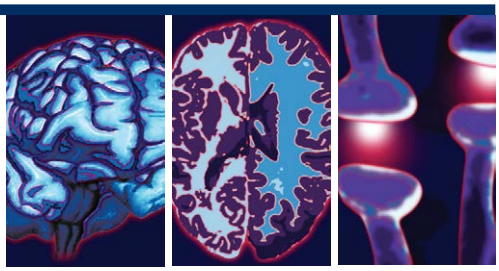

Female Wild-Type and *APP/PS1* Transgenic Mice Deficient in *Sort1* Are Prone to Anxiety-Like Behavior at Older Ages

Chun-Sheng Ruan[1,2,†], Larisa Bobrovskaya[1], Xin-Fu Zhou[1,†]

Highlights

- Aged female but not male *APP/PS1* mice showed hyperactivities and anxiety-like behavior
- Aged female but not male WT or *APP/PS1* mice deficient in *Sort1* showed anxiety-like behavior
- *Sort1* deficiency did not affect spatial cognition of aged WT or *APP/PS1* mice in any gender

Abstract

Alzheimer's disease (AD) and anxiety are two concurrent disorders often co-existing in older adults. Interestingly, women are more likely to experience anxiety than men in both general population and AD. To date, the mechanisms underlying the gender differences in the pathogenesis of AD and anxiety disorder are still not clear. Previously, we have found that the deletion of vacuolar protein sorting 10 protein (VPS10P)-domain containing receptor/sortilin (encoded by *Sort1* gene) increases anxiety-like behavior but does not affect cognition in both sexes of 3-month-old mice in an open field test and an elevated plus maze test; and females tended to show a more severe anxious phenotype. In this study, in order to examine the role of sortilin in the gender-dependent anxiety-like behavior with age under normal condition or physical illness like AD, we recruited both sexes of 9-month-old WT, *Sort1-/-*, *APP/PS1* (mouse model of AD), and *Sort1-/-APP/PS1* mice to perform the open field test and Morris water maze test. Our data showed that aged female but not male mice deficient in *Sort1* with or without the transgenes of *APP* and *PS1* displayed a significant increase in anxiety-like behavior as shown by decreased percentage of time in central zone in the open field test. Our data also showed that only female *APP/PS1* or *Sort1-/-APP/PS1* mice showed hyperactivities and anxiety-like behavior. *Sort1* deficiency did not affect cognitive behavior in both sexes of aged wild type or transgenic AD mice. The present study found a gender-specific role of sortilin in regulation of anxiety-like behaviors in aged mice, which could be a potential mechanism involved in gender differences of anxiety disorders.

Keywords

Sortilin, Anxiety, Cognition, Alzheimer's disease, Sex

Abbreviations

AD: Alzheimer's Disease; APP: Amyloid Precursor Protein; *APP/PS1*: *Appswe/PS1dE9* Double Transgenic Mice; BDNF: Brain-Derived Neurotropic Factor Precursor; Probdnf: BDNF Precursor; PS1: Presenilin-1; VPS10P: Vacuolar Protein Sorting 10 Protein; WT: Wild Type

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Introduction

Alzheimer's disease (AD) is the most common form of dementia, which is characterized by a number of hallmarks such as amyloid plaque (by abnormal accumulation of beta-amyloid peptide), neurofibrillary tangle (by abnormal accumulation of tau protein), brain atrophy and cognitive decline [1,2]. Patients diagnosed with AD often also show a variety of neuropsychiatric behaviors such as depression and anxiety [3].

The study of depression in connecting with AD has long been conducted, whereas the understanding of the relationship between anxiety and AD is still limited. Anxiety is an unpleasant emotion accompanied with nervous behavior [4]. It is commonly seen in the elderly people and significantly reduces the quality of their life [5]. Up to 70 per cent of AD patients are diagnosed with anxiety disorders [6]; and people with anxiety disorders are more susceptible to develop AD when they get old [7]. Anxiety and AD are concurrent disorders and each one may facilitate the development of another. To date, the molecular mechanism of anxiety in AD is poorly understood. In addition, gender difference in the onset of both AD and anxiety disorder has long been uncovered. Women are more vulnerable to develop AD [8] or anxiety [9] than men, which is most likely due to the involvement of female hormones (i.e. estrogen and progesterone) in the pathogenesis of these two diseases [10,11]. Still, the molecular mechanism of such gender differences in both AD and anxiety disorder is not well known.

