Original Article

Endemic fluorosis in Henan province, China: ERα gene polymorphisms and reproductive hormones among women

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Background and Objectives: The aim of this study was to explore the influence of fluoride exposure and *ERα* gene polymorphisms on reproductive hormone concentrations of women in accordance with endemic fluorosis residence. **Methods and Study Design:** A cross sectional study was conducted in Tongxu county, Henan Province, China. A total of 679 women were recruited using cluster sampling and each subject provided fasting blood and an associated urine sample. We measured the concentrations of serum gonadotropin releasing hormone (GnRH), follicle-stimulating hormone, luteinizing hormone, and estradiol and urinary fluoride. **Results:** In the defluoridation project group (DFPG), serum GnRH was lower in women carrying C/C genotype compared to in those carrying C/T and T/T genotypes of *ERα* gene rs3798577 (p<0.05). In the endemic fluorosis group (EFG), serum GnRH was lower in women carrying Pp genotype compared to in those carrying PP and pp genotypes of *ERα* PvuII (p<0.05). Serum GnRH in women from EFG who carried Pp, pp, Xx and xx genotypes in *ERα* gene PvuII and XbaI was lower than in those in the control group (CG) who carried same genotypes (p<0.05). Furthermore, serum GnRH in women from EFG was significantly lower than in those in CG, regardless of whether the women were carrying C/C, C/T or T/T genotypes of *ERα* rs3798577 (p<0.05). Serum estradiol concentrations in EFG were significantly lower than in CG when the women were carrying the Pp, Xx and T/T genotypes in *ERα* gene (p<0.05). **Conclusion:** Interaction of *ERα* gene and fluoride exposure may influence women's serum reproductive hormone concentrations.

Key Words: fluoride, ERα, gene polymorphism, women, reproductive hormones

INTRODUCTION

Fluorine, very active chemically, reacts with most metal elements to generate different fluorides widely found in nature.[1] Fluoride plays a key role in the prevention and control of dental caries. However, fluoride has a tendency to accumulate in organisms if the exposure persists over time, even at low concentration.[2] Dental and skeletal lesions are the major recognized effects of endemic fluorosis, caused by chronic persistent fluoride exposure through ingestion or inhalation and, most commonly, as a result of high fluoride levels in drinking water or industrial exposure from dust.[3,4] Excessive fluoride intake can also affect hormone secretion and soft tissues such as the liver, kidney, brain, reproductive organs and pancreas.[5-8] The relationship between fluoride ingestion and reproductive structure or function has also recived attention.[9-11] Fluoride exposure can increase the number of abnormal spermatozoa and reduce sperm quality and quantity in rats, mice and humans.[12-14] However, most reproductive studies have been animal experiments and mainly involved males; fewer studies have involved human populations and not reproductive physiology in women.

Estradiol (E_2) exerts its effects by binding to estrogen receptors (ER) which regulate the expression of multiple genes once activated. The ER gene consists of *ERα* and *ERβ*, which have different functions.[15] The human *ERα* gene is located on chromosome 6q25, comprise of 8 exons separated by 7 intronic regions and spans >140 kb pairs.[16] The *ERα* gene is one of the important candidate genes in determining peripheral E_2 concentration.[17,18] Genetic screening of *ERα* gene has revealed SNPs and variable-number tandem repeat polymorphisms and they have been studied in relation to postmenopausal osteoporosis, breast cancer, cardiovascular disease, Alzheimer's disease and so on.[19-22] The PvuII (rs2234693) caused by a T/C

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transition and XbaI (rs9340799) caused by a G/A transition SNPs in the first intron of *ERα* gene are widely studied.[23] Previous studies showed that both intronic polymorphisms PvuII and XbaI have been associated with bone mineral density variation, adiposity, dental fluorosis and so on.[16,18,24] The SNP rs3798577 is located in the 3'-untranslated region of the human *ERα* gene and it is suggested to be associated with circulating E_2 concentrations in African American women.[17]

Previous studies have shown that long-term exposure of female mice to NaF leads to adverse effects on the reproductive system and fertility[25] and that *ERα* gene polymorphisms could affect the concentrations of E_2 in postmenopausal women.[26] However, of the relationship between *ERα* gene polymorphisms and reproductive hormones in women with fluoride exposure is unknown. In consideration of the potential influence of fluoride and genetic susceptibility on women's reproduction, we conducted a cross sectional study of the interaction of the three potentially functional common variants in the *ERα* gene with fluoride exposure in drinking water on reproductive hormone concentrations in women.

