Name: Kentaro Hayashi

Email: hayashi3@wisc.edu

Major Professor: Craig S. Atwood, Ph.D.

Degree Objective: Ph.D. Endocrinology and Reproductive Physiology

Background: B.S., Biology Shinshu University, Nagano, Japan, M.Sc., Human Sciences Shinshu University, Nagano, Japan

Current Research Project:

Gene-Gene Interactions in the Steroidogenic Pathway in the Prediction of Alzheimer's Disease

Apolipoprotein E (APOE) ε4 allele is reported as the major risk factor for late-onset Alzheimer's disease (AD), though ~50% of AD patients do not carry the allele. The function of APOE is to transport cholesterol for luteinizing hormone (LH)-regulated steroidogenesis, and both LH and neurosteroids have been implicated in the etiology of AD. In our previous study, we scored AD DNA samples and age-matched control samples for APOE genotype and 14 single nucleotide polymorphisms (SNP) of LHB and LHCGR. Thirteen gene-gene interactions between the loci of *LHB*, *LHCGR*, and *APOE* were associated with AD. The most strongly supported of these interactions was between an *LHCGR* intronic polymorphism (rs4073366; lhcgr2) and *APOE* in males, which was detected using all three interaction analyses: linkage disequilibrium (LD), multifactor-dimensionality reduction, and logistic regression (LR).

The focus of our studies has centered on pathways that regulate steroidogenesis, since it is postulated that the endocrine dyscrasia associated with menopause, and andropause in men, is central to senescent changes leading to age-related diseases. Indeed, the incidence of a range of age-related diseases in both genders is elevated in those with lower circulating concentrations of sex steroids. Therefore, identifying the underlying genetic factors that regulate basal circulating sex steroid concentrations is of scientific, prognostic and diagnostic importance.

Correlation Study between Circulating Steroid Concentrations and AD in Post-menopausal Women and Andropausal Men

Post-menopause, and during andropause, the concentrations of sex steroids declines significantly. Since steroids have a great impact on body health and function, especially brain health and function, we analyzed (using LC/MS/MS for the measurement) the concentrations of 11 steroidogenic pathway members including progesterone, 11-DOC, aldosterone, 17α-OH-progesterone, cortisol, cortisone, DHEA, androstenedione, testosterone, estrone and 17β-estradiol in a total of 157 plasma samples from age-matched post-menopausal women (n = 76; age = 74.2 ± 7.9; mean ± S.D. (28 AD; age = 75.43 ± 10.37, 48 Control; age = 73.44 ± 6.11)) and elderly men (n = 81; age = 73.3 ± 8.7 (50 AD;

age = 73.82 ± 10.44, 31 Control; age = 72.35 ± 4.78)) collected from the Wisconsin ADRC and statistically analyzed the association between AD and those steroid concentrations. Our results indicate that aldosterone ($p < 0.01$), cortisone ($p < 0.01$), androstenedione ($p < 0.001$), and estrone ($p < 0.01$) concentrations in AD are significantly lower than in control in both genders. Moreover, the recursive partitioning (RP) analysis using the ratios of those steroids which have significant association with AD showed 64% of prediction with 100% of accuracy in both genders. Those results indicate that statistical analyses using ratio of hormone concentrations are better predictive models for AD.

Gene-Gene Interactions in the Steroidogenic Pathway in the Prediction of Circulating Sex Steroid Concentration

To address which genetic factors regulate basal circulating sex steroid concentrations, we obtained 132 matched serum and DNA samples from age-matched women (n = 64; age = 76.6 ± 7.04) and men (n = 68; age = 76.6 ± 7.04). These samples were analyzed for 17β-estradiol (E_2) and follicle-stimulating hormone (FSH) concentrations and 115 SNPs in genes that regulate sex steroid synthesis, catabolism, inactivation and elimination. Our data indicate a wide variation in the concentration of circulating sex steroids, including E_2, in both post-menopausal women (range: 12-42 pg/mL) and age-matched men (range: 12-70 pg/mL). Moreover, age-matched males had significantly higher circulating concentrations of E_2 than post-menopausal females (mean = 37.9 ± 12.1 pg/mL vs. 21.7 ± 8.4 pg/mL; $p<0.0001$). RP analyses of these results stratified by splitting the sample into either high or low circulating E_2 revealed that males (n = 33 high, 35 low) containing 1 or 2 T alleles in an *FSHR* exonic polymorphism and who also were T allele homozygous in an *HSD17B1* intronic polymorphism had lower circulating E_2 concentrations 100% of the time (n = 11).

