2006).

The fourth Apoer2 ICD mutant line, Apoer2[*Dab*⁻], has a three amino acid mutation in the cytoplasmic ICD just prior to the NPxY motif. Mutation of residues N893E, F894I, and D895G in the cytoplasmic tail of the receptor abrogates binding of the adaptor protein Disabled-1 (Dab1), an obligatory component of the Reelin signaling pathway. By preventing this interaction, the transmission of the Reelin signal through Apoer2 is disrupted. The NPxY motif is involved in the regulation of endocytosis of numerous proteins. Compared to other LDL receptor family members, the endocytosis coefficient of Apoer2 is very low. The three amino acid mutation so close to the NPxY motif affects, but does not completely abrogate, Apoer2 endocytosis (Beffert et al. 2006b). However, the mutation does severely impair synaptic plasticity further underscoring the importance of Apoer2 in the regulation of NMDA receptor function.

Burk and colleagues have previously shown a profound effect of selenium deficiency on synaptic function and neuronal survival, which is strikingly similar to the defects we have observed in the Apoer2 ICD mutants (Beffert et al. 2005; Beffert et al. 2006b; Beffert et al. 2006a; Peters et al. 2006; Burk et al. 2007; Valentine et al. 2008). This raised the possibility that the phenotypes of our Apoer2 ICD mutant mice might be, at least in part, caused by selenium deficiency and not by the disruption of neuronal signaling pathways. To exclude this possibility, the Apoer2 ICD mutants, along with wild type and Apoer2 KO controls, were fed a diet supplemented with a level of selenium that provides an adequate supply of this micronutrient to wild type mice. After four weeks the animals were euthanized and their brains, testes, and livers were collected for selenium level analysis. Consistent with previous reports, selenium levels were greatly reduced in both the brain (Figure 2A) and testis (Figure 2B) of Apoer2 KO mice (Hill et al. 2003; Olson et al. 2007). By contrast, none of the Apoer2 ICD mutants had significantly lower selenium levels in brain or testis, compared to wild type animals. As a control, selenium levels in the liver, where Apoer2 is not expressed, were comparable across all genotypes (Figure 2C). Although endocytosis in the Apoer2[*Dab*⁻] animals may be affected by the three amino acid mutation, it is not enough to decrease selenium levels in the brain or testis of these mice. We confirmed this conclusion by visualizing the localization of Sepp1 in the testis using immunohistochemistry. The WT animals and all of the Apoer2 ICD mutants showed vesicular Sepp1 staining at the basal lamina (Figure 3). As previously reported, there were no Sepp1 positive vesicles detected in the Apoer2 KO testes. These data indicate that the domains crucial for regulating synaptic function in the brain are not involved in the uptake of selenium in either of these tissues.

Although none of the Apoer2 ICD mutant mice had significantly decreased selenium levels in the testis, we observed decreased pregnancy rates and small litter size in the Apoer2[*Dab*⁻] ICD mutant animals. This impaired fertility in the Apoer2[*Dab*⁻] mice indicated a role for Apoer2 in spermatogenesis that is independent of selenium uptake into the testis. Therefore, we investigated the morphology of spermatozoa from wild type and Apoer2 mutant lines by isolating, fixing, and staining spermatozoa with DAPI to label the nucleus, with MitoFluor™ to stain the midpiece, and with a crossreacting polyclonal antibody against an unidentified sperm antigen that marks the acrosome and principal piece of the tail (Figure 4). The basic organization of the above mentioned sperm sections was identical between the spermatozoa collected from wild type mice and the Apoer2 mutants.