SUBJECTS AND METHODS

Subjects

A cross sectional study was conducted in seven villages of Tongxu county in Henan Province, China in 2011-2012 by random sampling according to fluoride concentration in the drinking water. The definitions of the endemic fluorosis group (EFG), the defluoridation project group (DFPG) and the control group (CG) have been described previously.[7] Two of the endemic fluorosis villages have had projects for the defluoridation of drinking water since the end of 2008. Women aged from 18 to 48 years old who were born, grew up or lived in the investigated villages for more than 5 years were recruited as subjects by a cluster sampling method. Subjects who work in other regions as migrant workers longer than one year, had history of chronic diseases, accepted hormone replaced treatment or were pregnant within the past year were excluded from this study. A total of 679 participants met the inclusion criteria in this study with a participation rate of 86.7%.

Collection of questionnaires and biological specimens

As described elsewhere,[7] with written consent, an in-person interview was conducted at the village clinics using a standardized and structured questionnaire. This allowed collection of demographic factors, growth and development, menstrual cycle, and reproductive history including the rate of spontaneous abortion, personal behaviours (such as diet, exercise, smoking and alcohol consumption), occupational history, medical conditions and medication use including hormone and vitamin supplementation. Each subject was venesected for 10 mL fasting blood and provided an associated 50 mL urine. After centrifugation, serum and white blood cells were separated and frozen at -80°C for subsequent analyses. All procedures were approved by the Institutional Review Board at Zhengzhou University in China.

Detection of the concentrations of urine fluoride and serum reproductive hormones

The urine fluoride concentrations were determined by a fluoride ion selective electrode.[27] Serum hormone concentrations were determined as described previously.[7] The serum concentration of gonadotropin releasing hormone (GnRH) was measured by ELISA (R&D Systems, Minneapolis, USA). Serum concentrations of E_2, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined using the chemiluminesence immunoassay (LUmo Luminometer, Autobio Labtec Instruments Co. Ltd., Zhengzhou, China). Each sample was run in duplicate and 15% of all samples were retested randomly. The intra- and inter-assay coefficients of variation were less than 10%.

Genotyping

PCR-RFLP

Genomic DNA was extracted using whole blood genomic DNA miniprep kits (Axygen Biosciences, Union City, USA). Genotyping procedures for the PvuII and XbaI polymorphisms in the *ERα* gene were carried out as follows. The forward primer (5-CTGCCACCCTATCTG TATCTTTTCCTATTCTCC-3) and the reverse primer (5-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA -3) were used in a polymerase chain reaction (PCR) to produce a 1300-base pair DNA fragment.[28] The absence and presence of the PvuII and XbaI restriction sites of the *ERα* gene were designated as P, p and X, x alleles, respectively. The concentrations of PCR reaction mixture and the PCR conditions (for PvuII and XbaI) have been described in detail previously.[27]

Taqman genotyping

The genetic analysis of *ERα* rs3798577 polymorphism was carried out by predesigned TaqMan primer and Taqman MGB probe sets (Applied Biosystems, Foster City, CA, USA). The reaction was performed in a 12.0 μL that contained 0.125 μL 40×predesigned TaqMan SNP Genotyping Assay (ID number: C_2823742_10; Applied Biosystems), 6 μL 2×TaqMan Genotyping Master Mix (Applied Biosystems), 1.0 μL of 50 ng genomic DNA, and 4.9 μL ultrapure PCR water. Cycling conditions comprised of an initial cycle at 95℃ for 10 mins, followed by 40 cycles of 92℃ for 15 s and 60℃ for 15 s. The fluorescent intensities of the PCR products were measured using the MX3000P Real-Time PCR System (Stratagene of Agilent Technologies Co. Ltd., Santa Clara, USA).