Vacuolar protein sorting 10 protein (VPS10P)-domain containing receptors (include sortilin, SorLA and SorCS1/2/3), which are important for intracellular transport and cell signalling [12], have been implicated in regulation of AD [13-15] and mood disorders (such as anxiety and bipolar disorder) [16-21]. Sortilin (encoded by the *Sort1* gene) is a 100-kDa type I membrane glycoprotein which is highly expressed in the brain [22]. It is the first receptor identified from the VPS10P family. As a sorting receptor, sortilin modulates the regulated secretion of brain-derived neurotropic factor (BDNF) which has been implicated with important role in anxiety disorder [23-25]. In addition, sortilin also acts as a receptor in signal transduction. It mediates the apoptotic effect of BDNF precursor (proBDNF) by forming a receptor complex with neurotrophin receptor p75 [26]. Moreover, it is also an important receptor to modulate the signalling pathway of neurotensin (Mazella, 2001) [27], which has been well-defined with a role in anxiety disorder [28,29]. Our previous study has shown that *Sort1* deficiency results in normal cognitive performance but increased anxiety-like behavior in both sexes of young mice (3 months old); interestingly, our data also suggest that young female mice deficient in *Sort1* are more prone to be anxious than males [21]. Given that females are more susceptible to experience anxiety and that anxiety disorders co-exist with AD, we propose that *Sort1* deficiency may cause more severe anxiety in aged female mice than male mice with or without the transgenes of *APP/PS1*. In this study, we tested the hypothesis by performing an open field test in 4 types of mice. We have also assessed the cognitive function in these mice by using the Morris water maze test.

Materials and Methods

Animals

To perform this study, wild-type (WT), *Sort1* knockout (*Sort1*-/-; Dr. Morales's Laboratory, McGill University, Canada), *APPswe/PS1dE9* (*APP/PS1*; Jackson Laboratory) and *Sort1*-/-*APP/PS1* (generated by *Sort1*-/- × *APP/PS1* crossing) mice (n = 6 each sex and each strain; all animals were C57BL/6 background) were generated at the same time and maintained in 12-h light/12-h dark cycles with free access to food and water in the Reid Animal Facility of University of South Australia.

Behavioral tests

At the age of 9 months old, all animals were subjected to an open field test followed by the Morris water maze test. The open field test was performed as previously described [21]. Animals were brought into an open arena (40 cm long × 40 cm wide × 40 cm high), and allowed to move freely for 5-min while being recorded with a digital camera linked to the ANY-maze software (Stoelting, USA). Traveling distance and percentage of mobility were considered as locomotor activities, and percentage of time in the central zone (central 24 cm × 24 cm area) was considered as anxiety-like behaviour. The Morris water maze test was performed as previously described [21]. Firstly, all animals were introduced to the water maze for acclimatization by allowing them to freely swim for 120 sec. On the second day, a platform was placed in one of the four divided quadrants (target quadrant)

at 0.5 cm level above the water surface. Each animal entered the water maze and was given a maximum 60 sec to find the platform with three trials (visible platform training). On the following days, the platform was hidden below the water surface (at 0.5 cm level), and the same approaches in visible platform training were applied every day (hidden platform training). The hidden platform training stopped when the WT mice showed a "well-trained" skill to find the platform within 15 sec. On the last day, the platform was removed and each animal was subjected to a 60-sec probe test. Escape latency to the platform and percentage of time spent in the target quadrant were recorded by ANY-maze software. All data are presented as mean ± SEM and analysed by IBM SPSS Statistics 21. One-way repeated measures ANOVA and two-way ANOVA were used for group comparisons. $p<0.05$ was considered statistically significant.