The Apoer2[*Dab*⁻] mice, like the Apoer2 KOs, however, produced a larger fraction of spermatozoa with abnormal tail morphology than wild type mice. We morphologically analyzed spermatozoa from multiple mice ($n \geq 3$) from each genotype. Spermatozoa were classified by the shape of the tail as straight, hairpin, angled, or curled (Figure 5). Straight spermatozoa were defined as those with an absence of a bend in the tail between the neck and the distal tip of the tail. Hairpin spermatozoa were bent back on themselves 180 degrees at the annulus (arrowhead in Figures 4E, 4F and 5C). Angled spermatozoa were also bent at the annulus at an angle between 40-120 degrees with a cytoplasmic droplet often residing at the bend (Figure 5B). The midpieces of curled spermatozoa were looped adjacent to or around the head of the spermatozoon, frequently forming a complete circle. This particular conformation, confirmed by scanning electron microscopy (Figure 5D), appears to coincide with the cytoplasmic droplet failing to migrate down the midpiece and, instead, remaining at the neck. This can cause the head to curl around the droplet. Table 1 shows the percent of the total population of spermatozoa in each of the four morphological conformations for all six mouse lines. As previously reported, we confirmed that the Apoer2 KO mice produce a considerably higher percentage of hairpin spermatozoa than wild type. The only Apoer2 ICD mutants that significantly differed from wild type with respect to tail morphology were the Apoer2[*Dab*⁻] mice. Like the KOs, they had few straight spermatozoa, however, the majority of the Apoer2[*Dab*⁻] spermatozoa were split between the hairpin (36.3%±4.1%) and the curled (35.0%±3.1%) conformations. By contrast, 66.3%±2.3% of Apoer2 KO spermatozoa showed the hairpin conformation. This suggests that in the Apoer2[*Dab*⁻] mice, migration of the cytoplasmic droplet may be impaired as it remains arrested at the base of the neck in a significant proportion of the spermatozoa, a phenotype not seen in any other Apoer2 ICD mutant line.

This abnormal morphology in the majority of the Apoer2[*Dab*⁻] spermatozoa could potentially affect sperm motility, and therefore cause impaired fertility in this mutant strain. To assess any defects in motility, spermatozoa were collected and various established parameters of sperm motility were measured (Table 2). Even though there was variability between animals, only the Apoer2 KO mice had a significantly lower percentage of motile spermatozoa compared to wild type. The motility of Apoer2[*ex19*] spermatozoa was essentially indistinguishable from wild type for all of the parameters tested. By contrast, spermatozoa from the Apoer2[*Δex19*] and Apoer2[*Stop*] lines, both of which lack the alternatively spliced exon 19, had velocities that were considerably reduced compared to wild type but not as much as the KO spermatozoa. This suggests that exon 19, which is essential for synaptic plasticity and neuronal survival, may also play a functional role in sperm motility. However, absence of exon 19 does not reduce motility enough to noticeably impair fertility. Although some of the velocity parameters of the Apoer2[*Dab*⁻] spermatozoa were identical to those of the Apoer2 KOs, a larger percentage of the Apoer2[*Dab*⁻] spermatozoa were motile compared to the KO mice. Even though fertility is impaired in the Apoer2[*Dab*⁻] mice, this modest increase in percent motility may be the factor preventing this Apoer2 ICD mutant line from being infertile like the Apoer2 KOs.

Discussion

The present study draws a clear distinction between two fundamentally different physiological functions of Apoer2: signal transduction and selenium transport. In the brain, Apoer2 relays the Reelin signal across the neuronal membrane and as a result controls neuronal migration, function, and survival. Here we have demonstrated that selenium uptake into the brains of mice expressing Apoer2 ICD mutations, which impair specific signaling functions in the brain, is not affected. Thus, the synaptic signaling role of Apoer2 is independent of its role in selenium uptake.

Using spermatogenesis as an independent model system, we further show that this separation between signaling and selenium uptake exists in the testis as well. None of the Apoer2 ICD mutants had lower testis selenium levels than wild type, yet we still observed significant defects in sperm morphology and motility in three of the Apoer2 ICD mutants. These data suggest that the intracellular domain regions that are crucial for normal brain function may also be relevant for determining sperm motility and maturation independent of selenium uptake.

The NPxY motif serves as a common endocytosis signal in numerous cell surface receptors. In the Apoer2 ICD it mediates the interaction with the PTB domain containing adaptor protein, Dab1. Mutation of the three amino acids prior to the NPxY motif marginally impairs endocytosis, however, it completely disrupts Dab1 binding to the ICD. This unique property of the Apoer2[*Dab*⁻] mouse line has allowed us to differentiate between the function of Apoer2 in selenium uptake and signal transduction. Our data show that testis selenium levels in the Apoer2[*Dab*⁻] mice are not lower than wild type. This indicates that selenium uptake remains unharmed by the mutation. However, even under normal selenium levels, the Apoer2[*Dab*⁻] line still produces spermatozoa with morphological and motility defects. These results suggest that Apoer2 may play a signaling role, through interaction with a PTB domain containing adaptor protein, which affects sperm maturation and function.