Data analysis

The database was established using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark) and all the data were double entered into the database by different people. Differences of fluoride concentration in the drinking water, urinary fluoride, body weight, height, mean marriage age and menstrual duration were examined using ANOVA. The serum concentrations of reproductive hormones in women were not well represented by a normal distribution although they were treated with logarithmically-transformation; therefore, the concentrations of reproductive hormones in different groups were analyzed using Kruskal-Wallis Test. The

Chi-square test was used to test for the differences in the rate of spontaneous abortion, abnormal menstruation and passive smoking among the three groups, as well as departures from the Hardy–Weinberg equilibrium of selected SNPs of *ERα* among controls. All of the SNP genotype frequencies in control subjects were consistent with the Hardy-Weinberg equilibrium (p=0.769 for PvuII, p=0.106 for XbaI, and p=0.172 for rs3798577). A p value of less than 0.05 was considered statistically significant. All analyses were performed using the SPSS software, version 21.0 (IBM SPSS In., Chicago, USA).

RESULTS

Distributions of selected variables in three groups

As shown in Table 1, the age, height, weight distributions and menstrual cycle, menstrual period were similar among the three groups (p>0.05 respectively). Urinary fluoride concentrations in women from both EFG and DFPG were significantly higher than in those from CG, and also higher in EFG than in DPFG participants (p<0.05 respectively). We also compared the rate of abnormal menstruation and spontaneous abortion among the three groups. No differences in abnormal menstruation or spontaneous abortion rate were found among EFG, DFPG and CG participants.

ERα gene PvuII, XbaI and rs3798577 polymorphisms and serum hormone concentrations in women from EFG, DFPG and CG

We compared the concentrations of serum GnRH, LH, FSH and E_2 in women from the same group who carried different genotypes of *ERα* PvuII, XbaI and *ERα* rs3798577. No statistically significant differences in serum GnRH, LH, FSH and E_2 were found in women from CG who carried different genotypes of the three SNPs (Table 2) (p>0.05 respectively). However, serum GnRH was lower in women carrying C/C genotype compared to in those carrying C/T and T/T genotypes of *ERα* rs3798577 in DFPG (Table 3) (p<0.05 respectively), and serum GnRH was lower in women carrying Pp genotype compared to in those carrying PP and pp genotypes of *ERα* PvuII in EFG (Table 4) (p<0.05 respectively).

Fluoride exposure, ERα gene PvuII, XbaI and rs3798577 polymorphisms and serum hormone concentrations in women from EFG, DFPG and CG

We also compared serum hormone concentrations in women from different groups who carried the same genotype of *ERα* PvuII, XbaI and rs3798577 (Table 5-Table 7). The concentrations of serum GnRH in women from EFG who carried Pp, pp, Xx and xx genotypes in *ERα* gene PvuII and XbaI were found to have significant differences compared to women from the CG group who carried the same genotypes (p<0.05 respectively). Serum GnRH concentrations in EFG women were significantly lower than DFPG and CG women, regardless of whether they carried C/C, C/T or T/T genotypes of *ERα* rs3798577 (p<0.05 respectively). The concentrations of serum E_2 in EFG women who carried Pp, Xx and T/T genotypes in *Erα* gene PvuII, XbaI and rs3798577 were found to be signifi-

Table 1. Distributions of selected variables in three groups

Indexes	CG (n=303)	DFPG (n=162)	EFG (n=214)	F/χ^2	p value
Age (years)	37.8±8.2	38.9±7.6	39.2±7.6	2.31	0.100
Height (cm)	158±5.3	157±4.4	158±6.3	2.81	0.061
Weight (kg)	61.7±9.0	62.4±9.1	61.8±9.2	0.732	0.312
Menstrual cycle (days)	29.4±3.3	29.5±6.4	29.9±4.8	0.629	0.533
Menstrual period (days)	5.1±1.6	5.0±1.6	5.1±1.7	0.249	0.780
Percentage of abnormal menstruation†	11.2% (34/303)	17.3% (28/162)	12.2% (26/214)	2.47	0.291
Percentage of spontaneous abortion‡	21.9% (66/302)	27.2% (44/162)	30.8% (65/211)	5.35	0.069
Urine fluoride (μmol/L)	52.6±26.3	73.7±52.6	142±78.9	164	<0.001

†Percentage of abnormal menstruation= (the number of abnormal menstruation/the total number) ×100%.
‡Percentage of spontaneous abortion= (the number of spontaneous abortion/the total number) ×100%.

