Investigating the Correlation between 2D: 4D Ratio and Risk for Alzheimer’s Disease

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Abstract
In mice, it has been observed that females are more susceptible to Alzheimer’s disease (AD) than males, with their pathology being visible more prominently and earlier than in males. However, it has also observed that the more feminized a male mouse is, the more vulnerable it appears to be to the onset of AD pathology, which could indicate that prenatal hormone exposure also affects vulnerability to the disease. To uncover if the same trend exists in humans, we are conducting a study to examine if exposure to different levels of testosterone in the womb correlates to predisposition to AD later in life. To determine prenatal testosterone exposure, we are using the length of the second finger (2D) compared to the fourth finger (4D) on study participants' hands, known as their 2D:4D ratio, which is affected by prenatal testosterone exposure. Lower 2D:4D ratio correlates to higher testosterone exposure in the womb, whereas a higher ratio correlates to lower testosterone exposure. We will compare the average 2D:4D ratio of two groups: AD patients and age-matched, healthy subjects.

Introduction
Women have higher incidence and prevalence of AD. The increased risk of AD in women has been largely attributed to the loss of protective estrogen actions resulting from ovarian hormone depletion at menopause. Estrogen depletion is similarly linked to increased risk of other disorders, including osteoporosis and cardiovascular disease. In mice, researchers have also observed that females are more susceptible to AD than males, with pathology visible more prominently and earlier than in males. However, researchers have also observed that the more feminized a male mouse is, the more vulnerable it appears to be to the onset of AD pathology, which has caused them to wonder if prenatal hormone exposure might also affect vulnerability to the disease.

Preliminary study of correlation between prenatal hormone exposure and AD severity in mice: To investigate this possibility, the Pike lab conducted a study in which they altered the relative prenatal hormone levels to which male and female triple-transgenic mice were exposed. Male triple-transgenic pups were treated with androgen receptor antagonist flutamide to desmasculinize them, while female triple-transgenic pups were treated with testosterone propionate (TP) to defeminize them. Disease severity was measured as compared to control triple transgenic mice in 2 ways: beta amyloid load was quantified in the subiculum, CA1 region of the hippocampus, and the frontal cortex in mice sacrificed at 7 months of age, and the mice were placed in a Y maze to assess their performance on a spontaneous alteration behavior task.

The results obtained in the female mice were inconclusive. Defeminized females had reduced beta amyloid load in the frontal cortex but an increase in the hippocampus as compared to controls, and no significant difference in spontaneous alteration behavior task performance was observed between the 2 groups. In male mice, however, the demasculinized males showed significantly worse disease pathology than controls in both assessments, with increased beta amyloid in the subiculum and the
hippocampus and significantly poorer performance on the spontaneous alteration behavior task. Thus this study concluded that decreased exposure to prenatal testosterone did appear worsen the pathology of AD in the male mice.

**Designing a study to examine the correlation between prenatal testosterone exposure and AD risk in humans:** In humans, it is well established that developmental patterns of estrogen and testosterone exposure induce numerous structural and functional differences between male and female brains. Importantly, men and women exhibit different vulnerabilities to several neurological disorders that occur prior to age-related hormone depletion, including post-traumatic stress disorder, schizophrenia, multiple sclerosis, autism, attention deficit disorder, and Tourette’s syndrome. The proposed research will provide novel insight into whether the increased risk of AD in women involves developmental effects of hormones, just as it does in mice.

Because we cannot directly measure levels of prenatal testosterone exposure, we will use 2D:4D ratio to measure this indirectly. Digit ratio has been examined extensively over the past decade as a method of determining prenatal testosterone exposure levels, or the amount of male sex hormones a fetus is exposed to while growing in the mother’s womb. Evidence suggests that the 2D:4D ratio is affected by these prenatal androgen levels. Lower exposure to testosterone in the mother’s womb correlates with a 2D:4D ratio near one, whereas higher levels of exposure correlate with a 2D:4D ratio significantly less than one. Thus, research to determine if a direct link does in fact exist between androgen hormone levels and the 2D: 4D digit ratio has increased over the past few years. These studies have largely concluded that a direct link is present.

