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Cytogenetic analysis of 531 Chinese women with premature ovarian failure

Xue Jiao[1,†], Chunrong Qin[2,†], Jie Li[3], Yingying Qin[1], Xuan Gao[1], Bo Zhang[4], Xiumei Zhen[5], Yun Feng[6], Joe Leigh Simpson[7], and Zi-Jiang Chen[1,*]

[1]Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, National Research Center for Assisted Reproductive Technology and Reproductive Genetics, The Key laboratory for Reproductive Endocrinology of Ministry of Education, Shandong Provincial Key Laboratory of Reproductive Medicine, 324 Jingwu Road, Jinan 250021, China [2]Maternal and Child Health Hospital in Shenzhen, Shenzhen, China [3]Reproductive Medical Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China [4]Center for Reproductive Medicine, Maternal and Child Health Hospital in Guangxi, Guangxi, China [5]Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China [6]Reproductive Medical Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China [7]Florida International University, College of Medicine, Miami, FL, USA

*Correspondence address. Tel: +86-531-85187856; Fax: +86-531-87068226; E-mail: chenzijiang@hotmail.com

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BACKGROUND: This retrospective cohort study was to determine the frequency and types of chromosomal abnormalities in Han Chinese women with well-documented premature ovarian failure (POF).

METHODS: Karyotype analysis and correlation to phenotypes were performed on 531 Chinese patients with proven POF (FSH > 40 mIU/ml) attending four reproductive centers in China. G-banded metaphase chromosomes were prepared and analyzed, with mosaicism excluded by counting up to 100 cells from lymphocytes.

RESULTS: Chromosomal abnormalities were present in 64 of 531 (12.1%) POF cases, of which 32 were X-structural aberrations (7 mosaic): 15 del(Xq), 2 del(Xp), 11 isochromosomes [6 i(Xp); 5 i(Xq)], 1 ring chromosome (mosaic), 1 inversion (mosaic), 1 isodicentric chromosome and 1 complex arrangement. Nine non-mosaic X-autosome translocations were detected, all but 1 involving Xq. Aneuploidy without a structurally abnormal X was found in 19 cases: 7 non-mosaic 45,X, 9 45,X mosaicisms and 3 47,XXX (1 mosaic with 46,XX line). Karyotypic abnormalities were more frequent in patients with primary amenorrhea (15/70, 21.4%) than those with secondary amenorrhea (49/461, 10.6%; $P = 0.01$). 45,X and 45,X/46,XX mosaicism were the complements most frequently associated with primary amenorrhea (46.7%). Two of the three cases with 46,XY or 45,X/46,XY karyotype presented with 'secondary amenorrhea'. One balanced autosomal Robertsonian translocation was also detected.

CONCLUSIONS: The overall prevalence of chromosomal abnormalities was 12.1% in this first large scale report of chromosomal aberrations in Chinese women with POF. In one of the largest samples of women with POF reported from any population, the prevalence of X-structural abnormalities, X-autosome translocations and X aneuploidy confirms the essential role X chromosomal abnormalities play in POF.

Key words: premature ovarian failure / X chromosome abnormalities / infertility / mosaicism / karyotype

Introduction

Premature ovarian failure (POF) may be characterized by primary amenorrhea or secondary amenorrhea for at least 4–6 months duration before the age of 40 years. Gonadotrophins are elevated (FSH > 40 mIU/ml) and estrogen is in the menopausal range (Coulam, 1982). POF occurs in ~1% of the general female population before 40 years old (Coulam *et al.*, 1986). The disorder is heterogeneous, with a wide spectrum of causes, including genetic, autoimmune, metabolic, infectious and iatrogenic. However, etiology remains to be elucidated in most cases (Shelling, 2010).

