

## **PCR assay for *FMR1* used to determine the incidence of expansions in early menopause in the Breakthrough Generations Study**

Claire Bennett<sup>1</sup>, James N. Macpherson<sup>2</sup>, Patricia A. Jacobs<sup>2</sup>, Nicholas Orr<sup>3</sup>, Andrew Hadd<sup>4</sup>, Anthony J. Swerdlow<sup>3</sup> and Anna Murray<sup>1</sup>

<sup>1</sup>Peninsula Medical School, University of Exeter, Exeter, EX1 2LU

<sup>2</sup>Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, SP2 8BJ

<sup>3</sup>Breakthrough Generations Study, Institute of Cancer Research, Sutton, SM2 5BR

<sup>4</sup>Asuragen Inc, Austin, Texas 78744

*FMR1* contains a polymorphic CGG repeat that has been associated with premature ovarian failure in subjects carrying 55-200 repeats or a 'premutation'. We aimed to determine the incidence of *FMR1* premutations in a large population based cohort of women with menopause  $\leq 45$  years. The Breakthrough Generations Study (BGS) is a prospective study of breast cancer that represents an unbiased female cohort of >100,000 women from which we selected over 2500 women with early menopause, plus matched controls.

Current methods for determining the number of CGG repeats are not amenable to high-throughput testing, particularly in females. The presence of a single allele by PCR, expected in approximately 15% of women, requires a Southern blot to confirm whether the individual is homozygous or carries an expansion not detected by PCR. We have evaluated a novel assay for estimating CGG repeat size that utilises FAM-labelled PCR products separated by capillary electrophoresis (Asuragen Inc).

DNA samples from females with *FMR1* CGG repeat sizes ranging from 20 to >200 were successfully detected in pilot studies, using a single PCR from only 10ng of genomic DNA. The method appears to be sensitive and reproducible. We will present preliminary data using this assay in the Breakthrough Generations study to determine the incidence of *FMR1* expansion mutations in women with early menopause.