Results

We first compared the locomotion and anxiety-like behaviors among WT, *Sort1-/-*, *APP/PS1*, and *Sort1-/-APP/PS1* mice with both sexes in open field test **(Figure 1)**. Travelling distances were plotted in **Figure 1B** (males) and **Figure 1E** (females). Two-way ANOVA analyses showed no main effect of *Sort1-/-* ($F_{(1,22)}$=2.43, p=0.14), no main effect of AD transgenes ($F_{(1,22)}$=0.25, p=0.62) and no *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=0.04, p=0.85) in the travelling distance in male mice; also showed a main effect of AD transgenes ($F_{(1,22)}$=14.24, $p<0.01$), but no main effect of *Sort1-/-* ($F_{(1,22)}$=0.14, p=0.71) and no *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=0.01, p=0.94) in the travelling distance in female mice. Percentages of mobile time were plotted in **Figure 1C** (males) and **Figure 1F** (females). Two-way ANOVA analyses showed no main effect of *Sort1-/-* ($F_{(1,22)}$=0.09, p=0.76), no main effect of AD transgenes ($F_{(1,22)}$=0.00, p=0.98) and no *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=0.39, p=0.54) in the percentage of mobility in male mice; also showed a main effect of the AD transgenes ($F_{(1,22)}$=7.29, $p<0.05$), but no main effect of *Sort1-/-* ($F_{(1,22)}$=0.99, p=0.33) and no *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=0.88, p=0.36) in the percentage of mobility in female mice. Percentages of time in central zone were plotted in **Figure 1D** (males) and **Figure 1G** (females). Two-way ANOVA analyses showed no main effect of *Sort1-/-* ($F_{(1,22)}$=0.25, p=0.63), no main effect of AD transgenes ($F_{(1,22)}$=0.71, p=0.41) and no *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=2.76, p=0.11) in the percentage of time in central zone in male mice; also showed a main effect of *Sort1-/-* ($F_{(1,22)}$=29.14, $p<0.0001$) and a main effect of the AD transgenes ($F_{(1,22)}$=13.10, $p<0.01$) and *Sort1-/-* × AD transgene interaction ($F_{(1,22)}$=6.06, $p<0.05$) in the percentage of time in central zone in female mice. These results taken together indicate that *Sort1* deficiency

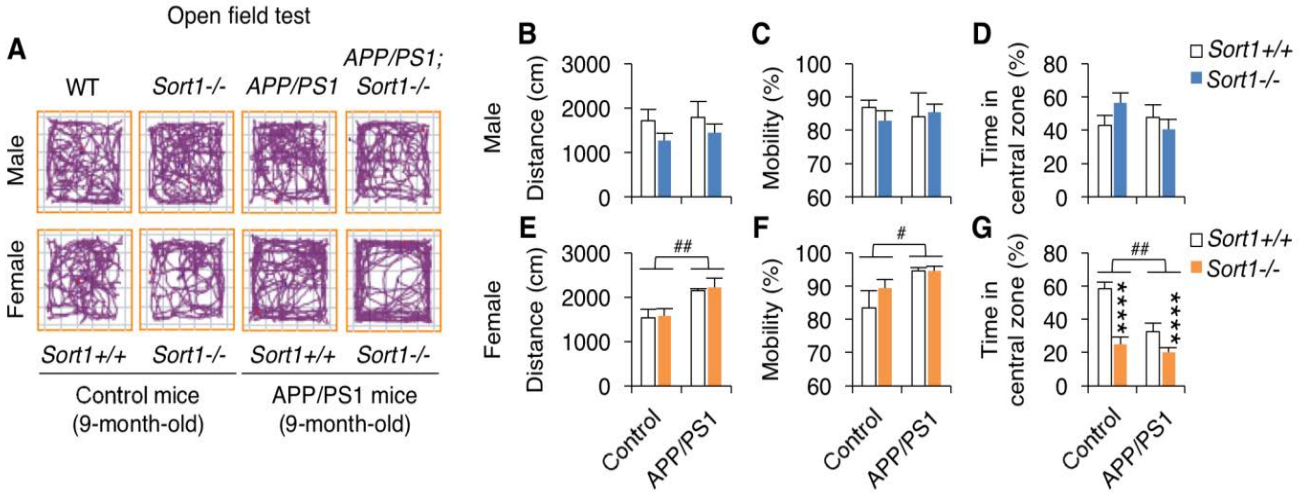

Figure 1: *Sort1* deficiency increased the anxiety-like behavior in aged female but not male WT and *APP/PS1* mice. A, Representative track plots in open field test from both males and females of 9-month-old WT, *Sort1-/-*, *APP/PS1* and *Sort1-/-APP/PS1* mice are presented. B-G, The traveling distances (B, male; E, female), the percentages of mobility (C, male; F, female) or the percentages of time spent in the central zone (D, male; G, female) in open field test were compared among WT (Control;*Sort1+/+*), *Sort1-/-* (Control;*Sort1-/-*), *APP/PS1* (*APP/PS1*;*Sort1+/+*) and *Sort1-/-APP/PS1* (*APP/PS1*;*Sort1-/-*) mice. n = 6 mice for each sex of each genotype. All data are presented as mean ± SEM. ****$p < 0.0001$; #$p < 0.05$; ##$p < 0.01$ (two-way ANOVA).