Table 2. Serum hormone concentrations and *ERα* SNPs (median (P_{25}, P_{75})) in women from CG

Genotypes		n	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
PvuII	PP	53	0.687 (0.589, 0.849)	7.8 (4.4, 17.7)	8.1 (5.2, 14.4)	0.194 (0.141, 0.362)
	Pp	136	0.629 (0.547, 0.740)	5.8 (3.4, 12.7)	6.9 (4.2, 13.4)	0.233 (0.145, 0.321)
	Pp	113	0.618 (0.512, 0.785)	7.5 (4.2, 11.6)	7.2 (5.0, 11.7)	0.206 (0.138, 0.292)
	H		4.42	4.25	1.47	1.04
	p value		0.110	0.120	0.481	0.595
XBaI	XX	35	0.621 (0.467, 0.767)	6.6 (4.1, 11.0)	7.1 (4.1, 10.4)	0.202 (0.138, 0.292)
	Xx	158	0.663 (0.578, 0.775)	7.3 (4.1, 14.0)	7.0 (4.7, 12.6)	0.214 (0.142, 0.338)
	Xx	109	0.610 (0.496, 0.785)	7.0 (3.8, 11.2)	7.4 (4.4, 13.5)	0.220 (0.138, 0.339)
	H		4.95	0.808	0.087	0.877
	p value		0.084	0.668	0.957	0.645
rs3798577	C/C	62	0.671 (0.533, 0.812)	6.5 (4.1, 14.0)	6.3 (4.3, 10.1)	0.246 (0.142, 0.365)
	C/T	158	0.629 (0.541, 0.759)	7.0 (3.8, 12.4)	7.5 (4.5, 13.3)	0.208 (0.138, 0.326)
	T/T	83	0.624 (0.517, 0.775)	7.3 (3.8, 11.3)	7.1 (4.7, 12.9)	0.229 (0.144, 0.312)
	H		1.19	0.491	0.814	0.709
	p value		0.552	0.782	0.666	0.702

Table 3. Serum hormone concentrations and *ERα* SNPs (median (P_{25}, P_{75})) in women from DFPG

Genotypes		*n*	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
PvuII	PP	24	0.621 (0.586, 0.706)	8.8 (6.2, 11.4)	7.1 (4.0, 15.9)	0.223 (0.113, 0.296)
	Pp	74	0.632 (0.563, 0.690)	7.8 (4.7, 15.0)	6.9 (4.7, 11.6)	0.225 (0.164, 0.337)
	Pp	62	0.658 (0.560, 0.756)	8.2 (4.9, 15.3)	8.2 (4.7, 12.5)	0.237 (0.146, 0.325)
	H		1.49	0.111	0.656	1.40
	p value		0.474	0.946	0.720	0.497
XBaI	XX	12	0.621 (0.462, 0.677)	7.5 (4.2, 19.3)	10.1 (3.6, 32.1)	0.162 (0.124, 0.296)
	Xx	116	0.645 (0.581, 0.722)	7.5 (4.8, 14.1)	6.7 (4.6, 11.0)	0.234 (0.168, 0.326)
	Xx	32	0.632 (0.539, 0.685)	10.4 (5.4, 23.1)	8.7 (5.6, 16.0)	0.200 (0.129, 0.341)
	H		2.17	3.47	3.42	2.21
	p value		0.338	0.176	0.181	0.331
rs3798577	C/C	32	0.594 (0.520, 0.663)	9.3 (6.2, 18.2)	8.2 (4.4, 13.0)	0.204 (0.131, 0.317)
	C/T	82	0.658 (0.578, 0.730)*	7.4 (4.7, 14.0)	6.6 (4.7, 10.4)	0.245 (0.161, 0.329)
	T/T	48	0.642 (0.594, 0.722)*	7.9 (4.6, 16.0)	8.2 (4.6, 14.0)	0.209 (0.145, 0.302)
	H		7.93	2.30	1.54	1.74
	p value		0.019	0.316	0.463	0.418

*$p<0.05$ compared to C/C.

