Array comparative genomic hybridization for the detection of submicroscopic copy number variations of the X chromosome in women with premature ovarian failure

Sir,

Evidence suggests that structural integrity of the X chromosome is important in the maintenance of ovarian function. Breakpoints of X chromosome rearrangements defined three critical regions for ovarian function between Xq13.3-q21.1, Xq26-28 and chromosome rearrangements defined three critical regions for the maintenance of ovarian function. Breakpoints of X chromosome with a resolution of 80 kb. The first clone (RP11-1051N9; 3.36–3.48 Mb) had a Cy5/Cy3 log2 ratio of 0.38 indicating this clone is partially duplicated while the other two clones (RP11-558O12; 3.46–3.66 and RP11-501G22; 3.64–3.72 Mb) had ratios of 0.58 and 0.49, respectively. The nearest neighboring clone on the array with a ratio within the normal interval (−0.04) was RP11-90D8 (3.95–4.13 Mb) demonstrating that the duplication is maximally 550 kb in size (3.40–3.95 Mb). This region only includes the gene PRKX (Protein Kinase, X-linked). In addition, it contains four non-coding RNAs; one ACA48-like, two U6-like and a hY1 RNA-like sequence. Analysis in the Database of Genomic Variants (http://projects.tcag.ca/variation/?source=hg18) revealed that the duplicated region has not been reported as a normal variant.

Case B entered menarche aged 15 and had raised gonadotrophins in the menopausal range by age 28. There was no family history of POF. Case B involved eight consecutive clones starting at 74.10 Mb (RP13-42E14) and finishing at 74.80 Mb (RP11-236O12), which correlate with a duplication at Xq13.3 of ~800 kb. Log2 ratios for these clones were 0.40 ± 0.08. The flanking ‘normal’ clones (0.04 ± 0.02) were RP11-130N24 (73.90 Mb) and RP11-324B6 (74.90 Mb). Within this region of copy number gain, the gene KIAA2022 (partially), ABCB7 (ATP Binding Cassette subfamily B member), UPRT (Uracil phosphoribosyltransferase Saccharomyces cerevisiae, homolog of) and ZDHHC15 (Zinc Finger DHHC Domain containing Protein 15) are contained. This duplication has not been reported as a normal variant in the Database of Genomic Variants.

Within this study, family members are unavailable to determine whether or not the detected duplications segregate with a history of POF. It is now clear that copy number variants are one of a number of genetic variations contributing to the phenotype of common disease (Shelling and Ferguson, 2007). The novel duplicated areas identified in two women within this cohort did contain genes of interest that could be sensitive to altered expression. Case A involved a duplication including the gene PRKX (located on Xp22.33). PRKX is expressed in a variety of adult tissues including the ovary (Blaschke et al., 2000), although the role of PRKX in normal ovarian function is unknown. Case B has a 632 kb duplication at Xq13.3 including the genes ABCB7 (ATP Binding Cassette subfamily B member) and ZDHHC15. ABCB7 is ubiquitously expressed while expression of ZDHHC15 is restricted to a few tissues (ear, pituitary gland, trachea), not including the ovary. Notably, the duplication is within a POF critical region. The genes within the duplicated regions may be potential candidate genes associated with POF. Another possibility is that defects in the X chromosome may affect the process of X

Comments of Dr Kvaskoff et al. are all of utmost interest and value. Some of the limits of our study were already mentioned in our original paper, but they are clarified in this letter better. Overall, we thus just thank the authors for their precious and constructive comments.

