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Second to fourth digit ratio (2D:4D) is associated with dementia in women

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Summary

Alzheimer's disease is characterized by sex differences that may involve sex hormone exposure during development. Finger length ratios, an indirect measure of prenatal androgen exposure, were found to significantly differ in women with and without dementia. This finding links a relatively feminine *in utero* development with vulnerability to dementia in women.

Keywords

Alzheimer's disease; dementia; prenatal androgens; sex differences; sexual differentiation

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is characterized by numerous sex differences. In particular, women show higher prevalence of AD than men, which may reflect in part a female bias in age-specific risks to the disease [1, 2]. The underlying causes of sex differences in AD and AD-related dementias (ADRDs) are uncertain. The most widely supported concept is that sex differences in ADRDs reflect sex-specific patterns in the age-related declines of primary sex steroid hormones, estrogens in women and androgens in men. Indeed, the loss of neuroprotective effects by sex hormones is strongly linked with the regulation of multiple components of AD pathogenesis as well as increased AD risk [3].

In addition to their activational effects in adulthood, sex hormones also exert profound organizational effects during development. In particular, increased levels of testosterone and

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Author contributions

JJ participated in the recruitment of subjects, collection and analysis of data, and revision of the manuscript. KY participated in the recruitment of subjects, collection and analysis of data, and approval of the manuscript. CJP conceptualized and designed the study, participated in data analysis, performed the statistical analyses, provided funding resources, and wrote and revised the manuscript.

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Declaration of interests
None.

estradiol during specific developmental periods are closely linked with sexual differentiation of the brain [4]. Organizational effects of sex steroid hormones yield neural variations along a spectrum of relatively more masculine versus feminine brains that may differ in their inherent vulnerability to neural disease or its expression. Consistent with this possibility, several neurological disorders exhibit significant sex differences in prevalence and/or presentation, including autism, schizophrenia, attention deficit disorders [5], and yet their onsets typically occur decades prior to age-related declines in sex steroid hormones. Unknown is whether sex differences in ADRDs may involve organizational sex hormone actions during early sexual differentiation.

One of only a few approaches to assess differences in the *in utero* sex hormone milieu that drives sexual differentiation is the ratio of the lengths of the second (digit 2) and fourth (digit 4) fingers (2D:4D) [6]. Prenatal androgens induce modest but significant changes in digit length resulting in 2D:4D that, on average, are higher in women than in men [7]. To investigate the possible contribution of organizational effects of sex steroid hormones to dementia risk, we assessed right hand 2D:4D in a community sample of elderly men and women with and without dementia. The data show that dementia in women, but not in men, is associated with significantly higher 2D:4D. This finding provides novel evidence that women with a relatively more feminine sexual differentiation may be at inherently greater risk for ADRDs.

2. Subjects and methods

2.1 Subjects

Subjects were recruited from community adult care centers and assisted living facilities. Under the approval and supervision of center personnel, all residents were informed of the study and welcomed to volunteer for a hand scan. Participants were asked to identify their gender, provide their age, and answer whether they had been diagnosed with significant memory impairment or dementia. Those with a positive history of dementia or significant memory impairment comprised the ‘demented’ group; all other subjects were classified as ‘non-demented’. In subjects with a positive diagnosis of dementia, there was an additional query about the type of dementia, if known. For some persons, requested information was provided by caregivers. No personal identifiers were collected. A total of 167 subjects – 81 men and 86 women – participated in the study. Exclusion criteria were age <65 years (11 excluded) or diagnosis of non-ADRD dementia (4 excluded). Further, subjects for which adequately focused hand images could not be attained were also excluded (13 excluded). The final subject pool for 2D:4D analysis consisted of 73 women (39 non-demented; 34 demented) and 66 men (31 non-demented; 35 demented). The study was conducted under a protocol (USC UPIRB # UP-13–00035) approved by an institutional review board.

2.2 Digit ratio determination

Images were collected of subjects’ hands using a scanner (Epson; Long Beach, CA). Subjects were instructed to place their hand on the scanner surface, palm side facing down, as flat as possible. Collected images were used to determine 2D:4D. Lengths of the second and fourth digits were made using a Vernier caliper with 0.01 mm sensitivity, measuring

from the midline of the basal, palmar crease to the tip of the finger. Each digit was measured twice, and the average of these values was used to calculate 2D:4D. All measurements were made by a single investigator who was blind to group designation during measurements and analyses. Because a meta-analysis of numerous 2D:4D studies demonstrated a much stronger effect of prenatal androgens on right hand 2D:4D [8], our analyses were restricted to right hand measurements.

2.3 Statistical analysis

Raw data were statistically analyzed using Prism software (version 8.3.1, GraphPad Software; San Diego, CA). The Kolmogorov-Smirnov test was used to assess normal distribution. Unpaired *t*-tests were used to compare sex differences in descriptive measures within the total subject pool. Pearson's *r* was used to correlate age and 2D:4D. Group differences in 2D:4D were compared using two-way ANOVA with sex and dementia status as the independent variables, followed by Tukey *post hoc* test for pairwise comparisons. Statistical significance was set at $p < 0.05$. Data are presented as means $\pm$ standard error of the mean.