Table 4. Association between serum hormone concentrations and *ERα* SNPs (median (P_{25}, P_{75})) in women from EFG

Genotypes		*n*	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
PvuII	PP	26	0.629 (0.517, 0.746)*	7.7 (4.4, 38.8)	8.6 (4.7, 56.1)	0.184 (0.117, 0.316)
	Pp	104	0.395 (0.162, 0.610)	6.0 (3.1, 17.0)	7.3 (4.9, 14.0)	0.168 (0.113, 0.344)
	Pp	84	0.565 (0.178, 0.727)*	6.1 (3.8, 11.5)	8.7 (5.3, 17.6)	0.177 (0.112, 0.320)
	H		13.2	2.12	1.56	0.021
	p value		0.001	0.347	0.458	0.989
XBaI	XX	26	0.605 (0.202, 0.706)	5.9 (2.5, 28.2)	7.7 (5.0, 28.4)	0.170 (0.091, 0.293)
	Xx	115	0.486 (0.170, 0.669)	6.0 (3.6, 14.9)	7.8 (4.9, 14.3)	0.167 (0.112, 0.330)
	Xx	73	0.517 (0.172, 0.679)	6.4 (3.9, 17.2)	7.9 (4.9, 19.5)	0.211 (0.125, 0.362)
	H		1.63	0.271	0.005	2.98
	p value		0.444	0.873	0.997	0.225
rs3798577	C/C	36	0.398 (0.186, 0.666)	7.2 (4.0, 16.1)	9.0 (4.6, 16.4)	0.206 (0.131, 0.343)
	C/T	114	0.523 (0.170, 0.671)	5.9 (3.6, 16.3)	8.0 (5.1, 17.1)	0.182 (0.116, 0.336)
	T/T	64	0.549 (0.172, 0.701)	7.1 (3.7, 18.4)	7.6 (4.9, 37.0)	0.141 (0.104, 0.315)
	H		0.679	0.502	0.069	3.29
	p value		0.712	0.778	0.966	0.193

*$p<0.05$ compared to Pp.

Table 5. Serum hormone concentrations and *ERα* PvuII genotypes (median (P_{25}, P_{75})) in women from different groups

Genotypes	Groups	*n*	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
PP	CG	53	0.687 (0.589, 0.849)	7.8 (4.4, 17.7)	8.1 (5.2, 14.4)	0.194 (0.141, 0.362)
	DFPG	24	0.624 (0.586, 0.690)	8.8 (6.2, 11.4)	7.1 (4.0, 15.9)	0.223 (0.113, 0.296)
	EFG	26	0.629 (0.517, 0.746)	7.7 (4.4, 38.8)	8.6 (4.7, 56.1)	0.194 (0.117, 0.316)
	H		3.46	0.139	2.00	1.36
	p value		0.177	0.933	0.369	0.508
Pp	CG	136	0.629 (0.547, 0.740)*	5.8 (3.4, 12.7)	6.9 (4.2, 13.4)	0.233 (0.145, 0.321)*
	DFPG	74	0.632 (0.563, 0.690)*	7.8 (4.7, 15.0)	6.9 (4.7, 11.6)	0.225 (0.164, 0.337)*
	EFG	104	0.395 (0.162, 0.610)	6.0 (3.1, 17.0)	7.3 (4.9, 14.0)	0.168 (0.113, 0.344)
	H		53.4	3.12	1.62	7.20
	p value		<0.001	0.211	0.445	0.027
Pp	CG	113	0.618 (0.512, 0.788)*	7.5 (4.2, 11.6)	7.2 (5.0, 11.7)	0.206 (0.138, 0.292)
	DFPG	62	0.658 (0.560, 0.756)*	8.2 (4.9, 15.3)	8.2 (4.7, 12.5)	0.237 (0.146, 0.325)
	EFG	84	0.565 (0.178, 0.727)	6.1 (3.8, 11.5)	8.7 (5.3, 17.6)	0.177 (0.112, 0.320)
	H		10.6	2.19	2.08	3.67
	p value		0.005	0.335	0.353	0.160

*$p<0.05$ compared to EFG.