Edgardo Somigliana and Paola Vigano*
Center for Research in Obstetrics and Gynecology Milano, Milan, Italy
*Correspondence address. E-mail: paola.vigano@crog.it
doi:10.1093/humrep/deq251
Advanced Access publication on September 30, 2010

Array comparative genomic hybridization for the detection of submicroscopic copy number variations of the X chromosome in women with premature ovarian failure

Sir,

Evidence suggests that structural integrity of the X chromosome is important in the maintenance of ovarian function. Breakpoints of X chromosome rearrangements defined three critical regions for ovarian function between Xq13.3-q21.1, Xq26-28 and Xp11.2-22.1; however, the majority of breakpoints within these regions have been mapped to gene-free genomic regions (Prueitt et al., 2002). One hypothesis is that chromosome dynamics on the X chromosome could be sensitive to structural changes, interfering with normal chromosome pairing during meiosis, leading to accelerated oocyte apoptosis (Burgoyne and Baker, 1984). A publication by Quilter et al. (2010), reported 15 novel copy number variations (CNVs) on the X chromosome in 20/42 (48%) of women with premature ovarian failure (POF). We have generated similar results that support the hypothesis that cryptic submicroscopic CNVs of the X chromosome may be important in the maintenance of ovarian function. Breakpoints of X chromosome with a resolution of 80 kb. Data normalization was performed against the median of the spot ratios of all clones. Cy5/Cy3 ratios for each clone were plotted in a log2 scale (Y-axis) relative to the position on the X chromosome (X-axis). Clones with log2 ratios outside the −0.3–0.3 interval were considered aberrant.

A microduplication on the X chromosome was detected in 3 of the 50 patients. Analysis in the Database of Genomic Variants (http://projects.tcag.ca/variation/?source=hg18) revealed that one of the three duplications was a polymorphic copy number variant. The polymorphism involved three consecutive flagged clones, RP11-359O20, RP11-294K6 and RP11-1M18.

Case A entered menarche aged 14 and had raised gonadotrophins in the menopausal range by the age of 37; her mother was reported to have undergone menopause before the age of 30 years. Case A harbored a microduplication at Xp22.33, corresponding to three consecutive clones RP11-1051N9, RP11-558O12 and RP11-501G22, covering the region from 3.42 to 3.68 Mb. The first clone (RP11-1051N9; 3.36–3.48 Mb) had a Cy5/Cy3 log2 ratio of 0.38 indicating this clone is partially duplicated while the other two clones (RP11-558O12; 3.46–3.66 and RP11-501G22; 3.64–3.72 Mb) had ratios of 0.58 and 0.49, respectively. The nearest neighboring clone on the array with a ratio within the normal interval (−0.04) was RP11-90D8 (3.95–4.13 Mb) demonstrating that the duplication is maximally 550 kb in size (3.40–3.95 Mb). This region only includes the gene PRKX (Protein Kinase, X-linked). In addition, it contains four non-coding RNAs; one ACA48-like, two U6-like and a hY1 RNA-like sequence. Analysis in the Database of Genomic Variants (http://projects.tcag.ca/variation/?source=hg18) revealed that the duplicated region has not been reported as a normal variant.

Case A entered menarche aged 14 and had raised gonadotrophins in the menopausal range by age 28. There was no family history of POF. Case B involved eight consecutive clones starting at 74.10 Mb (RP13-42E14) and finishing at 74.80 Mb (RP11-236O12), which correlate with a duplication at Xq13.3 of ~800 kb. Log2 ratios for these clones were 0.40 ± 0.08. The flanking ‘normal’ clones (0.04 ± 0.02) were RP11-130N24 (73.90 Mb) and RP11-324B6 (74.90 Mb). Within this region of copy number gain, the gene KIAA2022 (partially), ABCB7 (ATP Binding Cassette subfamily B member), UPRT (Uracil phosphoribosyltransferase Saccharomyces cerevisiae, homolog of) and ZDHHC15 (Zinc Finger DHHC Domain containing Protein 15) are contained. This duplication has not been reported as a normal variant in the Database of Genomic Variants.

Within this study, family members are unavailable to determine whether or not the detected duplications segregate with a history of POF. It is now clear that copy number variants are one of a number of genetic variations contributing to the phenotype of common disease (Shelling and Ferguson, 2007). The novel duplicated areas identified in two women within this cohort did contain genes of interest that could be sensitive to altered expression. Case A involved a duplication including the gene PRKX (located on Xp22.33). PRKX is expressed in a variety of adult tissues including the ovary (Blaschke et al., 2000), although the role of PRKX in normal ovarian function is unknown. Case B has a 632 kb duplication at Xq13.3 including the genes ABCB7 (ATP Binding Cassette subfamily B member) and ZDHHC15. ABCB7 is ubiquitously expressed while expression of ZDHHC15 is restricted to a few tissues (ear, pituitary gland, trachea), not including the ovary. Notably, the duplication is within a POF critical region. The genes within the duplicated regions may be potential candidate genes associated with POF. Another possibility is that defects in the X chromosome may affect the process of X
inactivation, or interfere with chromosomal pairing during meiosis (Shelling, 2000).