3. Results

Across all subjects, men and women exhibited similar age ranges, with men between 65–97 years and women 65–104 years. The mean age in women (84.5 ± 1.0 years) was slightly higher than in men (82.8 ± 1.1 years), though this difference was not statistically significant (Table 1). In comparing right hand 2D:4D across sex, we observed normally distributed ranges of values (Kolmogorov-Smirnov test, $p > 0.1$) that overlapped between sexes (Table 1). There was a significant difference in which women showed higher mean 2D:4D values than men (Table 1). To ensure that the observed sex difference in 2D:4D was not associated with the subtle sex difference in mean age, we compared correlations between age and 2D:4D in both sexes and found them to be statistically non-significant (Table 1).

Upon stratifying subjects by dementia status, we observed no statistically significant differences in mean ages between demented and non-demented groups in women (86.6 ± 1.4 vs. 82.8 ± 1.8 ; $t = 1.56$, $p = 0.12$) and men (82.9 ± 1.3 vs. 82.7 ± 1.7 ; $t = 0.07$, $p = 0.94$). In comparing right hand 2D:4D, we observed a significant interaction between sex and dementia ($F_{(1,135)} = 10.57$, $p = 0.001$) in which females with dementia exhibited a significantly higher 2D:4D than women without dementia ($p = 0.006$) (Fig. 1). Conversely, there was no significant difference in 2D:4D between men with and without dementia ($p = 0.57$).

4. Discussion

Although AD is characterized by numerous sex differences, the source(s) of these differences is not clearly known. There is strong evidence that sex-specific patterns in the age-related depletion of estrogens and testosterone differentially contribute to increased vulnerabilities of female and male brains, respectively, to AD pathogenesis [3]. There is also evidence that gender differences, including access to education, may impact sex differences in dementia risk [9]. Largely unstudied is whether well-established differences between male

and female fetuses in exposure to sex hormones impacts late life development and or manifestation of AD [3]. This study provides the novel finding that right hand 2D:4D, a marker of prenatal androgen levels [8], is significantly higher in women with dementia relative to women without dementia. The implications of this observation are that a relatively more masculine *in utero* development confers protection against AD for women and, relatedly, a more feminine *in utero* prenatal environment may predispose women to AD.

Sexual differentiation is initiated *in utero* in response to sexually dimorphic patterns of hormones, particularly the sex hormones testosterone and estradiol. Male fetuses exhibit relatively high testosterone levels that peak in the first third of gestation, whereas female fetuses show comparatively low testosterone but elevated estradiol that is highest in late gestation [10]. High prenatal androgens induce a wide range of permanent effects in brain and other tissues that have been experimentally studied in several species including primates [11]. One effect of prenatal androgens is to promote the growth of the fourth digit, which contributes to the lower 2D:4D characteristic of males, at least in mice [12]. Our finding of higher 2D:4D in women with dementia suggests that a relatively more masculine *in utero* hormonal milieu may yield a neural environment that is less conducive to development of ADRDs. Consistent with this possibility, prior observations in the 3xTg-AD mouse model of AD demonstrated that perinatal sexual differentiation affects adult development of neuropathology. Specifically, it was found that experimentally de-feminized (*i.e.*, relatively masculine) female mice showed brain regional decreases in β -amyloid deposition, whereas de-masculinized (*i.e.*, relatively feminine) male mice had brain-region specific increases in pathology [13].

Although dementia typically develops in late life, there is no *a priori* reason that vulnerability to ADRDs cannot at least partially involve events that occur during early development. Indeed, accumulating evidence indicates that numerous early life factors can impact vulnerability to dementia [14]. Our findings reinforce this position, emphasizing early biological regulators of relative masculinity-femininity as contributors to dementia risk. Consistent with this idea, conceptually parallel findings were recently reported that compared dementia risk in same-sex (SSDZ) versus opposite-sex (OSDZ) dizygotic twins [15]. In this approach, a female with a twin sister (SSDZ) is presumed to have a relatively more feminine *in utero* environment than a female with a twin brother (OSDZ). It was observed that dementia risk was significantly higher for women from SSDZ than OSDZ twin pairs [15], suggesting that relatively more feminine prenatal environments are associated with dementia in women. Interestingly, in both the present 2D:4D study and the recent twin study [15], dementia in males was not associated with a more feminine *in utero* environment; indeed in both studies males with dementia showed statistically non-significant trends of linkage with more masculine development. The meaning of these trends is unclear. One possibility is that for specific traits, the masculinizing effects of prenatal androgen exposure may exhibit a ceiling effect in which maximal effects are observed in all normal males [7], which would be consistent with the observed absence of a significant 2D:4D relationship with dementia in men. Further, in the context of the male pattern of early development, a modest shift towards a feminine pattern may provide advantages that reduce dementia risk in men. Perhaps in contrast to these observations relating dementia in women with feminine prenatal environments, a prior study found that dementia in women showed

no relationship with right hand 2D:4D and, perhaps surprisingly, was associated with significantly lower left hand 2D:4D (*i.e.*, relatively masculine) [16]. The reasons for the disparate findings of the latter study are uncertain, but may be related to the very limited size of the subject pool (N=40) and or the association only with left hand 2D:4D, which is not as clearly linked with prenatal androgens as right hand 2D:4D [8].