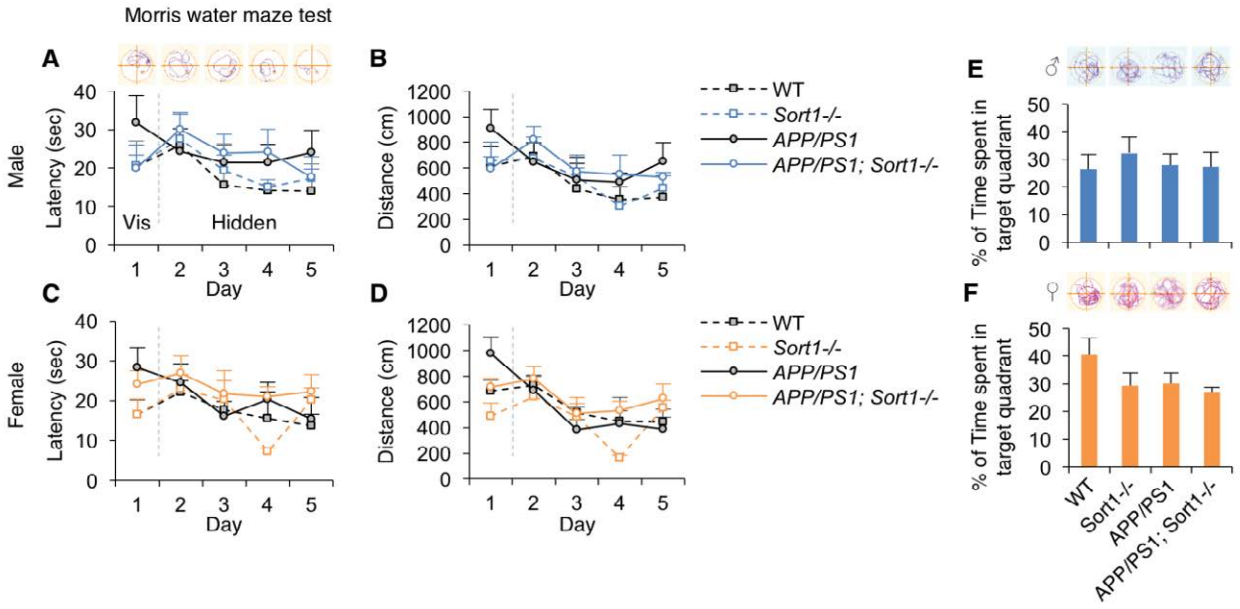

Figure 2: *Sort1* deficiency did not affect the spatial cognitive behaviors in both males and females of aged WT or *APP/PS1* mice. A-F, the latencies to platform (A, male; C, female), the traveling distances (B, male; D, female) or the percentages of time spent in target zoon (E, male; F, female) in Morris water maze test were compared among WT, *Sort1-/-*, *APP/PS1* and *Sort1-/-APP/PS1* mice. $n = 6$ mice for each sex of each genotype. Vis, visible platform; Hidden, hidden platform. All data are presented as mean ± SEM, and statistical analyses were performed by two-way ANOVA (E, F) and one-way repeated measures ANOVA (A-D).

specifically increases anxiety-like behavior in aged female mice with or without AD transgenes. Moreover, the results also indicate increases in locomotion activity and anxiety-like behaviors in aged female but not male *APP/PS1* mouse model of AD.

We further compared the spatial cognitive performance among these groups of animals in the Morris water maze test **(Figure 2)**. Escape latencies during training were plotted in **Figure 2A** (males) and **Figure 2C** (females); and one-way repeated measures of ANOVA analyses of these data showed no main effect of genotype in males ($F_{(3,19)}$=1.73, p=0.20) or females ($F_{(3,19)}$=1.44, p=0.26). Swimming distances during training were plotted in **Figure 2B** (males) and **Figure 2** (females); and one-way repeated measures of ANOVA analyses also showed no main effect of genotype in males ($F_{(3,19)}$=2.10, p=0.14) or females ($F_{(3,19)}$=1.75, p=0.19). Moreover, percentages of time spent in target quadrant during probe test were plotted in **Figure 2E** (males) and **Figure 2F** (females). Two-way ANOVA analytic results showed no main effect of *Sort1-/-* ($F_{(1,22)}$=0.27, p=0.61, males; $F_{(1,22)}$=2.64, p=0.12, females), no main effect of AD ($F_{(1,22)}$=0.12, p=0.73, males; $F_{(1,22)}$=2.13, p=0.16, females) and no *Sort1-/-* × AD interaction ($F_{(1,22)}$=0.39, p=0.54, males; $F_{(1,22)}$=0.91, p=0.35, females) in the percentage of time spent in target quadrant in males or females.

