International Journal of Hygiene and Environmental Health 239 (2022) 113879

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Effects of SNPs in *SOD2* and *SOD3* interacted with fluoride exposure on the susceptibility of dental fluorosis

Yuhui Du [a], Xiaoli Fu [a], Jing Jin [b], Zhiyuan Li [a], Kaihong Xu [a], Meng Guo [a], Xiangbo Hou [a], Zichen Feng [a], Limin Ding [a], Yongxiang Gong [a], Bin Liu [a], Fangfang Yu [a], Guoyu Zhou [a], Yue Ba [a,*]

[a] School of Public Health, Zhengzhou University, Zhengzhou, Henan, 450001, China
[b] Houzhai Center Hospital, Erqi District, Zhengzhou, Henan, 450001, China

ARTICLE INFO

Keywords:
Fluoride
Single nucleotide polymorphism
SOD2 gene
SOD3 gene
Dental fluorosis

ABSTRACT

A total of 649 children aged 7–13 years of age were recruited in a cross-sectional study in Tongxu County, China (2017) to assess the effects of interaction between single nucleotide polymorphisms (SNPs) in *SOD2* and *SOD3* gene and fluoride exposure on dental fluorosis (DF) status. Associations between biomarkers and DF status were evaluated. Logistic regression suggested that the risk of DF in children with rs10370 GG genotype and rs5746136 TT genotype was 1.89-fold and 1.72-fold than that in children with TT/CC genotype, respectively. Increased T-SOD activity was associated with a lower risk of DF (*OR* = 0.99). The rs2855262*rs10370*UF model was regarded as the optimal interaction model in generalized multifactor dimensionality reduction analyses. Our findings suggested that rs4880 and rs10370 might be useful genetic markers for DF, and there might be interactions among rs10370 in *SOD2*, rs2855262 in *SOD3,* and fluoride exposure on DF status.

1. Introduction

Multiple elements exposure in the environment might affect human health (Ceballos et al., 2021; Soler-Blasco et al., 2020; Wan et al., 2021). Excessive ingestion of fluoride can induce fluorosis. Dental fluorosis (DF) is one of the typical clinical features and is regarded as the first discovery of a clinical feature, suggesting the association between fluoride exposure and human health (Jha et al., 2011). DF is characterized by mottling, chalky spots (or stripes, plaques), yellowish to black stains, and even pitting defect on tooth or enamel surfaces. Considering its role as a biomarker of other health lesions caused by fluoride and effects on mental health, DF is an important clinical symptom (Molina-Frechero et al., 2017; Yu et al., 2018).

Excessive intake of fluoride during tooth formation has been confirmed as the main risk factor for DF (Moller, 1982; Pendrys and Stamm, 1990). The effects of fluoride on teeth are mediated through several ways. Fluoride reacts with calcium, depositing calcium fluoride in the developing tooth structure, disturbing normal mineralization of enamel, and causing anomalous spherical structure in the normal crystalline structure, with increased porosity and opacity (Everett, 2011). The mechanism of odontoblasts apoptosis induced by sodium fluoride (NaF) has also been confirmed in vitro (Li et al., 2013). In addition, fluoride exposure might have detrimental effects on transitional, early-secretory, and maturation stages of ameloblasts and the developing enamel matrix (DenBesten and Thariani, 1992; Lyaruu et al., 2006), significantly impacting fluoride-induced tooth damage. Oxidative stress, as an important mode of fluoride toxicity (Chouhan et al., 2010; Garcia-Montalvo et al., 2009; Zhang et al., 2007), might be responsible for several mechanisms above. Previous studies have confirmed that fluoride exposure induces oxidative stress, activating apoptotic pathway in cementoblasts and ameloblasts (Li et al., 2017; Ni et al., 2018; Wang et al., 2016). Superoxide dismutase isozymes (SODs), an important family of the oxidative stress system, are essential to protect the body from effects of O_2^-, including SOD1, SOD2 and SOD3. SOD1, also called Cu/Zn-SOD, is almost exclusively found in intracellular cytoplasmic spaces. SOD2 (Mn-SOD) and SOD3 (extracellular Cu/Zn-SOD) can allbe found in serum (Zelko et al., 2002). It has been reported that mutations in genes might be associated with changes in phenotypes and mutations in *SOD2*, and *SOD3* genes appear to be associated with changes in serum SOD activity (Lewandowski et al., 2020). The SOD activity is believed to be involved in several