Numerous mechanisms may independently or interactively contribute to how development of relatively more feminine phenotypes increases vulnerability to dementia. For example, changes in lifetime exposure to estrogens, which are affected by various aspects of reproductive function including ages of menarche and menopause, have been linked with both dementia risk [1, 9] and 2D:4D [17–20] in women, though these relationships are complex. Another possibility is microglia cells, the brain's macrophage and primary immune-related cell type [21]. Microglia have essential contributions to the sexual differentiation of the brain [22], show significant transcriptomic and functional sexual dimorphisms in adult brain [23], and are implicated as key regulators of AD pathogenesis [24]. Further, men and women exhibit significant differences in aspects of metabolic and inflammatory functions, both of which have roles in development of AD [25, 26]. Interestingly, there are also sex differences in the effects of apolipoprotein E allele 4 (APOE4), the predominant genetic risk factor for late-onset AD [27]. Deleterious APOE4-associated outcomes, including risk for AD [28–30], conversion from normal cognition to mild cognitive impairment [29–31] and even pathology in rodent models of AD [32, 33] are typically reported to be higher in females than in males.

There are limitations to this study and its implication that feminine patterns of early development are associated with dementia in women. First, while the female sex bias in dementia is associated primarily with AD [34], the subjects in this study showed a mixture of significant cognitive impairment that was not limited specifically to AD. Subjects with diagnoses that were not AD (*e.g.*, vascular dementia) were excluded. Nonetheless, the subjects assigned to the dementia group represent a broader group of ADRDs. Next, there are several factors that could have contributed to some of the findings that were not assessed, including education and socioeconomic status. There are also methodological limitations. 2D:4D is an indirect and imperfect indicator of prenatal hormonal milieu, though it is argued to be one of the few available tools suitable for this purpose [6]. Importantly, our 2D:4D findings are corroborated by a recent study of dementia risk in SSDZ and OSDZ twin pairs [15], a complementary approach for considering how subtle differences in prenatal environments can significantly impact sex differences across age. Finally, our findings are based on a modest sample size. Although the number of subjects is consistent with the 2D:4D literature, replication of our results with a larger, more completely characterized sample should be pursued in future work.

In summary, this study provides novel evidence that women's vulnerability to dementia is increased by a relatively more feminine *in utero* environment. Sex steroid hormones, in particular estradiol and testosterone, have numerous neuroprotective actions in adult human brain, the decline of which is implicated in increasing risk of women and men, respectively, to AD [3]. These well-established, age-related losses of activational sex hormone effects have been widely assumed to be the primary factors responsible for sexual dimorphisms in

AD [1]. Here, we demonstrate that organizational effects of sex hormones that occur *in utero* and are essential to normal sexual differentiation also significantly impact vulnerability to dementia, though this effect appears limited to women. Moving forward, understanding the mechanisms underlying this relationship promises to provide valuable insight into understanding AD etiology and developing therapeutic strategies.

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Highlights

- Women with dementia have higher second-to-fourth digit ratios than women without dementia.
- Prenatal sex hormone milieu may impact late life development of dementia in women.
- Sex differences in dementia risk may reflect in part developmental sexual differentiation.

Figure 1.

Comparison of second-to-fourth digit ratios (2D:4D) in elderly men and women without dementia (open circles; N= 31 men, 39 women) or with dementia (filled squares; N= 35 men, 34 women). Higher 2D:4D is an indicator of low prenatal androgen exposure and thus considered relatively feminine. Data show mean±SEM values. ns: not statistically significant; ** $p = 0.006$.

Table 1

Comparisons of age and right hand 2D:4D across total sample of women and men.

Women (N=73)	Men (N=66)	<i>t</i> Value	<i>p</i> Value
Age range: 65 – 104 years	Age range: 65 – 97 years		
^a Mean age: 84.5 ± 1.0 years	Mean age: 82.8 ± 1.1 years	1.09 (138)	0.28
2D:4D range: 0.877 – 1.105	2D:4D range: 0.839 – 1.028		
Mean 2D:4D = 0.964 ± 0.004	Mean 2D:4D = 0.948 ± 0.005	2.37 (138)	0.02 *
Age X 2D:4D correlation: $r = 0.04$, $p = 0.73$	Age X 2D:4D correlation: $r = -0.03$, $p = 0.78$		

^aMeans ± standard error of the mean

* Statistically significant