Chromosomal abnormalities have long been recognized as a frequent cause of POF, with widely varying percentages in reported series (Castillo *et al.*, 1992; Portnoi *et al.*, 2006; Ceylaner *et al.*, 2010; Janse *et al.*, 2010; Lakhal *et al.*, 2010; Baronchelli *et al.*, 2011). Numerous different karyotypic anomalies have been found, and

[†]These authors contributed equally to this work.

both X chromosomal abnormalities and autosomal rearrangements have been reported. However, few large cohorts have been studied (Lakhal *et al.*, 2010; Baronchelli *et al.*, 2011). Ethnic background is usually not well characterized, thus precluding taking into account the confounding effect that Mendelian causes of POF may be unusually common in selected populations. For example, in Finland FSH receptor mutations are a relatively more frequent explanation for ovarian failure and POF than elsewhere (Aittomaki, 1994, 1995). Thus, a proportionally lower percentage of karyotypic explanations might be expected in Finnish cases of POF. We have investigated 531 Chinese females with POF in order to determine the prevalence and type of cytogenetic anomalies in this ethnic group.

Materials and Methods

Patients

Between April 2003 and June 2011, women with POF were recruited from four clinical centers: Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University (Jinan), Department of Obstetrics and Gynecology, Peking University Third Hospital (Beijing), Maternal and Child Health Hospital (Shenzhen) and Center for Reproductive Medicine, Maternal and Child Health Hospital (Guangxi). Inclusion criteria included primary amenorrhea or secondary amenorrhea for at least 4 months duration prior to the age of 40 years and two serum FSH measures of >40 mIU/ml obtained at least 1 month apart. Patients with conditions known to induce POF (chemo- or radio-therapy, ovarian surgery or autoimmune diseases) were excluded. Patients with typical Turner stigmata were also excluded. Family history was obtained and considered 'positive' if another first- or second-degree relative had either POF or early menopause (menopause before 45 years old). The study was approved by the Ethical Committee of Reproductive Medicine of Shandong University. Written informed consent was obtained from all participants.

Cytogenetic analysis

Karyotype analysis was performed on GTG-banded metaphase chromosomes prepared from peripheral lymphocyte cultures, using a standard protocol that generated 400–450 band resolutions. A minimum of 30 metaphases per patient were analyzed. If any cell among the 30 showed a non-model cell (45,X or 47,XXX), an additional 70 cells were counted. The only tissue studied routinely was blood, for which reason this report is confined to lymphocyte analysis only. Chromosome polymorphisms, for example pericentic inversion of chromosome 9 and centromeric heterochromatin variants, were recorded but classified as normal.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences 19 statistical package for Windows. Student's *t*-test and Chi-square test were used where appropriate. A P value < 0.05 was considered statistically significant. All data are presented as mean ± SD.

Results

Demographic and clinical characteristics

In the 531 cases, 70 presenting with primary amenorrhea and 461 with secondary amenorrhea, the mean age at diagnosis was 29.0 ± 4.7 years old. Other demographic characteristics are summarized in Table I. In 8.8% of these, a first- or second-degree relative had either POF or early menopause. No specific somatic anomalies were found and height was routinely recorded (160.4 ± 5.6 cm).

Table I Characteristics of 531 Chinese women with POF.

Characteristic	Primary amenorrhea (n = 70)	Secondary amenorrhea (n = 461)
Age at diagnosis (years)	26.9 ± 3.4	29.3 ± 4.7
Age at menarche (years)		14.4 ± 1.8
Age at onset of menstrual dysfunction (years)		21.8 ± 5.9
Age of amenorrhea (years)		23.8 ± 5.6
Plasma FSH concentration (mIU/ml)	72.0 ± 25.45	77.0 ± 28.1

Chromosomal abnormalities

Chromosomal abnormalities were detected in 64 of 531 cases (12.1%). Of the 64, 60 (93.7%) involved the X chromosome; 1 (1.6%) involved autosomes only; 3 were non-mosaic 46,XY and 45,X/46,XY mosaicism. The frequency of karyotypic abnormalities in patients with primary amenorrhea (15/70, 21.4%) was significantly higher than that in patients with secondary amenorrhea (49/461, 10.6%; $P = 0.01$). Those patients with affected relatives showed a frequency of chromosomal abnormalities of only 4.3% (2/47), whereas in sporadic cases the frequency was 12.8% (62/484; $P = 0.09$). The distribution of abnormalities is detailed in Table II. Polymorphisms considered normal included four pericentic inversions of chromosome 9 and seven variants of centromeric heterochromatin.