Table 6. Serum hormone concentrations and *ERα* XbaI genotypes (Median (P_{25}, P_{75})) in women from different groups

Genotypes	Groups	*n*	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
XX	CG	35	0.621 (0.467, 0.767)	6.6 (4.1, 11.0)	7.1 (4.1, 10.4)	0.202 (0.138, 0.292)
	DFPG	12	0.621 (0.462, 0.677)	7.5 (4.2, 19.3)	10.1 (3.6, 32.1)	0.162 (0.124, 0.296)
	EFG	26	0.605 (0.202, 0.706)	5.9 (2.5, 28.2)	7.7 (5.0, 28.4)	0.170 (0.091, 0.293)
	H		1.37	0.576	0.895	1.69
	p value		0.505	0.750	0.639	0.430
Xx	CG	158	0.663 (0.578, 0.775)*	7.3 (4.1, 14.0)	7.0 (4.7, 12.6)	0.214 (0.142, 0.338)*
	DFPG	116	0.645 (0.581, 0.722)*	7.5 (4.8, 14.1)	6.7 (4.6, 11.0)	0.234 (0.168, 0.326)*
	EFG	115	0.486 (0.170, 0.669)	6.0 (3.6, 14.9)	7.8 (4.9, 14.3)	0.167 (0.112, 0.330)
	H		44.9	1.40	3.52	10.9
	p value		<0.001	0.496	0.172	0.004
xx	CG	109	0.610 (0.496, 0.762)*	7.0 (3.7, 11.2)	7.4 (4.4, 13.5)	0.220 (0.138, 0.339)
	DFPG	32	0.632 (0.539, 0.685)*	10.4 (5.4, 23.1)	8.7 (5.6, 16.0)	0.200 (0.129, 0.341)
	EFG	73	0.517 (0.172, 0.679)	6.4 (3.9, 17.2)	7.9 (4.9, 19.5)	0.221 (0.125, 0.362)
	H		11.5	4.49	2.23	0.449
	p value		0.003	0.106	0.328	0.799

*$p<0.05$ compared to EFG.

Table 7. Serum hormone concentrations and *ERα* rs3798577 genotypes (Median (P_{25}, P_{75})) in women from different groups

Genotypes	Groups	*n*	GnRH (nmol/L)	LH (IU/L)	FSH (IU/L)	E_2 (nmol/L)
C/C	CG	62	0.671 (0.533, 0.812)*	6.5 (4.1, 14.0)	6.3 (4.3, 10.1)	0.246 (0.142, 0.365)
	DFPG	32	0.594 (0.520, 0.663)*	9.3 (6.2, 18.6)	8.2 (4.4, 13.0)	0.204 (0.131, 0.317)
	EFG	36	0.398 (0.186, 0.666)	7.2 (4.0, 16.1)	9.0 (4.6, 16.4)	0.206 (0.131, 0.343)
	H		16.4	4.29	2.28	1.06
	p value		<0.001	0.117	0.320	0.587
C/T	CG	158	0.629 (0.541, 0.759)*	6.9 (3.8, 12.4)	7.5 (4.5, 13.3)	0.208 (0.138, 0.326)
	DFPG	82	0.658 (0.578, 0.730)*	7.4 (4.7, 14.0)	6.6 (4.7, 10.4)	0.245 (0.161, 0.329)
	EFG	114	0.523 (0.170, 0.671)	5.9 (3.6, 16.3)	7.9 (5.1, 17.1)	0.182 (0.116, 0.336)
	H		29.8	1.62	2.88	5.10
	p value		<0.001	0.444	0.237	0.078
T/T	CG	83	0.624 (0.517, 0.775)*	7.3 (3.8, 11.3)	7.1 (4.7, 12.9)	0.229 (0.144, 0.312)*
	DFPG	48	0.642 (0.594, 0.722)*	7.9 (4.6, 16.0)	8.2 (4.6, 14.0)	0.209 (0.145, 0.302)*
	EFG	64	0.549 (0.172, 0.701)	7.1 (3.7, 18.4)	7.6 (4.9, 37.0)	0.141 (0.104, 0.315)
	H		11.8	0.273	0.836	7.03
	p value		0.003	0.872	0.662	0.030

