

ABSTRACT

Approximately 1% of women will experience menopause before the age of 40, a condition known as premature ovarian failure (POF). The goal of this thesis was to identify genetic causes of POF by examining a number of candidate factors in POF patients and control women. Carriers of *FMR1* premutations (55-200 CGG repeats) are known to be at increased risk of POF. A higher prevalence of alleles between 35-54 repeats was found among POF patients ($p=0.01$), suggesting that risk for POF may extend outside the classic premutation range. There was no evidence for any difference in *FMR1* promoter methylation or gene expression between cases and controls. Allele distributions of gene polymorphisms in the androgen receptor (*AR*), estrogen receptor β , sex hormone binding globulin, and FSH receptor genes did not differ between POF patients and controls. However, haplotype at the estrogen receptor α gene, *ESR1*, was found to be associated with POF in a simple dominant manner (RR=9.7; 95% CI=2.6-35.6). Although the functional effect of this haplotype could not be confirmed, it may confer a more active promoter that influences risk by increasing the rate of follicular atresia. X-chromosome inactivation (XCI) skewing can indicate an abnormal X chromosome and may therefore be increased in POF patients. There was no increase in skewed XCI $\geq 90\%$ in patients with secondary amenorrhea, however, there was a significant increase in 4 POF patients with primary amenorrhea ($p=0.001$). No X-chromosome abnormalities were detectable by high resolution DNA microarray, and skewed XCI may be explained by a trisomic rescue event causing reduced follicular pool. Age-related chromosome factors were assessed to determine if POF patients demonstrate an increased rate of cellular aging. With age, XCI skewing and *AR* methylation increase and telomere length decreases. There was no difference in skewing or methylation between patients and controls. Surprisingly telomere length was increased in POF patients ($p=0.04$), a finding that may be explained by abnormal estrogen exposure. Genotype at the longevity-associated *APOE* gene was not associated with POF. In conclusion, these findings have illuminated several new areas of research in this field and provide background for future research into POF pathogenesis.

BIOGRAPHICAL NOTES

Born: February 1, 1978, Terrace, British Columbia

Academic Studies: B.Sc. University of Calgary, 2000

GRADUATE STUDIES

Field of Study: Genetics and Women's Reproductive Health

Courses

MEDG520	Advanced Human Molecular Genetics	Dr. E. Simpson
MEDG530	Human Genetics	Dr. J. Friedman
MEDG540	Seminar	Dr. F. Dill
MEDG545	Current Topics in Medical Genetics	Dr. C. Brown
MEDG548	Directed Studies	Dr. W. Robinson
INDS502	Ethics of Research Involving Humans	Dr. M. McDonald

Instructors

AWARDS

CIHR Institute of Gender and Health, Doctoral Research Award
Michael Smith Foundation for Health Research, Senior Graduate Scholarship
UBC Medical Genetics, James Miller Award
Interdisciplinary Women's Reproductive Health Training Program, Studentship
CIHR Institute of Genetics, Doctoral Research Award
Michael Smith Foundation for Health Research, Junior Graduate Scholarship
Natural Science and Engineering Research Council, Postgraduate Scholarship A

PUBLICATIONS

- Bretherick KL**, Hanna CW, Currie LM, Fluker MR, Hammond GL, Robinson WP (2007). Survey of endocrine related gene variants reveals that estrogen receptor α polymorphisms are associated with idiopathic premature ovarian failure. *Fertility and Sterility* 89(2):318-24.
- Bretherick KL**, Metzger DL, Chanoine JP, Panagiotopoulos C, Watson SK, Lam WL, Fluker MR, Brown CJ, Robinson WP (2007). Skewed X-chromosome inactivation is associated with primary but not secondary ovarian failure. *American Journal of Medical Genetics A* 143(9):945-51.
- Bretherick KL**, Fluker MR, Robinson WP (2005). *FMR1* repeat sizes in the grey zone and high end of the normal range are associated with premature ovarian failure. *Human Genetics* 117(4):276-282.
- Bretherick KL**, Gair JL, Robinson WP (2005). The association of skewed X-chromosome inactivation with aneuploidy in humans. *Cytogenetic and Genome Research* 111(3-4):260-265.