* Corresponding author.
E-mail addresses: 694078176@qq.com (Y. Du), xlfu66@126.com (X. Fu), 670030787@qq.com (J. Jin), lizhiyuan@zzu.edu.com (Z. Li), kaihhongong@163.com (K. Xu), 15754307599@163.com (M. Guo), 1142077913@qq.com (X. Hou), 2313888516@qq.com (Z. Feng), 1617738851@qq.com (L. Ding), 1610991676@qq.com (Y. Gong), 2507656299@qq.com (B. Liu), yufangfang@zzu.edu.cn (F. Yu), zhouguoyu@zzu.edu.cn (G. Zhou), byyue@zzu.edu.cn (Y. Ba).

https://doi.org/10.1016/j.ijheh.2021.113879
Received 11 August 2021; Received in revised form 11 October 2021; Accepted 28 October 2021
Available online 2 November 2021

Abbreviation

DF	dental fluorosis
SNPs	single nucleotide polymorphisms
GMDR	generalized multifactor dimensionality reduction analyses
SODs	Superoxide dismutase isozymes
AMBN	*ameloblastin*
TFIP11	*tuftelin interacting protein 11*
TUFT1	*tuftelin*
COL1A2	*type I collagen alpha 2 chain*
ESR	*estrogen receptor*
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CV	coefficient of variant
T-SOD	total superoxide dismutase
UF	Urinary fluoride
UCr	urinary creatinine
OR	odds ratio
CI	confidence interval
TBA	testing balanced accuracy
CVC	cross-validation consistency

fluoride-induced health effects, including reproductive damage, neural impairment, and hepatic anomalies (Adelakun et al., 2021; Cao et al., 2019; Lu et al., 2017). A study of Li et al. (2017) indicated that SOD activity also played an important role in the mechanism of NaF-induced ameloblast apoptosis. Therefore, SOD activity might correlate with the DF status.

It is noteworthy that genetic factors, such as gene polymorphisms, have attracted considerable attention concerning DF susceptibility. Previous studies (Charone et al., 2019; Dalledone et al., 2019; Kuchler et al., 2018) found that the polymorphisms of *ameloblastin* (*AMBN*), *tuftelin interacting protein* 11 (*TFIP11*), *tuftelin* (*TUFT1*), *Ambn*、*Col14a1*, *Mmp20* and *estrogen receptor 1* (*ESR1*) might have a relationship with the risk of DF. Our previous studies showed that *type* I *collagen alpha* 2 *chain* (*COL1A2*) gene *Pvu*II and *ESR* gene polymorphisms were also related to DF development (Ba et al., 2011; Huang et al., 2008). Considering the important role of SOD activity in the DF process, variations in SOD genotypes would be associated with the prevalence of DF. Besides, a study demonstrated that the health effects of fluoride exposure had interactions with genetic factors (Ma et al., 2017). However, differential susceptibility of genetic factors to DF and the effects of interactions between fluoride exposure and genetic factors on DF development remains unclear.

Given the above, a cross-sectional study was conducted in a rural area located in Tongxu County of Henan Province. Five single nucleotide polymorphisms (SNPs) were selected from *SOD2* and *SOD3* genes to assess the effects of genetic polymorphisms and interactions with fluoride exposure on the prevalence of DF in school-age children to provide useful genetic marker for differential risk of DF and epidemiological evidence for prevention of DF.

2. Materials and methods

2.1. Location and subjects

As described in our previous study (Wang et al., 2021), a cross-sectional study was conducted in rural areas of Tongxu County of Henan province, China, an endemic fluorosis area of drinking water type due to natural geological structure in 2017. Four local primary schools were randomly selected according to fluoride concentration, one of which was located in the fluorosis area and the other three were located in the non-fluorosis area. Students in grades 2–6 from the four schools were selected by the cluster sampling method. From which we selected students who were born and resided locally. They did not expose to other fluoride sources from brick tea and industry dust. Children who were non-local residents or had received calcium and phosphorus supplements were excluded. Children with diseases affecting calcium and phosphorus metabolism, digestive diseases, thyroid-related disease, and neuropsychiatric disorders were also excluded. All the 649 children aged 7–13 years recruited in the study were boarding-school students and had similar living conditions, living habits and dietary patterns. There is no fluoride pollution from coal combustion and industry in the investigated area, and the local residents had no habit of drinking brick tea. Therefore, drinking water is the main source of fluoride exposure. Children and their guardians agreed to participate in the study and signed informed consent forms after being informed of the study procedure. The protocol of this research was approved by the Ethics Committee of Zhengzhou University (ZZUIRB, 2017–018).

