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 associated with POF; however, our detection rate of two novel CNVs in 2/50 (4%) of women with POF using a similar resolution X chromosome tiling pathway was lower.

For our study, patients were women with POF consecutively referred to the Department of Obstetrics and Gynaecology in Auckland; their ages ranged from 15 to 39 years (average 27 years). Ethical approval was previously described (Shelling, 2000). The diagnosis of POF was made following the cessation of menses for 6 months or more before the age of 40 years, coincident with FSH concentrations greater than 40 IU/l. All women with POF were 46, XX with normal FMR1 CGG repeats. Women with an identifiable cause of menopause were excluded. A personal and family history were taken; physical examination by a clinical geneticist excluded features of a syndrome. The X chromosome tiling path array is described elsewhere (Froyen et al., 2007). There were 1875 genomic clones from Saccharomyces cerevisiae (Uracil phosphoribosyltransferase, 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 (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 revealed that the duplicated region has not been reported as a normal variant.

Case B involved nine consecutive flagged 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.

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 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 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 genes 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 at 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.

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**References**


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**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.

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