The most common abnormality was a structural abnormality involving an X chromosome. We detected 17 terminal deletions [15 del(Xq); 2 del(Xp)], of which 3 also had a 45,X line; 11 isochromosomes [6 i(Xp); 5 i(Xq)], 2 of which were 45,X mosaic; 1 ring X and 1 inv X, both with a 45,X cell line; 1 non-mosaic isodicentric and 1 complex X arrangement. In addition, nine X-autosome translocations were detected. All but one involved Xq, usually in regions Xq22–Xq24. Autosomes involved in translocations included 2, 4, 5, 6, 12, 14 and 19.

Aneuploidy without a structurally abnormal X was found in 19 cases. The most frequent non-mosaic aneuploidy was 45,X ($n = 7$), followed by 47,XXX ($n = 2$). Cases with non-mosaic 45,X monosomies showed short stature (149.0 ± 3.5 cm) and hypoplastic uterus but otherwise few somatic features of Turner syndrome. In 10 other cases, X mosaicism was present: 45,X/46,XX ($n = 4$), 45,X/47,XXX ($n = 3$), 45,X/46,XX/47,XXX ($n = 2$) and 46,XX/47,XXX ($n = 1$). 45,X and 45,X/46,XX mosaicisms were the complements most frequently associated with primary amenorrhea (7/15, 46.7%).

In three cases, 46,XY was present, one 45,X/46,XY and two non-mosaic 46,XY. Menstruation in one of the latter two was not the result of a known hormone-producing tumor; however, the exact

Table II Prevalence and distribution of karyotype abnormalities in Chinese women with POF.

Abnormal karyotypes (64/531, 12.1%)								
Autosome (1/64, 1.6%)	**X chromosome (60/64, 93.7%)**						**46,XY line (3/64, 4.7%)**	
	X-numerical abnormalities (19/60, 31.7%)	**No.**	**X-structural abnormalities (32/60, 53.3%)**	**No.**	**X-autosomal translocations (9/60, 15.0%)**	**No.**		
45,XX,-13,-14,t(13;14)	45,X	3[a] + 4	Terminal deletions	17	46,X,t(x;2)(q13;q36)	1	46,XY	1[a] + 1
	47,XXX	2	46,X,del(X)(q13)	1[a]	46,X,t(X;4)(q22;q21)	1	45,X[95]/46,XY[5]	1
	Mosaicism	10	46,X,del(X)(q21)	3	46,X,t(X;5)(q24;q22)	1		
	45,X/46,XX	4[a]	46,X,del(X)(q22)	1[a] + 3	46,X,t(X;6)(q24;q25)	1[a] + 1		
	45,X/47,XXX	3	46,X,del(X)(q24)	3	46,X,t(X;12)(q22;p24)	1		
	45,X/46,XX/47,XXX	2	46,X,del(X)(q25)	1	46,X,t(X;14)(p10;p10)	1		
	46,XX/47,XXX	1	46,X,del(X)(q27)	2	46,X,t(X;14)(q22;q32)	1		
			45,X[72]/46,X,del(X)(p11)[28]	1	46,X,t(X;19)(q22;q13)	1		
			45,X[34]/46,X,del(X)(p21)[66]	1				
			45,X[2]/46,X,del(X)(q26)[18]/46XX[80]	1				
			Isochromosome	11				
			46,X,i(X)(p10)	5				
			46,X,i(X)(q10)	2[a] + 2				
			45,X[61]/46,X,i(X)(p10)[39]	1[a]				
			45,X[46]/46,X,i(X)(q10)[34]	1[a]				
			Ring chromosome	1				
			45,X[89]/46,X,r(X)(p22-q25)[11]					
			Inversion	1				
			45,X[57]/46,X,inv(X)(q12q26)[43]					
			Dicentric chromosome	1				
			46,X,psu idic(X)(q28)					
			Complex rearrangement	1				
			46,X,der(X),t(X;X)(p22;p22)					

No. number of secondary amenorrhea
[a]No. number of primary amenorrhea

cause remains to be elucidated. Irrespectively, this phenotypic female presented with ostensible 'POF'. A single balanced autosomal Robertsonian translocation was also detected [t(13q;14q)]. No reciprocal translocations of autosomes were found.