2.2. Collection of general information

Demographic data were obtained through a structured questionnaire by a face-to-face interview, including age, gender, class, grade, health status, paternal and maternal information, etc. Both children and their guardians joined in and provided information.

The body mass index (BMI) was used to assess children's developmental status. All the subjects were examined by skilled medical professionals from Kaifeng Centers for Disease Control and Prevention (CDC) to measure height and weight. Height (cm) and weight (kg) were measured in duplicate by a standard measurement device (V. BODY HBF-371; OMRON, Kyoto, Japan). Precisions were 0.1 cm and 0.1 kg, respectively, and the mean value was used for calculation. The BMI was calculated using height and weight values with the formula: BMI (kg/m^2) = weight/height^2.

2.3. Assessment of dental fluorosis

DF was also examined by the public medical professionals from Kaifeng CDC. Prior to the examination, medical experts were trained in diagnostic criteria and achieved unified examination skills. Then the examinations were performed in a brightly lit room. After tooth cleaning and drying, all the buccal surfaces of permanent teeth were examined with the help of artificial lights. Dean's fluorosis index (WS/T 208–2011) was used to determine the presence of DF. Each child was examined twice by two independent medical professionals. The accordant results of double measurements were recorded. In case of disagreement, the third expert joined in and performed the third assessment, and the final diagnosis was recorded by collating the three diagnostic results.

2.4. Measurement of urinary fluoride and urinary creatinine

Urinary fluoride (UF) concentrations were determined as the internal exposure levels and urinary creatinine (UCr) levels were used to correct for variations in urine dilution, in this study. All the children provide more than 50 mL of early-morning urine samples for measuring UF and UCr concentrations. UF levels were measured by the fluoride ion-selective electrode method (Shanghai Exactitude Instrument, Shanghai, China), with 0.01 mg/L of the detection limit. All the samples were measured twice, and mean values were used. The coefficient of variant (CV) values of repeated determinations were all <10%. UCr contents were determined using the picric acid method and all the assays were performed according to the kit protocol (Creatinine Assay Kit C011-1-1, Jiancheng Bioengineering Institute, Nanjing, China), which were used to correct for variations in urine dilution (Bashash et al., 2017). Each sample was measured twice and average values were used. In addition, 15% of urine samples from different plates were randomly

selected to repeat the measurement. The CV values of repeated tests were <10%.

2.5. Assessment of SOD activity

Peripheral fasting blood samples were collected, and serums were separated. Each serum sample was used to assess the total superoxide dismutase (T-SOD) activity. All the assays were performed according to the manufacturer's instructions. Ten percent of serum samples were randomly selected for duplicate measurements. The CV coefficients ranges was <10%, and mean values of repeated measurement were ultimately used.

The T-SOD activity was assessed with the Xanthine Oxidase Method (Total SOD Assay Kit A001-1, Jiancheng Bioengineering Institute, Nanjing, China). In this method, the xanthine-xanthine oxidase system was used as a superoxide generator. SOD can inhibit the generation of nitrite by the reaction between hydroxylamine and superoxide. The reduction of absorbance at 550 nm was measured at room temperature (RT). One unit of SOD was defined as the amount of enzyme that causes 50% inhibition.

2.6. Identification of genotypes

Five SNPs in *SOD2* and *SOD3* were selected from Haploview software, meeting the following criteria: (1) The minor allele frequencies of these SNPs were all >0.1; (2) these SNPs were reported in previous studies; (3) The retained SNPs in the same gene was not strong in linkage disequilibrium (pairwise $r^2 < 0.8$). According to the criteria, 5 SNPs from the *SOD2* and *SOD3* genes were retrieved, including rs10370 (in *SOD2*), rs4880 (in *SOD2*), rs5746136 (in *SOD2*), rs13306703 (in *SOD3*) and rs2855262 (in *SOD3*) (see Table S1).

Genomic DNA miniprep kits (LifeFeng Biotechnology, Shanghai, China) were used to extract the genomic DNA from the peripheral blood samples. The genotyping of the 5 SNPs was performed by operators blinded to subjects' information using a custom-by-design 48-Plex SNPscan™ Kit (Cat#: G0104; Genesky Biotechnologies Inc., Shanghai, China). The kit was developed according to the patented SNP genotyping technology of Genesky Biotechnologies Inc., based on double ligation and multiplex fluorescence PCR. The reaction was carried out in an ABI2720 thermal cycler (Chen et al., 2012). Approximately 4% of the samples were repeatedly genotyped for quality control, and the consistency rate was >96%. The identified genotypes were TT/TG/GG for rs10370, GG/GA/AA for rs4880, CC/CT/TT for rs5746136, CC/CT/TT for rs13306703 and TT/TC/CC for rs2855262.