Discussion

Overall, the present study examined the role of *Sort1* deficiency in the gender-specific anxiety behaviors and cognition in aged mice with or without the AD transgenes. It showed that *Sort1* deficiency increases the anxiety-like behaviors in aged female but not male mice in normal aging or in the presence of AD transgenes. It also showed that only female *APP/PS1* mouse model of AD showed hyperactivities and anxiety-like behaviors. Finally, it showed that *Sort1* deficiency did not change cognitive performance in any gender of aged mice with or without AD transgenes. The present study confirmed the role of sortilin in regulation of anxiety-like behavior and revealed a gender-specific regulation of these behaviors in aged female mice.

The study first showed that the 9-month-old female but not male mice deficient in *Sort1* had anxiety-like behaviors. As we previously have discussed [21], the most likely mechanism under '*Sort1*-deficiency induced anxiety-like behavior' is probably due to *Sort1* deficiency-

induced dysfunctional regulation of proBDNF [23] or neurotensin [27] which contributes to the pathogenesis of anxiety disorders [24,28]. In regard to the gender-dependent anxiety caused by *Sort1* deficiency in aged mice in the open field test, this is consistent with our previous results which showed that young female mice deficient in *Sort1* tended to be more anxious than males in both the open field test and elevate plus maze test [21]. The results suggest that deletion of sortilin may alter the metabolism of female hormones (estrogen and progesterone) or their receptors. Given that sortilin is a 'broad-spectrum' intracellular sorting receptor, it is likely that lack of sortilin may affect the secretion of the anxiolytic female hormones [30,31] or their uptakes or their cellular transport; and lower level or deregulated locations of these hormones result in the development of anxiety-like behavior. Another possibility is due to the interaction of estrogen (or progesterone) and BDNF (or neurotensin) [32-36]. The deregulated BDNF (or neurotensin) system, which is caused by lack of sortilin, may in turn affect the stabilization of these female hormones. The study also showed that 9-month-old female but not male mice deficient in *Sort1* showed anxiety-like behavior under Alzheimer's condition, which is consistent with the above findings in normal aging. In addition, a significant effect of interaction between *Sort1* deficiency and AD transgenes suggests that *Sort1* deficiency worsens the anxiety-like symptoms in the mouse model of AD. It is known that the prevalence of anxiety disorders declines in patients with age [37], which is likely to occur due to increased capability in coping with negative life impact due to social skills gradually developed over time [38]. Although we did not observe a significant decrease in the anxiety-like behavior in 9-month-old (this study) *vs* 3-month-old (Ruan *et al.*, previous study [21] WT mice of both males ($t_{(11)}$=1.08, p=0.30) and females ($t_{(11)}$=0.84, p=0.42), we observed male but not female *Sort1*-/- mice showed a significant decline in this behavior (males: $t_{(11)}$=4.48, p<0.001; females: $t_{(11)}$=-1.21, p=0.25; Student t-test), which further confirms the female-specific role of sortilin in the regulation of anxiety-like behaviors. Furthermore, the present study did not show anxiety-like behavior in the aged male *APP/PS1* mice in open field test, which is consistent with the studies conducted in 9-month-old [39] or 12-month-old [40] male *APP/PS1* mice without showing anxiety-like behavior in both open field test and elevated plus maze test. However, the present study showed hyperactivities and anxiety-like behavior in the aged female *APP/PS1* mice in open field test. Two other studies which were conducted in 9-month-old or 18-month-old [41] *APP/PS1* mice also showed anxiety-like behavior in elevated plus or zero maze test; however, in these studies the mice gender was not specified [42] or female mice included together with male mice [42]. Thus, further studies are required to confirm the increased anxiety-like behaviors in the aged female *APP/PS1* mice. Our study also showed that both male and female of 9-month-old mice deficient in *Sort1* showed normal cognitive skills in normal aging or Alzheimer's condition. These findings are consistent with our previous data showing no cognitive deficit in 3-month-old mice with *Sort1* deficiency [21]. Why there was no cognitive decline in 9-month-old *APP/PS1* mice and *Sort1-/-APP/PS1* mice is not known, but it is most likely because these mice were not very old. The finding is consistent with studies conducted in 9-month-old *APP/PS1* mice with both normal learning and memory behaviors [42], and 12-month-old *APP/PS1* mice with normal memory behavior [40].

To conclude, the present study shows a gender difference in the development of anxiety disorders due to genetic modifications. Although this study has a limitation as we did not perform other behavioral assessments such as elevated plus/zero maze test, the anxiety-like behavior detected in the open field test in this study is consistent with the behavior in elevated plus maze test as described in our previous study [21]. In addition, open field test has been well examined as a robust paradigm to assess anxiolytics [43]. We will continue to investigate the underlying mechanism involved in the gender-specific regulation of sortilin in anxiety-like behavior in our future studies.

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