Discussion

This is the largest series of cytogenetic studies ever performed on Chinese women with POF, revealing the prevalence of chromosomal abnormalities to be 12.1%. Structural abnormalities involving the X chromosome were most frequently found, sometimes but not always mosaic (45,X). The necessity of two intact X chromosomes in normal ovarian development and function was confirmed.

Among several different structural abnormalities of the X chromosome, Xq was involved more than Xp (73.8 versus 26.2%). Terminal deletions of Xq were overall the most frequent (14 non-mosaic and 3 additional mosaic). This is of interest because previous cytogenetic and molecular characterization of X rearrangements has defined two critical regions (CRs) for POF: CR I Xq13-Xq21 and CR II Xq23-Xq27 (Therman and Susman, 1990; Toniolo, 2006). Perturbations presumably confer haploinsufficiency or interrupt pivotal genes in these regions. Non-specific defective meiotic pairing or a position effect on contiguous genes is also possible (Simpson, 2008; Persani *et al.*, 2009). Heterochromatin rearrangements have further been proposed, given that Xq21 lies in a 'gene desert' region where X-autosome translocation breakpoints have been frequently associated with ovarian failure. This suggests epigenetic down-regulation of oocyte-expressed autosomal genes translocated to CR I (Rizzolio *et al.*, 2006, 2007, 2009). The other relatively common X structural abnormalities found here were isochromosomes ($n = 11$), involving both Xq and Xp.

In our series, 8 of the 9 X-autosome reciprocal translocations involved Xq. Breakpoints on X chromosome were Xp10 ($n = 1$), Xq13 ($n = 1$), Xq22 ($n = 4$) and Xq24 ($n = 3$). Our distribution differs somewhat from a previous report, which showed 80% of Xq breakpoints in X-autosome balanced translocations to involve the gene-poor region Xq21 (Rizzolio *et al.*, 2006). X-autosome translocations and X-terminal deletions in women with POF have been used to localize genes or gene regions involved in ovarian maintenance. Genes on the X chromosome identified by translocations include DIAPH2 (Xq22; Bione *et al.*, 1998), XPNPEP2 (Xq25), DACH2 (Xq21.3; Prueitt *et al.*, 2002), POF1B (Xq21.1; Bione *et al.*, 2004), CHM (Xq21.1; Lorda-Sanchez *et al.*, 2000), PGRMC1 (Xq24; Mansouri *et al.*, 2008), COL4A6 (Xq22.3; Nishimura-Tadaki *et al.*, 2010) and NXF5 (Xq22.1; Bertini *et al.*, 2010). However, evidence is robust for most of these genes except for PGRMC1 and COL4A6. The perhaps dozen autosomal genes which are known to cause POF and are disrupted by X-autosome translocations were also not investigated for molecular perturbations.

In seven non-mosaic cases of aneuploidy, 45,X was the only complement detected. X monosomy without mosaicism is more typically found in primary amenorrhea; however, in our series only three of the seven cases encountered had primary amenorrhea. 45,X presenting as secondary amenorrhea is also well known. Simpson (1975) reported that 3% (5/178) of 45,X patients menstruated. Occasional menstruation occurs in monosomy X, however, consistent with the pathogenesis of ovarian failure in 45,X involving increased germ cell attrition compared with that in the 46,XX fetus (Jirasék, 1976).

Non-mosaic 47,XXX was found in two POF cases, an association reported previously (Tungphaisal and Jinorose, 1992; Holland, 2001; Skalba *et al.*, 2010) but still of uncertain significance (Tartaglia *et al.*, 2010). The presence of three X chromosomes could lead to meiotic disturbance and plausibly could lead secondarily to ovarian failure. However, the incidence of 47,XXX is 1:800 females at birth, whereas that of POF is ~1%; thus, coincidental occurrence of 47,XXX and POF is not unexpected. Goswami *et al.* (2003) reported the prevalence of 47,XXX in 52 POF women to be 3.8%, whereas in our series the prevalence was 1.5% (8/531) when including 6 mosaic cases (5 with 45,X; 1 with 46,XX; Table II). None of the cases with a 47,XXX cell line had known autoimmune disease, an association previously reported (Holland, 2001; Goswami *et al.*, 2003). In addition to potential meiotic perturbations, overexpression of genes that escape X-inactivation could cause POF in 47,XXX. However, specific genes remain to be further defined (Tartaglia *et al.*, 2010).