PRESENTATIONS

Hanna CW, **Bretherick KL**, Gair JL, Stephenson MD, Brown CJ, Lansdorp PM, Robinsonson WP (2008). Shorter telomere length in women with recurrent



miscarriage. 1st Annual Canadian Human Genetics Conference, St Sauveur, Quebec, April 9-12, 2008.

- Bretherick KL**, Hanna CW, Fluker MR, Robinson WP (2007). Telomere length is increased in women with premature ovarian failure. American Society of Human Genetics 57th Annual Meeting, San Diego, Oct 23-27, 2007.
- Hanna CW, **Bretherick KL**, Gair JL, Stephenson M, Robinson WP (2007). Telomere length and skewed XCI in mothers of a trisomic pregnancy. American Society of Human Genetics 57th Annual Meeting, San Diego, Oct 23-27, 2007.
- Bretherick KL**, Fluker MR, Robinson WP (2007). *FMR1* expression is not altered in premature ovarian failure patients who lack a premutation allele. 13th International Workshop on Fragile X and X-Linked Mental Retardation, Venice, Oct 3-7, 2007.
- Bretherick KL**, Fairbrother N, Harbord SH, Avila L, Robinson WP (2006). Fertility 101: A survey of university women's knowledge of reproductive ageing. Canadian Fertility & Andrology Society 52nd Annual Meeting, Ottawa, Nov 15-18, 2006.
- Bretherick KL**, Fluker MR, Brown CJ, Robinson WP (2006). X chromosome DNA methylation: a new quantitative assay and preliminary results in women with premature ovarian failure. American Society of Human Genetics 56th Annual Meeting, New Orleans, Oct 9-13, 2006.
- Bretherick KL**, Currie LM, Fluker MR, Robinson WP (2005). Estrogen receptor polymorphisms and premature ovarian failure. American Society of Human Genetics 55th Annual Meeting, Salt Lake City, Oct 25-29, 2005.
- Bretherick KL**, Fluker MR, Robinson WP (2005). Hormone receptor variants and premature ovarian failure. European Human Genetics Conference, Prague, May 7-10, 2005.
- Bretherick KL**, Fluker MR, Stephenson M, Robinson WP (2004). *FMR1* repeat length in premature ovarian failure and mothers of trisomy. American Society of Human Genetics 54th Annual Meeting, Toronto, Oct 26-30, 2004.
- Bretherick KL**, Fluker MR, Beever CL, Anderson CL, Brown CJ, Robinson WP (2003). Skewed X chromosome inactivation in premature ovarian failure. American Society of Human Genetics 53rd Annual Meeting, Los Angeles, Nov 4-8, 2003.

SUPERVISORY COMMITTEE

Dr. Wendy Robinson (Medical Genetics)
 Dr. Carolyn Brown (Medical Genetics)
 Dr. Geoff Hammond (Obstetrics and Gynecology)
 Dr. Barbara McGillivray (Medical Genetics)

PROGRAMME

The Final Oral Examination
 For the Degree of

DOCTOR OF PHILOSOPHY
 (Medical Genetics)

KARLA LUCIA BRETHERICK

B.Sc. Cellular Molecular & Microbial Biology, University of Calgary, 2000

Monday, April 7, 2008, 9:00 am
 Room 200, Graduate Student Centre

“Genetic Factors in Premature Ovarian Failure”

EXAMINING COMMITTEE

Chair:

Dr. Basil Ho Yuen (Reproductive and Developmental Science)

Supervisory Committee:

Dr. Wendy Robinson, Research Supervisor (Medical Genetics)
 Dr. Carolyn Brown (Medical Genetics)

University Examiners:

Dr. Dan Rurak (Obstetrics and Gynecology.)
 Dr. Dixie Mager (Medical Genetics)

External Examiner:

Dr. Stephanie Sherman
 Department of Human Genetics
 Emory University
 Atlanta, Georgia
 USA