Of interest is the lower copy number detection rate in our study (4%) compared with the detection rate of 48% in the recent publication by Quilter et al. (2010). Quilter et al. report that one of the 15 women with a CNV had primary amenorrhea. Possibilities to explain the differences in detection rate compared with this study may be differences in the age of onset of POF, or the presence of a positive family history of POF. Quilter et al. (2010) does not provide these demographic details. Further studies in larger numbers of POF patients clinically characterized by age of onset and the presence of a positive family history would help clarify the association between X chromosome CNV and POF.

Funding

This work was supported by the Hunter Medical Research Institute.

References


T.E. Dudding1*, O. Lawrence2, I. Winship3, G. Froyen4, J. Vandewalle5, R. Scott2,5,6 and A.N. Shelling2

1HMRI, Mothers and Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia
2Division of Genetics, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia
3Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Melbourne, VIC, Australia
4Human Genome Laboratory, Center for Human Genetics, Department of Medical and Developmental Genetics, K.U. Leuven, Leuven, Belgium
5Information Based Medicine Program, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW 2305, Australia
6School of Biomedical Sciences, Faculty of Health, University of Newcastle, Newcastle, NSW 2308, Australia
7Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

*Correspondence address. E-mail: tracey.dudding@hnehealth.nsw.gov.au

doi:10.1093/humrep/deq284

Advanced Access publication on October 16, 2010

Reply: Array comparative genomic hybridization for the detection of submicroscopic copy number variations of the X chromosome in women with premature ovarian failure

Sir,
The study described by Dudding and colleagues confirms the results of our own work (Quilter et al., 2010), which suggested that submicroscopic copy number variants (CNVs) of the X chromosome may be of significance for the aetiology of premature ovarian failure (POF). They have used both an array of comparative resolution and a patient cohort of similar size to our study. The two micro-duplications they detected were in Xp22.33 and Xq13.3. Although the former is not consistent with our results it is within 5 Mb of one of our novel CNV at Xp22.31, present in two of our patients with micro-duplications. The latter is within another of our reported CNV at Xq13.3–Xq21.33, present in one patient with a micro-duplication. These findings are important as they support the need for more detailed future investigations of the contribution of CNV, and the candidate genes within them, to the development of POF.

The main difference between the two studies was that in ours the frequency of CNV detected was higher (48%) compared with Dudding’s (4%). Although this is a big difference we did carry out Q-PCR to validate our results. This frequency discrepancy may be attributed to differences between the two patient cohorts with ours coming from the UK and theirs from New Zealand. The majority of our patients came from a regional hospital covering a rural area, so it is possible that there may be ancestral relationships of which we are unaware and unfortunately access to family history or samples, was not available for our study. In a comparative unpublished study on a more diverse population carried out within our laboratory, we found 9 of 40 patients with CNV. Of these, six were considered to be non-polymorphic after comparison with the database of common variants and four were on the X chromosome. This illustrates that a more diverse population can give results that are comparable with Dudding et al. (2010) and Aboura et al.’s (2009) results, and is worth taking into consideration for any future studies. In addition, 6 of 42 of our patients had primary amenorrhea and 36 had secondary amenorrhea but further clinical information was limited. Dudding et al. were able to record more clinical details, including age of onset of POF and family history. We agree with these authors that any future studies on large numbers of POF patients should include the recording of extensive clinical details, including where possible family histories and samples, so that patients can be subtyped where necessary. It may then become clearer if a pattern emerges linking CNVs to certain subcategories of ovarian failure.

Funding

This work was funded by the Department for Environment, Food and Rural affairs (DEFRA).