Mosaicism with a 45,X line was common overall. In addition to arising from mitotic non-disjunction, a monosomy X line can arise secondarily if an X-structural abnormality exists, especially a dicentic chromosome or isochromosome. The presence of a monosomy X line did not result in a significant difference in the age of onset in our POF cases, either overall or in any stratified group. However, the cell sample was small.

Few autosomal translocations—Robertsonian or reciprocal—have been reported in POF. We detected a single Robertsonian translocation and no reciprocal translocations. Robertsonian translocation t(13;14) has been found in sporadic cases of POF in Belgian, American and Japanese women (Hens *et al.*, 1989; Orczyk *et al.*, 1989; Kawano *et al.*, 1998). It is plausible that critical ovarian loci on acrocentric chromosomes might be disrupted, resulting in premature exhaustion of ovarian follicles in the case we studied. However, the association could also merely be coincidental given the not uncommon incidences of both Robertsonian translocations and POF. Alternatively, any translocation may non-specifically predispose to meiotic breakdown and, hence, POF.

The presence of Y-bearing cell was, unexpectedly, found in 3 'POF' patients. All three had female external genitalia, hypoplastic uterus and no visible gonads, while the 45,X/46,XY case and one non-mosaic 46,XY case presented with secondary amenorrhea. Such cases have been reported in a recent study, one having a known SRY deletion (Ceylaner *et al.*, 2010). In our 45,X/46,XY case, menstruation could plausibly have been caused by the 45,X line. Less likely is the presence of an undetected 46,XX line in ovaries. However, menstruation in the non-mosaic 46,XY sex reversal requires another explanation. One possibility is that uterine bleeding in 46,XY was the result of a hormone-producing tumor, such as gonadoblastoma. However, clinical evaluation revealed no definitive evidence for such a tumor. Other potential explanations include undisclosed ingestion of hormones or xenobiotic estrogens.

Most X-chromosomal abnormalities lead to infertility, however, some X-deletions show less severe perturbations of ovarian function and tend to reduce, but not necessarily obliterate, reproductive capacity. These deletions may be transmitted to offspring as reported (Fitch *et al.*, 1982; Veneman *et al.*, 1991; Tharapel *et al.*, 1993; Zinn *et al.*, 1997; Lakhal *et al.*, 2010). Unfortunately, in this study we

Table III Summary of frequency of chromosomal abnormalities in different population studies of POF.

Reference	Frequency of CA (%)	No. of CA	Sample size	Clinical characteristics	Population
Present study	12.1	64	531	PA, SA	Chinese
Baronchelli *et al.* (2011)	10.0	27	269	PA, SA, EM	Italian
Lakhal *et al.* (2010)	10.8	108	1000	PA, SA	Tunisian
Ceylaner *et al.* (2010)	25.3	19[a]	75	SA	Turkish
Janse *et al.* (2010)	12.9	19	147	SA	Dutch
Portnoi *et al.* (2006)	8.8	8	90	PA, SA	French
Zhang *et al.* (2003)	12.5	13	104	POF	Chinese (Chongqing)
Devi and Benn. (1999)	13.3	4	30	SA	American
Davison *et al.* (1998)	2.5	2	79	PA, SA (FSH >20 IU/l)	English
Castillo *et al.* (1992)	32.0	15	47	POF	Chilean
Rebar and Connolly (1990)	25.4	16	63	PA, SA	American

CA, chromosomal abnormalities; PA, primary amenorrhea; SA, secondary amenorrhea; EM, early menopause.
[a]Including two Swyer syndrome.

could not clarify the familial or *de novo* origin of the X chromosome aberration because parental karyotyping was not available. Although X-abnormalities usually lead to infertility, this is not always so. Thus, some X-deletions maybe transmitted to daughters. In addition, a few cases of familial POF were reported to be associated with heritable Xq interstitial/terminal deletions (Krauss *et al.*, 1987; Maraschio *et al.*, 1996). We found no difference in the incidence of chromosomal abnormalities between sporadic and familial POF patients, consistent with an earlier report (Janse *et al.*, 2010).