*$p<0.05$ compared to EFG.

cant lower than in CG women who carried the same genotypes ($p<0.05$ respectively). However, no statistically significant differences in serum LH, FSH were observed among the three groups when women carried the same genotypes for these three SNPs ($p>0.05$ respectively).

DISCUSSION

Endemic fluorosis is a major public health concern in China due to the excessive consumption of fluoride in drinking water, brick tea, and inhalation with coal-burning. The defluoridation project for drinking water has been carried out since the 1980s in northern China because most cases of endemic fluorosis in such areas are caused by high fluoride exposure in the drinking water. In the past, the focus has been on health problems, both in humans and animals, known to be attributable to fluoride exposure, like dental and skeletal disease, and, to some extent, soft tissue like the kidney, brain, and male reproductive tissues.[29-31] Yet it has been possible that high fluoride exposure would lead to reproductive and endocrine dysfunction, and even changes in sexual orientation in children.[32,33] Our previous publications showed that chronic fluoride exposure can alter reproductive hormone status for FSH and E_2 in males.[7] However, little has been known about personal susceptibility and the interaction between fluoride exposure and genetic susceptibility in regard to human reproductive function. To this end, the current study explored the impact of both fluoride exposure and *ERα* gene polymorphisms on women's hypothalamic-pituitary-ovarian axis (HPO axis) by way of GnRH, FSH, LH and E_2.

GnRH is a ten peptide hormone produced and released by hypothalamus.[34] It plays an important role in regulating reproductive function. It can promote the hypophysis to secrete gonadotropins which regulate ovarian function and ovulation.[35] Serum GnRH concentrations in EFG women were significantly lower than those in CG women when they were had different genotypes for selected SNPs of the *ERα* gene, with the exception of PvuII PP and XbaI XX genotypes. The change of serum GnRH in those in EFG, therefore, may be more closely related to fluoride exposure. On the other hand, serum GnRH concentration may be less susceptible to fluoride exposure

when women carry the PP genotype of PvuII and XX genotype of XbaI in *ERα* gene because no significant differences were observed in serum GnRH concentrations among the different groups when they carried these two genotypes. Previous studies have demonstrated that advanced ovarian aging may be related to *ERα* rs3798577 genotypes.[36] However, we found no significant association of serum concentrations of LH and FSH with fluoride exposure if study subjects carried the same genotypes of selected SNPs in *ERα* gene. The study also showed that there were no significant differences in the serum concentrations of LH and FSH among women who carried different genotypes of *ERα* gene if they were exposed to the same concentration of fluoride. This suggests that serum FSH and LH concentrations might not be associated with both fluoride exposure and *ERα* polymorphisms. The secretion of LH and FSH are regulated by GnRH produced by the hypothalamus.[34] and also subject to the negative feedback regulation by ovarian hormones. These complicated regulation mechanisms may require FSH and LH to be maintained at relatively stable concentrations. E_2, a hormone secreted by the ovaries, is modulated by FSH and is an indicator of ovarian function[37] also related to ER. The associations of *ERα* gene polymorphisms with sex steroid concentrations are well-documented. Sowers et al suggested that the prevalence of women carrying C/C genotype of *ERα* rs3798577 is relative low among African American women, while there is no association in Chinese women.[17] The current findings are consistent with this with no association to be found between serum concentrations of E_2 and genotypes of selected *ERα* gene in women form CG. There may be no modification of selected *ERα* SNPs to serum E_2 concentrations of women in the child-bearing age. The association of *ERα* gene polymorphisms with reproductive disease has been studied, with inconsistent findings.[38-40] Corbo et al demonstrated that *ERα* PvuII and XbaI gene polymorphisms were correlation with fertility rate and the number of abortions.[41] Yoon et al also observed that the genotype distributions or allele frequencies of the PvuII polymorphism was significantly different between an idiopathic premature ovarian failure group and a control group.[42] The current study has showed that the concentrations of serum E_2 in EFG in those who carried Pp genotype of PvuII, Xx genotype of XbaI, as well as T/T genotypes of rs3798577 were significantly lower compared to those in CG and DFPG women who carried the same genotypes. It indicates that women who carry these three genotypes may be more susceptible to fluoride exposure. Since serum hormone concentrations of HPO axis hormones vary with ovulation, we compared the population distributions of menstrual cycles including the ovulatory period, follicular phase and luteal phase in the three groups. No statistical differences were observed in the distributions of the menstrual cycle among the three groups (Table 1).