2.7. Statistical analyses

The dataset was created using the Epidata3.0 software (Epidata Association Odense, Denmark) and all data were put in independently by two operators.

Means and standard deviations were displayed for age, BMI, UCr concentration, UF levels, T-SOD activity, and percentages/proportions for gender, gene types, and allele types. The chi-square test and student's *t*-test were used for difference testing in continuous and categorical variables between children with and without DF. In trend testing, new variables were generated based on the UF concentrations and the T-SOD activity, taking the median value of each tertile range as every value in this tertile range. Multiple logistic regressions were employed to explore associations between DF and biomarkers, with or without covariates adjustments, and reported as the odds ratio (*OR*) with the 95% confidence interval (*CI*).

Furthermore, the associations between T-SOD activity and SNPs were also evaluated, reporting β value with the 95% *CI*. The statistical analyses above were performed by SPSS 21.0 (SPSS Inc., Chicago, USA). In addition, taking previous studies as references, multivariable linear or logistic regressions were adjusted by confounding factors such as age, gender, BMI and UCr (Abanto Alvarez et al., 2009; Narwaria and Saksena, 2013; Zhou et al., 2019a). To further explore the associations between interactions of genetic factors and fluoride exposure and the risk of DF, the generalized multifactor dimensionality reduction (GMDR) method was performed with 10-fold cross-validation. A dichotomous variable of fluoride exposure, generated by UF concentrations taking 1.4 mg/L as the cutoff value, was used in the GMDR analyses. The models were assessed by the testing balanced accuracy (TBA), cross-validation consistency (CVC) scores, and *P* values. The TBA was used to evaluate the accuracy of the interaction to predict DF status. The CVC scores are measures of the consistency degree defined as the times of a given combination regarded as the optimal model in a particular validated run. The *P* values were used to assess the significance of an identified model. The GMDR analyses were performed using GWAS-GMDR 1.0 beta software (http://ibi.zju.edu.cn/software) (Xu et al., 2016). The test was regarded statistically significant at $P < 0.05$.

3. Results

3.1. The distributions of general characteristics and biomarkers in children with and without DF

A total of 649 subjects aged 7–13 years were recruited in the present study dataset, including 337 (51.9%) girls and 312 (48.1%) boys. Totally, 178 (27.4%) children were suffering from DF. As shown in Table 1, the children were divided into two groups by DF status: the non-DF and DF groups. The mean ages in the DF group was significantly higher than in the non-DF group ($P = 0.026$). The gender, BMI and UCr in the DF group were comparable to the non-DF group. Compared with children in the non-DF group, children in the DF group exhibited higher UF concentrations and lower T-SOD levels. The DF prevalence in children with high fluoride exposure (UF > 1.4 mg/L) was 33.4%, while it was 22.6% in children with low fluoride exposure (UF ≤ 1.4 mg/L).

3.2. The associations between DF and biomarkers

The ancestral allele types are T for rs10370, G for rs4880, C for rs5746136, C for rs13306703 and T for rs2855262 (see Table S1). The genotype distributions of SNPs are presented in Table 2. We found that children with the GG genotype of rs10370 had a 1.89-fold (95%*CI* for *OR*: 1.20, 2.96) DF risk than children with the TT genotype, after adjusting for the control factors. An increased risk of DF was also observed in children carrying the TT genotype of rs5746136 (*OR*: 1.72, 95% *CI* for *OR*: 1.09, 2.69). In terms of alleles, compared with children carrying the T/C alleles, children carrying the G allele of rs10370 (*OR* = 1.31) or T allele rs5746136 (*OR* = 1.26) had a higher risk of DF.

The associations between UF and DF are presented in Table 3. The prevalence of DF showed an upward trend across tertiles of UF (all *P* <

Table 1
General characteristics of the study population.

variables	non-DF ($n = 471$)	DF ($n = 178$)	*t*/	*P* value
Age (year)[a]	9.96 ± 1.31	10.22 ± 1.26	2.230	**0.026**
Gender[b]			1.339	0.247
Boys	233(74.7)	79(25.3)		
Girls	238(70.6)	99(29.4)		
BMI (kg/m^2)[a]	17.65 ± 3.17	17.40 ± 2.34	1.087	0.278
UCr (mg/L)[a]	972.32 ± 660.51	1009.68 ± 679.68	0.630	0.529
UF (mg/L)[a]	1.31 ± 0.85	1.59 ± 0.88	3.634	**<0.001**
UF group[b]			9.550	**0.002**
UF ≤ 1.4 mg/L	278(77.4)	81(22.6)		
UF > 1.4 mg/L	193(66.6)	97(33.4)		
T-SOD (U/ml)[a]	115.56 ± 21.05	111.32 ± 20.61	2.324	**0.021**

Abbreviation: BMI, body mass index; DF, dental fluorosis; T-SOD, total superoxide dismutase; UCr, urinary creatinine; UF, urinary fluoride.