The overall frequency of chromosomal abnormalities in our study (12.1%) was similar to that reported from a cohort of 1000 cases in Tunisian women (10.8%; Lakhal *et al.*, 2010), 269 cases in Italian women (10.0%; Baronchelli *et al.*, 2011), 147 cases in Dutch women (12.9%; Janse *et al.*, 2010), 104 cases in Chinese women (12.5%; Zhang *et al.*, 2003) and an American cohort of 30 cases (13.3%; Devi and Benn, 1999). The prevalence in our study was slightly higher than that reported in French (8.8%; Portnoi *et al.*, 2006) and also English (2.5%) women (Davison *et al.*, 1998). Although the prevalence observed was higher in Turkish women (25.5%; Ceylaner *et al.*, 2010), and in one American (25.4%; Rebar and Connolly, 1990) and Chilean (32.0%; Castillo *et al.*, 1992) report, selection biases probably account for different prevalences in these studies. The prevalence of chromosomal abnormalities in previous studies is summarized in Table III.

Our study was restricted to traditional G-banded karyotype in lymphocytes. Low-level mosaicism, cryptic chromosomal aberrations and premutations in fragile X mental retardation 1 were not sought routinely. More subtle abnormalities might easily be missed by conventional karyotype analysis alone, and analysis of other tissues would doubtless detect additional mosaic cases. Indeed, molecular cytogenetic approaches such as fluorescence *in situ* hybridization and array comparative genomic hybridization (array CGH), are now being used to detect low-grade 45,X mosaicsm, define precisely breakpoints and identify micro-deletion and -duplication [or copy number variation (CNV)]. A few reports have revealed *de novo* or more precise rearrangements on the basis of conventional karyotyping and identification of plausible causative genes (Han *et al.*, 2006; Tachdjian *et al.*, 2008; Bertini *et al.*, 2010; Giacomozzi *et al.*, 2010). Therefore, further defining of breakpoints by the above approaches is of importance in exploring the genetic etiology of POF.

Four array CGH studies have identified plausible CNVs associated with POF when compared with Database of Genomic Variants (http://projects.tcag.ca/variation). Aboura *et al.* (2009) identified 8 known CNVs (7 on autosomes) in 99 French patients with POF, among which 5 potential candidate genes were involved in female reproduction—DNAH5, NAIP, DUSP22, NUPR1 and AKT1. Using a complete X chromosome tiling path array, Quilter *et al.* (2010) found 15 novel discrete X chromosome intervals in 20/42 (48%) women with POF in the UK, whereas Dudding *et al.* (2010) detected only two micro-duplications (Xp22.33 and Xq13.3) and a low frequency (4%) in patients from New Zealand. Another array CGH study involving 74 German patients with POF/ovarian dysgenesis identified 44 private losses and gains that might be potentially causative for POF (Ledig *et al.*, 2010). Further replication in a second independent cohort and functional experiments are warranted to demonstrate their plausible causative roles in POF.

In summary, this report of the largest cohort of Chinese women yet studied found the prevalence of chromosomal abnormalities in POF to be 12.1%, most cases involving X-structural abnormalities or X-aneuploidy. This confirms a major role for X chromosome abnormalities in POF, highlighting the importance of routine assessment of chromosomal abnormalities. Chromosomal studies thus provide valuable clinical information for reproductive management and genetic counseling. In addition to providing an etiologic explanation for the individual patient with POF, the cases facilitate identification of genes responsible for POF.

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Authors' roles

X.J., C.R.Q., Y.Y.Q., X.M.Z. and B.Z. recruited subjects and conducted data analysis. C.R.Q., X.G., X.M.Z. and B.Z. coordinated and performed karyotype analysis. X.J. and C.R.Q. drafted the manuscript. J.L., Y.Y.Q., Y.F. and J.L.S. contributed to critical revision. Z.-J.C. designed the study, supervised experiments and revised the manuscript. All authors critically reviewed the article and approved the final manuscript.

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Conflict of interest

None declared.

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