Several limitations in this study must be recognized. Firstly, the study sample size was relatively small; thus chance findings cannot be excluded. However, the selected population was well characterized, with assessments of different variables affecting hormone concentrations including dietary intake, exercise, smoking, alcohol consumption and supplemental vitamins. Chance findings, thus, may be controlled to a large extent. Secondly, as potential confounders, variables mentioned above should be adjusted. However, all of the participants did not have habits like smoking, drinking and vitamin supplementation, and had the similar dietary habit. Adjustment of these variables did not result in material changes for the observed differences, and thus were not included in the final model. Finally, it is necessary to explore further whether there is any interaction of *ERα* polymorphisms with fluoride on women's reproductive hormones during the menstrual cycle, although we found no differences in the cycle distributions between the three study groups. Future studies should be based on larger samples, and should explore in greater detail the combined effects of gene and environment involved in the HPO axis pathway and the putative environmental factors which may relate to reproductive function together with or separately from fluoride.

In conclusion, the study provides limited evidence that gene-environmental interactions could influence serum reproductive hormones in women. In particular, with endemic fluorosis, these may be interactions of fluoride and *ERα* polymorphisms which might influence the HPO axis and its reproductive functions. Future studies are needed to confirm these findings in large populations in order to increase he understanding of how fluoride interacts with gene polymorphisms to alter reproductive function in women.

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AUTHOR DISCLOSURES
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Original Article

Endemic fluorosis in Henan province, China: ERα gene polymorphisms and reproductive hormones among women

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中国河南省氟病区女性 ERα 基因多态性与生殖激素的关系

背景与目的：探讨氟暴露及 *ERα* 基因多态性对氟病区女性生殖激素浓度的影响。**方法与研究设计：**按照饮用水中氟浓度分为氟病区组、改水组和对照组。采用整群抽样的方法选择中国通许县居住的 679 名成年女性为研究对象，采集研究对象空腹静脉血及晨尿样本，并测量血清促性腺激素释放激素（gonadotropin releasing hormone, GnRH）、卵泡刺激素、促黄体生成素、雌二醇和尿氟浓度。**结果：**改水组携带 *ERα* rs3798577 C/C 基因型女性血清 GnRH 浓度低于携带 C/T 和 T/T 基因型女性（$p<0.05$）。氟病区组携带 *ERα* PvuII Pp 基因型女性血清 GnRH 浓度低于携带 PP 和 pp 基因型女性（$p<0.05$）。携带 *ERα* PvuII Pp、pp 和 *ERα* XbaI Xx、xx 基因型的女性血清 GnRH 浓度低于对照区携带相同基因型的女性（$p<0.05$）。此外，无论其携带的 *ERα* rs3798577 基因型是 C/C 、C/T 或 T/T，氟病区组女性血清 GnRH 浓素均低于对照区女性（$p<0.05$）。携带 *ERα* PvuII Pp, *ERα* XbaI Xx 和 *ERα* rs3798577 T/T 基因型的氟病区女性血清雌二醇浓度低于携带相同基因型的对照区女性（$p<0.05$）。**结论：***ERα* 基因和氟暴露的交互作用可能影响女性血清生殖激素的浓度。

关键词：氟、ERα、基因多态性、女性、生殖激素