Importantly, these results makes biological sense since a change in *FSHR* signaling induced by this missense mutation (Ala → Thr, position 281) and the intronic-induced changes in 17β-HSD expression, which converts E_1 and androstenedione/T into E_2, would be anticipated to modulate E_2 concentration. In females (n = 32 high, 32 low), those heterozygous (G/C) for an intronic SNP in *LHR* were 82% likely to have lower circulating E_2 concentrations. These results support the utility of identifying gene-gene interactions in identifying complex human traits such as circulating sex steroid concentration.

Gene-Gene Interactions in the Steroidogenic Pathway in the Prediction of AD

GWAS has reported various SNPs associated with AD; however, the predictive powers of those SNPs are still less than 1%. It is because GWAS has analyzed only single main-factor effect. Therefore we hypothesized that gene-gene interaction is the better predictor for AD. To examine whether genetic factors regulating circulating hormone concentrations predict AD, we used Caucasian 839 DNA samples obtained for age- and gender-matched AD and control subjects from NCRAD and ADRC, and genotyped 146 SNPs in genes that regulate sex steroid synthesis,

catabolism, inactivation and elimination and 21 SNPs which have recently been reported the association with AD by GWAS. Our single main-factor effect analysis indicates that *APOE* and *TOMM40* are significantly associated with AD as same as previously reported. None of 21 SNPs reported by GWAS were significantly associated with AD in our cohort except *APOE*. Then gene-gene interaction analyses, RP analysis using whole DNA samples indicated that 1) 61.5% of individuals with *APOE* genotype epcilon 4 (ε4) positive, 2) 71.4% of individuals with *APOE* ε4 negative, C/C and T/T alleles at *LDLR*_3, C/T allele at *SORL1*_2, C/G allele at *FSHR*_13 were classified as AD. Conversely, 85.4% of individuals with *APOE* ε4 negative, C/T allele at *LDLR*_3 were protected from AD. RP analysis using female DNA samples indicated that 1) 60.2% of individuals with *APOE* ε4 positive and 2) 77.8% of individuals with *APOE* ε4 negative, T/C allele at *ESR*_2, T/T allele at *SULT2A1*, T/T allele at *MS4A4A*, C/C allele at *CYP46A1*_2 were classified as AD. Conversely, 88.5% of individuals with *APOE* ε4 negative and T/T and C/C alleles at *ESR*_2 were protected from AD. RP analysis using male DNA samples indicated that 81.8% of individuals with *APOE* ε4 positive and G/G allele at *HSD17B1*_1 were classified as AD. Conversely, 100% of individuals with *APOE* ε4 negative and A/A allele at *PGR*_6 were protected from AD. We also performed RP analysis only with 21 SNPs reported by GWAS; however, the predictive power was less than the RP result using all of our genotyped SNPs data. These results indicate that *APOE* genotype is the most important factor to predict AD, and gene-gene interaction analyses are better predictive models for AD.

Utilization of Fibroblast Steroidogenesis as a Predictive Marker for Alzheimer's Disease

Peripheral tissues may be a useful marker for determining post-reproductive sex steroid synthetic capacity. We have therefore focused on tissues that produce sex steroids. Fibroblasts are one such tissue cell type that constitute connective tissue and are easily acquired. The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Fibroblasts produce the ingredient of dermis called collagen, elastin, hyaluronic acid and so on. In 2004, Slominski et al. reported the expression of p450scc protein in human skin, suggesting that fibroblast might produce sex steroids. However, this has never been reported, and so our initial studies have examined the steroidogenic potential of fibroblasts. First, we measured the secretion of progesterone (P_4) from fibroblasts treated with LH and human chorionic gonadotropin (hCG). Both LH and hCG induced P_4 secretion from fibroblasts. On the other hand, pregnenolone (P_5) treatment did not increase the P_4 secretion compare to the control.