[a] Continuous variables were presented by mean ± standard deviation.

[b] Categorical variables were presented by number (proportion/percentage).

Table 2
The association between SNPs and the risk of DF.

SNPs	non-DF ($n = 471$)	DF ($n = 178$)	*OR* (95% *CI*)[a]	*P* value for genotypes	*P* value for alleles
SOD2					
rs10370				**0.008**	
TT	142 (30.1)	46 (25.8)	reference		
TG	241 (51.2)	81 (45.5)	1.07 (0.73,1.57)	0.741	
GG	88 (18.7)	51 (28.7)	1.89 (1.20,2.96)	**0.006**	
T	525 (55.7)	173 (48.6)	reference		
G	417 (44.3)	183 (51.4)	1.31 (1.04,1.64)		**0.021**
rs4880				0.496	
GG	13 (2.8)	4 (2.2)	reference		
GA	126 (26.8)	45 (25.3)	1.58 (0.48,5.17)	0.452	
AA	332 (70.5)	129 (72.5)	1.81 (0.57,5.74)	0.316	
G	152 (16.1)	53 (14.9)	reference		
A	790 (83.9)	303 (85.1)	1.17 (0.85,1.62)		0.333
rs5746136				**0.034**	
CC	134 (28.5)	42 (23.6)	reference		
CT	238 (50.5)	84 (47.2)	1.08 (0.73,1.60)	0.683	
TT	99 (21.0)	52 (29.2)	1.72 (1.09,2.69)	**0.019**	
C	506 (53.7)	168 (47.2)	reference		
T	436 (46.3)	188 (52.8)	1.26 (1.01,1.58)		**0.044**
SOD3					
rs13306703				0.646	
CC	326 (69.2)	125 (70.2)	reference		
CT	135 (28.7)	47 (26.4)	0.89 (0.62,1.29)	0.552	
TT	10 (2.1)	6 (3.4)	1.39 (0.52,3.71)	0.514	
C	787 (83.5)	297 (83.4)	reference		
T	155 (16.5)	59 (16.6)	0.97 (0.72,1.32)		0.858
rs2855262				0.460	
TT	198 (42.0)	81 (45.5)	reference		
TC	223 (47.3)	76 (42.7)	0.83 (0.59,1.16)	0.270	
CC	50 (10.6)	21 (11.8)	1.06 (0.62,1.81)	0.838	
T	619 (65.7)	238 (66.9)	reference		
C	323 (34.3)	118 (33.1)	0.98 (0.78,1.25)		0.893

[a] Adjusted by age, gender, BMI, UCr and UF.

0.05). Compared with children in tertile 1, children in tertile 2 and in tertile 3 had 1.85-fold and 2.47-fold DF risk, respectively. A 1.53-fold (95%*CI* for *OR*: 1.22, 1.93) risk of DF was observed for each 1 mg/L increment in UF concentrations after adjusting for potential confounding factors.

The associations between T-SOD activity and DF levels are presented in Table 4. The risk of DF showed upward trends across tertiles of T-SOD (*P* for trend <0.05) after adjusting for covariates. The risk of DF in children decreased 0.1% with an increase in per-unit T-SOD activity, suggesting that increased T-SOD activity was a protective factor for the incidence of DF.

Table 3
The association between UF and DF.

UF (mg/L)	DF			
	Crude, *OR* (95% *CI*)	*P* value	Adjusted, *OR* (95% *CI*)[a]	*P* value
Tertile 1(≤0.88)	Reference		Reference	
Tertile 2 (0.89–1.64)	1.72(1.10,2.69)	**0.018**	1.85(1.17,2.93)	**0.008**
Tertile 3(>1.64)	2.30(1.48,3.57)	**<0.001**	2.47(1.52,4.02)	**<0.001**
Trend test		**<0.001**		**<0.001**
Increase per 1 mg/L	1.43(1.18,1.74)	**<0.001**	1.53(1.22,1.93)	**<0.001**

[a] Adjusted by age, gender, BMI and UCr.

Table 4
The association between T-SOD and DF.

T-SOD (U/mL)	DF			
	Crude, *OR* (95% *CI*)	*P* value	Adjusted, *OR* (95% *CI*)[a]	*P* value
Tertile 1(≤105.37)	Reference		Reference	
Tertile 2 (105.38–123.35)	0.95(0.69,1.33)	0.778	1.01(0.71,1.46)	0.933
Tertile 3(>123.35)	0.60(0.42,0.85)	**0.004**	0.64(0.43,0.95)	**0.029**
Trend test		**0.005**		**0.034**
Increase per 1 mg/L	0.99(0.98,0.99)	**0.004**	0.99(0.98,0.99)	**0.014**

[a] Adjusted by age, gender, BMI, UCr and UF.

3.3. The associations between SNP types and T-SOD activity

Simple and multiple linear regressions were used to explore the associations between SNPs and T-SOD activity. After covariates adjustments, significant associations were observed between the T-SOD activity and polymorphisms of rs10370 and rs4880. Compared with children carrying the TT/GG genotypes, children with the GG genotype of rs10370 and the AA genotype of rs4880 exhibited 4.24 U/mL and 4.25 U/mL reductions in T-SOD activity, respectively. No associations were observed between rs5746136, rs13306703, and rs2855262 genotypes and the T-SOD activity. Compared with children carrying the T allele, children carrying the G allele of rs10370 exhibited significant reductions in T-SOD activity (adjusted $\beta = -3.87$, 95%*CI* for β: −6.03, −1.71), after adjusting for confounding factors. Similarly, children carrying the A allele of rs4880 and the T allele of rs5746136 also exhibited significantly reduced T-SOD activity compared to children carrying the G/C allele (Table 5).

3.4. The effects of interactions between gene types and fluoride exposure

GMDR analyses were performed to explore the effects of interactions between fluoride exposure and *SOD2* and *SOD3* polymorphisms on the risk of DF. The possible interaction models are summarized in Table 6. After covariate adjustments, the GMDR analyses suggested three interaction models of UF*rs10370, UF*rs2855262*rs10370, and UF*rs2855262*rs10370*rs4880 with statistically significant differentiation (all $P = 0.0107$), indicating potential gene-environment interactions among UF, rs10370, rs2855262, and rs4880. Based on TBA and CVC, UF*rs2855262*rs10370 model was regarded as the optimal model, showing a good CVC of 10/10 and TBA of 58.66%. The high-risk and low-risk distributions of the optimal model are illustrated in Fig. 1. We found the GG genotype of rs10370, higher UF concentrations (>1.4 mg/L) combined with the TT genotype of rs2855262 took the highest DF risk with the highest sum score in the optimal model. The combinations of rs10370 = GG*UF > 1.4 mg/L and rs4880 = AA*rs10370 = GG*UF ≤ 1.4 mg/L*rs2855262 = TT had the maximum risks in the other two statistically significant models (Figs. S1 and S2), respectively.

Table 5
The associations of SNPs and T-SOD activity.

SNPs	T-SOD activity			
	Crude β (95% *CI*)	*P*-value	Adjusted β (95% *CI*)[a]	*P*-value
SOD2				
rs10370				
TT	reference		reference	
TG	−2.06(−5.01,0.884)	0.170	−2.22(−5.29,0.84)	0.155
GG	−2.86(−6.53,0.82)	0.127	−4.24(−8.06,−0.43)	**0.029**
T	reference		reference	
G	−2.89(−4.97,−0.80)	**0.007**	−3.87(−6.03,−1.71)	**<0.001**
rs4880				
GG	reference		reference	
GA	−0.07(−10.41,10.26)	0.989	−5.77(−15.83,4.29)	0.259
AA	−3.82(−7.12, −0.52)	**0.023**	−4.25(−7.65, −0.84)	**0.015**
G	reference		reference	
A	−3.27(−6.20, −0.33)	**0.029**	−3.80(−6.80,−0.80)	**0.013**
rs5746136				
CC	reference		reference	
CT	−2.09(−5.04,0.85)	0.164	−2.39(−5.45,0.67)	0.126
TT	−2.17(−5.73,1.39)	0.231	−3.25(−6.97,0.46)	0.086
C	reference		reference	
T	−2.54(−4.62, −0.46)	**0.017**	−3.43(−5.59, −1.27)	**0.002**
SOD3				
rs13306703				
CC	reference		reference	
CT	1.67(−1.64,4.98)	0.322	1.77(−1.70,5.25)	0.317
TT	−10.05(−18.41, −1.68)	**0.019**	−3.91(−13.60,5.79)	0.429
C	reference		reference	
T	−0.72(-3.50,2.06)	0.611	−0.66(−2.26,3.59)	0.657
rs2855262				
TT	reference		reference	
TC	2.65(−0.30,5.60)	0.079	1.69(−1.38,4.76)	0.281
CC	−0.98(−5.84,3.88)	0.692	−1.37(−6.31,3.58)	0.588
T	reference		reference	
C	1.08(−1.13,3.29)	0.339	0.35(−1.93,2.63)	0.763

[a] Adjusted by age, gender, BMI, UCr and UF.