The significant number of spermatozoa with curled midpieces produced by the Apoer2[*Dab*⁻] strain is not commonly observed. To our knowledge only one other mutant mouse line resembles this phenotype, the GOPC (Golgi-associated PDZ- and coiled-coil motif-containing protein) deficient mice (Suzuki-Toyota et al. 2004). The spermatozoa of these animals have rounded heads with the midpiece curled around the head. The proposed reason for this curl is the lack of a posterior ring that separates the cytoplasmic droplet from the rest of the perinuclear cytoplasm. Thus, as the cytoplasmic droplet migrates down the midpiece, the head of the spermatozoon is dragged along curling the tail around itself. The GOPC KO mice are more severely affected than the Apoer2[*Dab*⁻] mouse line as the Apoer2 mutants do not have rounded heads. Furthermore, there is no evidence for the lack of a posterior ring as the cytoplasmic droplet seems to form normally and only a third of the spermatozoa display the curled phenotype.

This lack in the severity of phenotype does not rule out the possibility that the migration of the cytoplasmic droplet could play a role in the curled morphology of the Apoer2[*Dab*⁻] spermatozoa. To date, the cause and reason for the migration of the cytoplasmic droplet is unknown. However, it is accepted that in many species the droplet will migrate down the midpiece as the spermatozoon travels through the epididymis and matures (Cooper and Yeung 2003). Because we find that a significant fraction of spermatozoa that have migrated to the cauda part of the epididymis retain their cytoplasmic droplets proximal to the head, Apoer2[*Dab*⁻] spermatozoa may fail to mature properly. Not only do we observe a proximal droplet, we also see the midpiece curled around it. When given an osmotic shock, immature sperm tails are more likely to curl than mature spermatozoa because they have not yet assembled the necessary molecular machinery for proper cell volume regulation (Yeung et al. 1999). These

observations support a role for Apoer2 signaling through the intracellular domain of the receptor not only controls synaptic transmission in the brain, but similar pathways appear also to be important for sperm maturation in the epididymis. Further molecular definition of this novel signaling function may reveal new rational targets for male contraception and infertility therapies.

Materials and Methods

Animals

Generation of Apoer2 KO (Trommsdorff et al. 1999), Apoer2[*ex19*] (Beffert et al. 2005), Apoer2[*Δex19*] (Beffert et al. 2005), Apoer2[*Stop*] (Beffert et al. 2006), Apoer2[*Dab⁻*] (Beffert et al. 2006) mice has been previously described. All mice were housed under a 12 h: 12 h light dark cycle and fed a normal chow diet unless otherwise stated. All animals were euthanized via inhalation of isoflurane according to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the UT Southwestern Animal Care and Use Committee.

Tissue selenium level analysis

Mice were weaned at three weeks of age and fed a *Torula* yeast diet containing 0.25 mg/kg selenium as sodium selenite (Burk et al. 2007). The diet was purchased from Harlan-Teklad (Madison, WI, USA). Mice consumed this diet for 4 weeks, after which they were euthanized and exsanguinated. Tissues were collected and selenium levels were measured as previously described (Olson et al. 2007).

Immunocytochemistry

Testis—Testes were fixed in 4% paraformaldehyde in PBS for 1 h at 4°C and cryoprotected in 20% sucrose in PBS overnight. Frozen sections (5 μm) from these testes were washed with TBST (20 mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 0.025% sodium azide, 0.05% Tween 20) and incubated in blocking buffer (1% BSA and 1% glycine in TBST) for 1 h. Sepp1 monoclonal rat antibody (Olson et al. 2007) diluted in blocking buffer was added to the slices for 1 h at room temperature. The slides were washed 3 × 5 min with TBST and incubated with Alexa Fluor 594 chicken anti-rat fluorescent secondary antibody (Invitrogen, Carlsbad, CA, USA, 1:200) diluted in blocking buffer for 1 h. Sections were washed in TBST and coverslips were mounted using ProLong Gold with DAPI mounting medium (Invitrogen). Pictures were taken with a Zeiss Axioplan 2 (Thornwood, NY, USA) fluorescence microscope using identical exposure settings. At least three animals were analyzed per genotype.

Sperm—Adult males were euthanized, and cauda and caput sections of the epididymis were dissected free of connective tissue and fat deposits. The tissue was minced in 0.1 M sodium phosphate buffer (pH 7.4) and the spermatozoa were allowed to swim up for 10 min. The suspension was spun at 1,000 × *g* to remove any remaining tissue. Spermatozoa were fixed in 4% paraformaldehyde overnight at 4°C. The fixed spermatozoa were resuspended and streaked onto slides, dried, and permeabilized with ice cold methanol for 15 min. Slides were washed, blocked for 1 h at room temperature (blocking buffer: 0.1% BSA, 10% goat serum, 1×PBS), and incubated overnight at 4°C with the primary antibody (1:200 dilution of a crossreacting polyclonal antibody against an unidentified sperm antigen). Slides were washed in PBS followed by the addition of Alexa Fluor 488 goat anti-rabbit fluorescent secondary antibody (Invitrogen, 1:200) for 1 h at room temperature in blocking buffer. The slides were washed in PBS and incubated with MitoFluor™ (Invitrogen, 1:200 in PBS) for 10 min at room temperature. Finally, the slides were washed in PBS and coverslips were mounted using ProLong Gold with DAPI mounting medium (Invitrogen). Pictures were taken with a Zeiss Axioplan 2 fluorescence microscope.

Scanning Electron Microscopy

Adult Apoer2 ICD mutant spermatozoa were collected as described for immunocytochemistry. After centrifugation at $800 \times g$ for 5 min the spermatozoa were fixed with 4% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4°C. Spermatozoa were pelleted and resuspended in 0.1 M cacodylate buffer for plating on Poly-L-Lysine coated coverslips. After incubation in osmium tetroxide, graded ethanol dehydration, and treatment with hexamethyldisilazane (HMDS), the coverslips were sputter coated in a Denton DV--502A evaporator (Moorestown, NJ, USA) with a gold/palladium target. Scanning electron images were taken with a FEI XL30 ESEM (Hillsboro, Oregon, USA), spot 4.0, 20.0kV.

Morphological analysis

Mice 3-6 months of age were euthanized and epididymides were dissected free of connective tissue and fat deposits. Using fine scissors, a few small incisions were made in a small section of the cauda epididymis and the tissue was incubated in 1 ml of Biggers-Whitten-Whittingham (BWW) medium (osmolarity, 310 mosmol/L) supplemented with 12 mg/ml BSA (Andersen et al. 2003). Spermatozoa were allowed to swim up for 10 min at 37°C. Tissue was removed from the medium and spermatozoa were fixed in 4% paraformaldehyde overnight at 4°C. Spermatozoa were streaked onto slides, covered with coverslips, visualized using normal light microscopy, and categorized according to tail morphology. One hundred spermatozoa were analyzed for each of three mice from every genotype. Percentages were averaged and statistical analysis was performed using the Student's t-test.

Sperm motility analysis

Spermatozoa, from adult male mice 3-6 months of age, were collected in the same manner as for morphological analysis, tissue was removed from the medium, and the spermatozoa were immediately placed into an 80 μ m slide well warmed to 37°C. Sperm motility was analyzed according to the methods previously described (Quill et al. 2003). Briefly, the slide was placed in The IVOS Sperm Analyzer (Version 12, Hamilton Thorne Research, Beverly, MA, USA) for motility analysis. Images were collected for 0.5 s at 60Hz. For each sample (three mice per genotype, one sample per mouse), 100 spermatozoa tracks were selected for unobstructed motion during the entire recording time and the following parameters were analyzed: % motility, curvilinear velocity (μ m/s), average path velocity (μ m/s), straight line velocity (μ m/s), amplitude of lateral head displacement (the largest distance the head reaches away from the averaged path, μ m), beat cross frequency (Hz), linearity (%).

Acknowledgements

We thank Priscilla Rodriguez and Isaac Rocha for outstanding technical assistance and for mouse colony maintenance and Amy Motley for analysis of selenium levels. This study was supported by grants from the National Institutes of Health (HL63762, HL20948, ES02497).

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Figure 1. Apoer2 ICD mutant knockin mice

Apoer2[*ex19*] mice constitutively express the full length receptor containing the alternatively spliced exon 19. Apoer2[Δ*ex19*] mice constitutively express the full length receptor without the alternatively spliced exon 19. Apoer2[*Stop*] mice express a truncated receptor lacking the C-terminal ICD after the NPXY domain. Apoer2[*Dab⁻*] Mice express the full length receptor with a mutation in the Dab-1 binding site.

Figure 2. No significant difference in tissue selenium levels of Apoer2 ICD mutant mice from wild type

Tissue selenium values were measured from mice of all six genotypes after four weeks on a 0.25 mg/kg selenium diet. Only Apoer2 KO mice presented with significantly lower selenium levels than wild type mice in the brain (A) and testes (B); $^{***}P < 0.001$. Liver selenium levels were comparable among all genotypes (C).

Figure 3. Vesicular Sepp1 staining observed in all Apoer2 ICD mutant mice

Fixed mouse testes were stained for Sepp1. Vesicular Sepp1 staining (arrows) is apparent in WT mice and all Apoer2 ICD mutants. As previously reported, there is no Sepp1 vesicular staining in Apoer2 KO testes. Scale bar = 10 μ m.

Figure 4. Sperm immunostaining reveals normal organization of spermatozoa in all Apoer2 mutant mice

Fixed spermatozoa were immunostained with MitoFluor™ (midpiece; red), a crossreacting polyclonal antibody against an unidentified sperm antigen (acrosome and principal piece; green), and DAPI (nucleus; blue). (A) wild type (B) Apoer2[ex19] (C) Apoer2[Δex19] (D) Apoer2[Stop] (E) Apoer2[Dab^{-/-}] (F) Apoer2 KO. Pictures are representative of the major morphological sperm population for each genotype. Arrows indicate location of annulus. Scale bar = 10 μm.

Figure 5. Sperm classification into four morphological categories

The majority of spermatozoa observed fell into one of four different morphological categories: straight, hairpin (sperm tail is bent back on itself at the annulus), angled (sperm tail is slightly bent at the annulus creating an angled tail), and curled (the tail is curled proximal to the head of the spermatozoon). Asterisks mark sperm head. Arrow marks annulus

Table 1

Quantitative analysis of sperm morphology

	% Straight	% Hairpin	% Angled	% Curled
WT	64.7 ± 4.4	23.7 ± 5.0	6.3 ± 1.2	5.3 ± 1.2
<i>Apoer2[ex19]</i>	63.3 ± 4.4	23.3 ± 4.1	7.7 ± 1.2	5.7 ± 2.3
<i>Apoer2[Δex19]</i>	62.0 ± 4.2	18.7 ± 1.3	3.0 ± 1.7	16.3 ± 5.4
<i>Apoer2[Stop]</i>	57.7 ± 6.4	20.0 ± 7.1	10.3 ± 1.3	12.0 ± 0.6
<i>Apoer2[Dab⁻]</i>	17.7 ± 5.4 *	36.3 ± 4.1	11.0 ± 1.5	35.0 ± 3.1 *
<i>Apoer2</i> KO	11.0 ± 1.7 *	66.3 ± 2.3 *	6.0 ± 0.0	16.7 ± 3.4

One hundred spermatozoa from each of three animals per genotype were scored according to morphological category. (mean ± S.E.)

* $P < 0.005$

Table 2

Motility analysis of wild type and Apoer2 mutant sperm

	WT	Apoer2[ex19]	Apoer2[lex19]	Apoer2[Stop]	Apoer2[Dab]	Apoer2 KO
% Motile	69.0 ± 5.6	46.3 ± 10.3	30 ± 7.1	48.3 ± 7.5	32.7 ± 9.4	18.7 ± 7.3 *
Curvilinear velocity, µm/s	304.6 ± 4.0	283.1 ± 11.2	233.0 ± 2.7 *	242.6 ± 7.4 *	145.3 ± 7.1 *	160.4 ± 25.0 *
Average path velocity, µm/s	140.0 ± 0.4	142.1 ± 2.3	114.2 ± 3.6 *	116.4 ± 2.8 *	85.0 ± 6.2 *	80.1 ± 9.9 *
Straight line velocity, µm/s	103.2 ± 2.6	116.6 ± 1.6	87.1 ± 5.2	87.9 ± 5.0	66.9 ± 7.8	54.8 ± 7.5 *
Amplitude of lateral head displacement, µm	16.6 ± 0.2	14.0 ± 0.6	12.3 ± 0.2 *	12.8 ± 0.2 *	7.7 ± 0.4 *	9.2 ± 1.6
Beat cross frequency, Hz	24.7 ± 1.4	26.3 ± 1.1	28.2 ± 0.3	28.5 ± 0.7	30.2 ± 1.6	30.5 ± 1.3
Linearity, %	34.6 ± 0.6	42.6 ± 1.8	39.1 ± 1.8	37.1 ± 2.0	48.0 ± 4.9	37.5 ± 0.8

Spermatozoa, 150 from each of three animals per genotype, were analyzed for percent motility and various motility parameters, (mean ± S.E.)

* $P < 0.01$