We next identified the expression of steroidgenic proteins in fibroblasts - StAR, p450scc and GnRHR proteins. LH increased the expression of the active form of StAR and GnRHR, indicating that LH promotes cholesterol transport into the mitochondrion for sex steroid synthesis. Interestingly, P_4 and P_5 decreased the expression of the active form of StAR, and only P_4 increased StAR inactive form. Both P_4 and P_5 decreased p450scc and GnRHR. These results suggest that P_4 and P_5 negatively feedback to decrease the GnRH signaling, cholesterol transport into the mitochondria, and cholesterol utilization for P_4 production. These results indicate both positive and negative feedback in fibroblasts as has also been demonstrated in the brain.

Honors:

Grants Received:

Publications:

- Atwood, C.S. and **Kentaro Hayashi** (2010) Is a Therapeutic Answer to Alzheimer's Disease Already Sitting in Our Lap? (Alzheimer's Research Forum)
- Sivan Vadakkadath Meethal, **Kentaro Hayashi**, Craig S. Atwood (2013) Human Stem Cell Proliferation and Differentiation: Lessens From a Lost Era of Research

National Presentations:

- Kentaro Hayashi and Atwood, C.S. (2014) Identification of Gene-Gene Interactions in the Steroid Metabolic Pathway That Predict Circulating Sex Hormone Concentrations (Poster presentation) ICE/ENDO 2014, 16th International Congress Endocrinology and The Endocrine Society's 96th Annual Meeting & EXPO MON-0434.

Other Presentations:

- Hayashi, K. and Atwood C.S. (2010) Identification of SNPs in Genes of the Steroidogenic Pathway that Predict Alzheimer's Disease. 22nd Annual Colloquium for the Institute on Aging.
- Hayashi, K. and Atwood C.S. (2010) Identification of SNPs in Genes of the Steroidogenic Pathway that Predict Alzheimer's Disease. ERP Research Symposium.
- Hayashi, K. and Atwood C.S. (2010) Identification of SNPs in Genes of the Steroidogenic Pathway that Predict Alzheimer's Disease. Department of Medicine Research Day.
- Hayashi, K. and Atwood C.S. (2011) Identification of SNPs in Genes of the Steroidogenic Pathway that Predict Alzheimer's Disease. ERP Research Symposium.

- Hayashi, K. and Atwood C.S. (2011) Identification of SNPs in Genes of the Steroidogenic Pathway that Predict Alzheimer's Disease. Department of Medicine Research Day.

- Hayashi, K. and Atwood C.S. (2012) Utilization of Fibroblast Steroidogenic Capacity as a Predictive Marker for Alzheimer's Disease. ERP Research Symposium.

- Hayashi, K. and Atwood C.S. (2012) Utilization of Fibroblast Steroidogeic Capacity as a Predictive Marker for Alzheimer's Disease. 24th Annual Colloquium for the Institute on Aging.

- Hayashi K and Atwood, C.S. (2013) Identification of Genetic Variants in the Steroid Metabolism Pathway that Regulate Circulating Sex Hormone Concentration. Endocrinology and Reproductive Physiology Program Annual Research Symposium.

- Hayashi K and Atwood, C.S. (2013) Identification of Genetic Variants in the Steroid Metabolism Pathway that Regulate Circulating Sex Hormone Concentration. Endocrinology and Reproductive Physiology Program Annual Research Symposium.

- Hayashi K, Gonzales T.K., Vadakkadath Meethal S. and Atwood, C.S. (2014) Identification of Genetic Variants in the Steroid Metabolism Pathway that Regulate Circulating Sex Hormone Concentration. Inaugural Alzheimer Research Day.

- Kentaro Hayashi and Atwood, C.S. (2014) Identification of Gene-Gene Interactions in the Steroid Metabolic Pathway That Predict Circulating Sex Hormone Concentrations (Poster presentation) Endocrinology and Reproductive Physiology Program Annual Research Symposium

- Hayashi K, James A. Yonker, Vadakkadath Meethal S., Gonzales T.K., and Atwood, C.S. (2014) Altered Sex Steroid Flux in Alzheimer's Disease (Oral presentation) Endocrinology and Reproductive Physiology Program Annual Research Symposium

ERP Service: