ERβ gene *Rsa*I polymorphism and children's dental fluorosis

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Abstract

Objective. To explore the distribution of ER RsaI genotype in children who lived in the areas with or without high fluoride in drinking water, and investigate the relationship between ER gene RsaI polymorphisms and children's dental fluorosis. Methods. We conducted a case-control study among children aged 8 to 12 years old with (n=74) or without (n = 163) dental fluorosis in two counties in Henan Province. The RsaI polymorphisms in the ER gene were genotyped using the PCR-RFLP procedure. Results. The frequency distribution of ER RsaI genotype was rr 60.8% (45/74), Rr 27.0% (20/74), RR 12.1 % (9/74) in children with dental fluorosis, rr 73.9% (51/69), Rr 20.2% (14/69), RR 5.8% (4/69) in children without dental fluorosis from high fluoride areas, and rr 63.8% (60/94), Rr 34.0% (32/94), RR 2.1% (2/94) in the children without fluorosis from control areas respectively. There were no significant differences in the three groups (P>0.05). Conclusion. There were no correlation between ER RsaI genotype and the dental fluorosis, and the further study is needed. [Life Science Journal. 2010; 7(1): 51 – 55] (ISSN: 1097 – 8135)

Keywords: estrogen receptor; dental fluorosis; gene polymorphism; fluoride

1 Introduction

Fluoride plays a key role in the prevention and control of dental caries. Dental fluorosis is one of the important performances of skeletal lesion of fluorosis. Excessive fluoride intake has also been shown to affect dentin and cementum mineralization throughout life, diminishing bone density and adversely impacting bone health. Previous study showed that not all the children with high fluoride exposure suffer from dental fluorosis. Therefore, we subsequently hypothesized that the genetic susceptibility would be associated with dental fluorosis status[1,2]. Estrogen plays an important role in stimulating osteoblast activity and promote the deposition of calcium and phosphate in bone[3,4]. It combines with specific receptor and thereby regulates a series of gene expression[5]. A number of studies have reported that there is the relationship between *ER* gene polymorphism and the occurrence of osteoporosis and other diseases, it shows that *ER* polymorphism would be associated with bone metabolism and bone mineral density. However, no study has assessed the relationship between *ER* gene polymorphisms and dental fluorosis. We conducted a case-control study to investigate the relationship between *ER Rsa*I gene polymorphisms and Children's dental fluorosis.

2 Materials and Methods

2.1 Location and population

A case-control study was conducted in four villages of two counties (Kaifeng and Tongxu) in Henan Province. Both counties include one endemic fluorosis village (EFV) and one non-endemic fluorosis village (NEFV). EFV was defined as a village with fluoride levels exceeding 2.0 mg/l in drinking water. NEFV was defined as a village with fluoride levels of less than 1.0 mg/l in drinking water. In addition to different water fluoride concentrations, both EFV and NEFV have the similar levels of nature condition, economy condition, construction of population, kind of food, or life style and so on. Calcium ion and magnesium ion concentration in drinking water were found no significant differences in EFV and NEFV areas. Children aged 8 to 12 years old, born and raised in the four villages were recruited excluding those who have received drug treatment in the form of bisphosphonates, calcitonin, fluoride, or hormone replacement therapy and/or who had hip fractures. A total of 237 children participated in this study. All participants were examined for dental fluorosis using the Dean's Method[6]. Children who were diagnosed as grade 0 or 1 were classified as non-dental fluorosis, whereas those who were diagnosed as grade 2, 3, 4, or 5 were classified as dental fluorosis. Each child provided about 6 ml of fasting venous blood and 10 ml of instant urine samples for the polymorphism analysis and determination of urinary fluoride respectively. All procedures were approved by the Institutional Review Board at Zhengzhou University, China (IRB00006861, FWA00014064).

2.2 Method

2.2.1 Detection of urine fluoride

The urine fluoride level in samples of children were detected by fluoride ion selective electrode (Shanghai Exactitude Instrument Company, China).The high-loaded fiuoride statue was classified when the urine fluoride concentrations exceeding 1.5 mg/l.

2.2.2 Children's dental fluorosis inspection

Dental and public health practitioners diagnose dental fluorosis in accordance with the Dean's classified method[6]. Dental fluorosis prevalence (DF) and community fluorosis index (CFI) as statistical indicator.

DF%= (the number of who were diagnosed as grade 2, 3, 4, or 5 / the total number of checked) ×100%

CFI={(the number of questionable ×0.5)+(the number of very mild×1)+(the number of mild×2+(the number of moderate×3)+(the number of severe×4)}/ the total number of checked

2.3 Genotyping

DNA was extracted using whole blood genomic DNA miniprep kits (Axygen Biosciences, Union City, USA). DNA was genotyped at the following markers: the RsaI RFLP (in exon 5) inside the ER gene, the forward primer (5'-TCTTGCTTTCCCCAGGCTTT-3'), and the reverse primer (5'-ACCTGTCCAGAACAAGATCT-3') were used in polymerase chain reaction (PCR) to produce a 156-base pair (bp) DNA fragment[7]. The PCR reaction amplification was conducted in reaction mixtures each containing 13μl of ddH_2O, 2.5μl of 10×buffer, 1.5mM of $MgCl_2$, deoxynucleotide triphosphate (dNTP; 200uM each), 2.5 U of Taq Polymerase (TaKaRa, Japan), 0.25μM each of the two primers for each marker, and 300 ng of genomic DNA. PCR amplification conditions: predegeneration at 94 ℃ for 5min, 35 cycles for degeneration at 94 ℃ for 20s, annealing at 53℃ for 30s , extension at 72℃ for 30s, final extension at 72 ℃ for 5min . After the amplification, PCR products were digested with endonuclease enzyme *Rsa*I (MBI, Lithuania) at 37℃ for 2.5 h and then the samples were electrophoresed in 2.5% agarose gels and stained by 0.3μg / ml of ethidium bromide. Gels were then visualized on a transilluminator under UV light and photographed. The absence and presence of the RsaI restriction sites of the ER gene were designated as R and r alleles respectively.

2.4 Data analysis:

Differences of fluoride in urine among cases and controls were examined using the analysis of variance (ANOVA) method. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated using multivariate logistic regression analysis. Potential confounding variables, such as age and gender, were adjusted for in the regression analysis. Adjustment for urine fluoride level did not result in a substantial change of the observed results. Therefore, the urine fluoride level was not included in the final model. Chi square test was used to test for departures from the Hardy-Weinberg equilibrium among controls. The SNP genotype frequencies in control subjects were consistent with the Hardy-Weinberg equilibrium (P=0.617). All tests of significance were two-sided. A P-value of less than 0.05 was considered statistically significant. All analysis were performed using the SPSS software, version 12.0.

3 Results

3.1 Distributions of select variables in dental fluorosis cases and controls (Table 1)

Table 1. Distributions of select variables in dental fluorosis cases and controls

	Cases (n=74)	Controls in EFV (n=69)	Controls in NEFV (n=94)
Age* (years)	9.96±1.31	8.25±1.31	9.93±1.28
P-value		0.01	0.18
Gender (n)			
Boys	33	41	40
Girls	41	28	54
P-value		0.076	0.19
High-loaded fluoride status (n)			
Yes	66	55	9
No	8	14	85
P-value		0.67	<0.01
Dean's scoring			
0 Normal	0	57	90
1 Questionable	0	12	4
2 Very mild	11	0	0
3 Mild	26	0	0
4 Moderate	24	0	0
5 Severe	13	0	0

* Values are means ± SD.

There were 74 subjects who were diagnosed with dental fluorosis. All of them lived in EFV, the prevalence of dental fluorosis was 51.7% (74/143) and dental fluorosis index was 1.35 in EFV and 0.021 in NEFV respectively. There were nearly 89.2% (66/74) of cases whose urinary fluoride levels exceeding 1.5mg/L. The exceeded rate of urinary fluoride was 84.6% (55/69) and 9.6% (9/94) in controls of EFV and NEFV respectively. The dental fluorosis cases were older than controls from EFV ($P<0.05$).

3.2 The distribution of genotypes for the *ER RsaI* polymorphism in peripheral blood and its relationship with children's dental fluorosis

Figure 1 shows the target fragments of ER RsaI. No significant differences were found in the distribution of genotypes for the *ER RsaI* polymorphism among these three groups ($P > 0.05$; Table 2). No significant differences were found in the prevalence rate of dental fluorosis for different gender Children in EFV ($P>0.05$; Table 3). The distribution of genotypes for the ER RsaI polymorphism in boys and girls with dental fluorosis was no significant difference ($P > 0.05$; Table 4). Urinary fluoride of children in these three groups have statistically significant differences ($P<0.05$). The level of urine fluoride was significantly lower in controls from NEFV compared with cases and control from EFV($P<0.05$ respectively) However, no significant difference was observed in the level of urinary fluoride between cases and controls from EFV ($P=0.668$; Table 5). When comparing the *ER RsaI* genotype of children who has high-loaded fluoride status, no significant difference was found in children with or without dental fluorosis ($P>0.05$; Table 6).

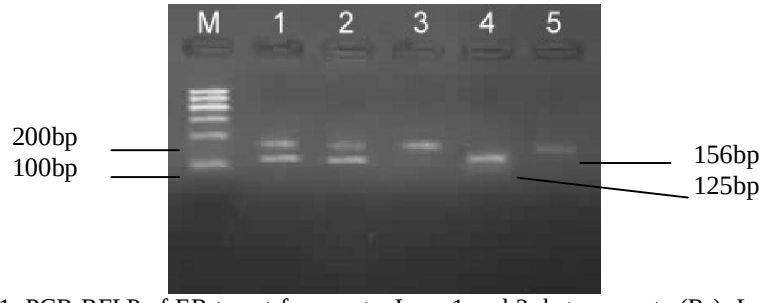

Figure 1. PCR-RFLP of ER target fragments. Lane 1 and 2: heterozygote (Rr); Lane 4: mutant homozygote (RR); Lane 3 and 5: wild-type homozygote (rr).

Table 2. Association between dental fluorosis and *ER RsaI* polymorphisms

Polymorphisms	Cases N=74	EFV controls		NEFV controls	
		N=69	OR(95%CI)	N=94	OR(95%CI)
rr	45(0.608)	51(0.739)	1.00	60(0.638)	1.00
Rr	20(0.270)	14(0.202)	1.705(0.452-6.435)	32(0.340)	3.955(0.761-20.541)
RR	9(0.121)	4(0.058)	1.235(0.290-5.263)	2(0.021)	5.294(0.960-29.193)

Adjusted for age and gender.

Table 3. The comparison of dental fluorosis in boys and girls(%)

gender	Cases	Controls in EFV	Prevalence	χ^2	*P*
boys	33	41	44.6%	3.14	0.076
girls	41	28	59.4%		

Table 4. Comparison of ER RsaI polymorphisms in boys and girls with dental fluorosis

gender	rr	Rr	RR	total	χ^2	*P*
boys	22(0.667)	6(0.182)	5(0.151)	33	1.757	0.415
girls	23(0.561)	13(0.317)	5(0.122)	41		
total	45	20	9	74		

Table 5. Comparison of urine fluorosis in groups

Groups	n	The scope of urinary fluoride	median	*P*
Cases	74	1.049~5.706	2.445	0.000
EFV controls	69	1.143~5.135	2.419	
NEFV controls	94	0.110~2.299	0.702	

Table 6. Comparison of ER RsaI genotypes in high-loaded fluoride status children with or without dental fluorosis

Groups	rr	Rr	RR	Total	χ^2	*P*
Cases	44(0.667)	16(0.242)	6(0.091)	66	0.275	0.872
Non-dental fluorosis	39(0.709)	12(0.218)	4(0.072)	55		
Total	83	28	10	121		

4 Discussion

Endemic fluorosis is a major public health concern in Henan province due to the excessive consumption of fluoride in drinking water. To date, the role of genetic susceptibility in relation to fluorosis, particularly dental fluorosis, has been unclear. Huang et al. found that children with homozygous P allele of COL1A2 PvuII had about five times the risk of dental flouorosis compared to children with homozygous p allele after adjusting for age and gender [8]. The results above indicated that genetic factor plays a role in the formation of Children's dental fluorosis. Estrogen plays an important role in stimulating osteoblast activity, promoting the deposition of calcium and phosphate in bone. Therefore, *ER* genetic polymorphism may be have an impact on the combination of estrogen and biological activity. There are some similarities between the formation of teeth and bones. Furthermore, the pathological change of dental fluorosis is the same as skeletal fluorosis, and it related to osteoblast activities, secreted structural matrix proteins and multiple proteases, and fluid composition including calcium ions and fluoride ions. As such, we speculate that *ER* gene polymorphism may be associated with pathological changes in bone, at the same time it may influence the formation of Children's dental fluorosis in Endemic fluorosis areas.

*Rsa*I polymorphism of *ER* gene is located on the codons 328, and resulting in synonymous mutation[9]. Studies suggested that the synonymous mutation may affect the expression of mRNA at synthesis, editing, sophisticated, transit, translation, degradation, etc, and thus may have an impact on protein function [10-12]. Sundarrajan et al.[13]suggested that ERß gene RsaI polymorphisms may be associated with ovulatory defects in some patients. On the other hand, Lau and Bagger also reported that *ER* gene *Rsa*I polymorphism was not associated with bone mineral density[14,15]. In the current study, no relationship was found between ER gene Rsa Ⅰ polymorphism and dental fluorosis although children with dental fluorosis had about two times higher of the proption of carrying homozygous H allele of *ER Rsa*I compared to children without dental fluorosis. The above results indicated that *ER* gene polymorphism may not be obvious associated with dental fluorosis..

Our study showed that no significant differences were found in urinary fluoride levels between cases and controls from the EFV (*P*>0.05). It means that, in the endemic fluorosis area, nearly half of children (45.5%) with higher interior exposure levels (urinary fluoride>1.5mg/L) did not suffer from dental fluorosis. The result shows that there has individual susceptibility under the same exposure conditions. However, no statistically significant difference in the distribution of *ER Rsa*I genotype among these groups (*P*>0.05). The reasons here, on the one hand, *ER* gene RsaI polymorphism may be not the fluoride-sensitive polymorphism marker. On the other hand, dental fluorosis is a complex disease and it is likely that several genes influence dental malformations. Therefore, further investigation on other polymorphisms of *ER* gene and other candidate genes related to calcium-metabolism may be useful.

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