Table 6
The summary information for GMDR.

model	TBA	*P* value[a]	CVC
rs10370	0.5365	0.3770	8/10
UF*rs10370	0.5429	**0.0107**	9/10
UF*rs2855262*rs10370	0.5866	**0.0107**	10/10
UF*rs2855262*rs10370*rs4880	0.5497	**0.0107**	9/10
UF*13306703*rs2855262*rs10370*rs4880	0.5115	0.3770	9/10
UF*13306703*rs2855262*rs10370*rs4880*rs5746136	0.5056	0.8281	10/10

[a] Adjusted by age, gender, BMI and UCr.

4. Discussion

The relationships between biomarkers (UF, T-SOD, and SNPs) and DF were explored in the present study. The associations between T-SOD activity and SNPs in *SOD2* and *SOD3* genes were also tested. Then the associations between gene-environment interactions and the risk of DF were investigated with GMDR analyses. We found that children with the GG genotype of rs10370 and the TT genotype of rs5746136 had a 1.89-fold and 1.72-fold risk of DF, respectively, than children carrying the TT/CC gene types, and compared with children carrying the T/G/C alleles, children carrying the G allele of rs10370, the A allele of rs4880 or the T allele of rs5746136 had a higher risk of DF. In addition, increased T-SOD activity was associated with a lower risk of DF. Besides, interactions of rs2855262, rs10370 and UF were regarded as the optimal model in the risk of DF, in which, the children carrying the GG genotype of rs10370, the TT genotype of rs2855262 and having higher UF concentrations (>1.4 mg/L) had the highest risk of DF.

Given the number of DF cases and affected areas, the drinking water type endemic fluorosis is still a major public health concern in China, especially in the north-central region. There were still 1055 counties regarded as drinking water type endemic fluorosis regions, and 12.41 million suffered from dental fluorosis until the end of 2019 according to the National Health Commission of the P.R. China (http://www.nhc.gov.cn/guihuaxxs/s10748/202006/ebfe31f24cc145b198dd730603ec4442.shtml). Therefore, it is essential to identify the susceptible populations to prevent DF. Studies have widely investigated the importance of fluoride exposure, including the duration, ingested amount, and the stage of fluoride exposure, in the incidence of DF (DenBesten and Thariani, 1992; Robinson and Kirkham, 1990). Consistent with previous studies (Yu et al., 2018; Zhou et al., 2019a), the positive associations between the risk of DF and UF concentrations in children aged 7–13 years were also observed in this study. Recent researches have directed attention toward the importance of oxidative stress in the mechanisms of fluoride-induced DF (Li et al., 2017; Zhou et al., 2019b). A significant decrease in SOD activity in NaF-treated ameloblasts has been reported (Li et al., 2017). In this study, reductions in the risk of DF were associated with T-SOD activity, suggesting that increased T-SOD activity might be a protective factor for DF. Studies have shown that mutations in *SOD3* and *SOD2* gene might be associated with changes in corresponding SOD levels (Bresciani et al., 2015; Folz et al., 1994). The significant associations between mutations in *SOD2* gene and T-SOD activity observed in this study indicate that mutations in rs10370 and rs4880 in *SOD2* gene were correlated with decreased T-SOD activity in serum. The decreased T-SOD activity might lead to its impaired catalytic ability to convert the superoxide radical to hydrogen peroxide (Wang et al., 2018).