*Figure 1. Histograms of the distributions of the 2D:4D ratio for 400 females and 400 males. Age range is 2–25 years. The female distribution has a mean of 1.00 and the male a mean of 0.98. Figure courtesy of Manning et al. 1998*

The effect of sex steroid hormones on 2D:4D ratio is thought to be modulated through the presence of receptors sensitive to either testosterone or estrogen in both the second and fourth digits of the hand. These receptors, which are present in far greater number in the fourth digit, control the rate of chondrocyte differentiation in the fingers of a fetus. Testosterone increases the rate of chondrocyte differentiation, making the finger grow faster, while estrogen slows this differentiation. Because the fourth digit has a greater concentration of these hormone receptors, a higher ratio of prenatal testosterone to estrogen exposure will cause
the fourth digit to grow more quickly than the second, yielding a lower 2D:4D ratio\textsuperscript{12}.

Because androgen levels affect development and differentiation of many tissues, they can be predictive of increased or decreased risk of several conditions or diseases. Our aim is to determine whether or not they can serve as a marker for risk of developing AD later in life.

\textbf{Materials and Methods}
Participants for both the control group and the AD group were recruited through the Orange County Chapter of the Alzheimer’s Association. Participants and/or their caretakers were given the following instructions on how to make photocopies of both their right and left hands, using the procedure of Manning \textit{et al}\textsuperscript{13}.

1) Place hand palm down on the photocopier with fingers together and fully extended. Make sure the hand is as flat as possible, and then make a photocopy. Repeat for other hand. Please make sure that all finger creases are visible on both photocopies.

2) Staple the photocopies together, then label with the age and gender of the participant.

The participants will either mail or send scanned versions of their photocopies back to our lab. Each photograph will be marked with an individual, multi-digit code that allows it to be placed in the correct study group later, but ensures that all measurements are made blind to the status of the individual (i.e. whether they are in the AD or control sample). Then, the second and fourth finger on each photocopy will be measured from the basal crease to the tip of the finger using a digital caliper with 0.01-millimeter resolution. The measurement obtained for the second finger will be divided by the measurement obtained for the fourth finger to calculate the 2D:4D ratio of each individual photocopy. This method has been shown to have a high degree of repeatability\textsuperscript{14}.

\textbf{Results}
After all measurements are made, the photocopies will be divided into 4 groups: female AD patients, male AD patients, female control, and male control. The average 2D:4D ratio of each group will be calculated for both the right and left hand. The averages of both hands for the two female groups and the two male groups will be compared to test the hypothesis. Independent T-tests will be performed to determine statistical significance.
Discussion
As AD becomes increasingly prevalent, research that provides insight into the mechanisms of the disease is more important than ever before. Previous efforts to treat and prevent AD in humans with hormone therapy have been largely ineffective, and although new treatment strategies tested in mice and rats have had greater success, they are far from a cure to the disease. If our hypothesis is correct, meaning we find that the control groups have a lower average 2D:4D ratio than the AD groups, this indicates that higher levels of prenatal testosterone exposure may lower the risk for AD later in life. Though this result purely indicates correlation between high prenatal testosterone and low AD risk, 2D:4D could still potentially serve as an additional diagnostic tool for physicians trying to assess AD risk.

This study aims to bring us a step closer to understanding how brain development affects disease pathology. The next step would be to investigate what specifically about the organizational effects of prenatal hormone exposure on the brain makes someone more or less vulnerable to AD. Though difficult to conduct, a longitudinal study that directly measures prenatal hormone exposure and correlates this to risk for AD later in life would also be a viable next step, as 2D:4D ratio is an imperfect measure of prenatal hormone exposure. The more insight that can be gained about the exact causes and mechanisms of Alzheimer’s disease, the greater the likelihood that a cure can one day be developed.

References
8 Cosgrove, Kelly P., Carolyn M. Mazure, and Julie K. Staley. "Evolving knowledge of sex differences in brain structure, function,