Genetic factors, especially gene polymorphisms, have been implicated in the mechanism of DF recently. Previous studies have explored the associations between *matrix metalloproteinases* (*MMPs*), *catalase* (*CAT*) rs769217, *paraoxonase 1* (*PON1*) rs662, vitamin D receptor gene CDX2, *COL1A2*, *osteocalcin*, *ESR* and *PTH Bst* BI polymorphisms and DF status, providing evidence of the associations between of *COL1A2, CAT* rs769217, *PON1* rs662 and *ESR* gene polymorphisms and the risk of DF in children with high-load fluoride (Ba et al., 2009, 2011; Huang et al., 2008; Liu et al., 2018, 2019; Romualdo et al., 2019; Wen et al., 2012; Zhang et al., 2010). Subsequently, Kuchler et al., 2017, 2018 suggested that the polymorphisms of *tissue inhibitors of metalloproteinase1*, *distal-less1, DLX2, AMBN, TFIP11*, and *TUFT1* genes involved in enamel development might be useful genetic markers for the differential risk of DF. Furthermore, the study conducted by Abbasoglu et al. (2020) indicated that the polymorphisms of rs4284505 in microRNA17 were associated with the DF status. In the present study, there were significant associations between polymorphisms of *SOD2* (rs10370 and rs5746136) and the risk of DF, which might be due to decreased T-SOD activity caused by mutations in the *SOD2* gene, resulting in impaired clearance of reactive oxygen species and then leading to oxidative stress. Then the imbalance in the oxidative and anti-oxidant system during oxidative stress might mediate the ameloblast apoptosis and other cellular events and ultimately cause DF (Li et al., 2012, 2017).

It is well established that the effects of a single gene on the phenotypic trait of DF might be too small to be noticed sometimes. For instance, Huang et al., Jarquin-Yneza et al., and Rahila et al. (Huang et al., 2008; Jarquin-Yneza et al., 2018; Rahila et al., 2019) reported significant associations between polymorphisms in *COL1A2 Pvu*II (rs214777) and the incidence of DF, whereas Escobar-Garcia et al. and Saha et al. (Escobar-Garcia et al., 2016; Saha et al., 2021) did not observe such associations. These differences do not appear to be explained only by regions, fluoride exposure, and race. The process of DF might result from a complex interaction of genetic aspects and environmental exposure. Interactions between fluoride exposure and genetic factors in phenotypes have been reported. Zhao et al. (2021)

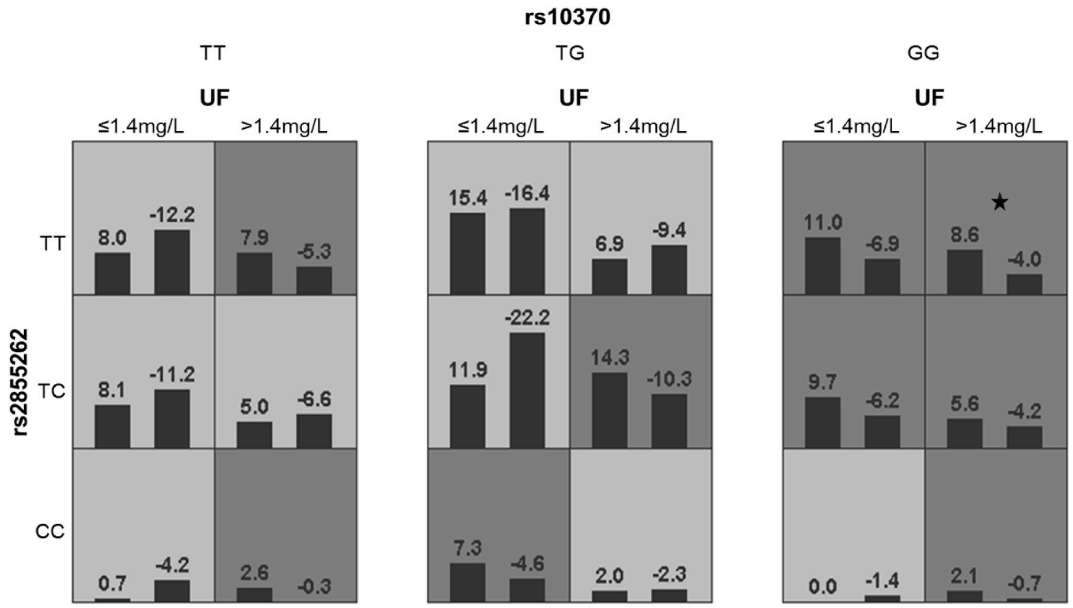

Fig. 1. The interaction pattern among rs10370, rs2855262 and UF. In each cell, the left bar represents a positive score, and the right bar a negative score. Dark and light gray cells correspond to the high-risk and low-risk interaction combinations, respectively. High-risk cells are indicated by dark shading, low-risk cells by light shading. White cells are unclassified. The ★ represents that the GG genotype of rs10370, higher UF concentrations (>1.4 mg/L) combined with the TT genotype of rs2855262 got the highest sum score in this model.

observed the effects of interactions between fluoride exposure and dopamine relative genes on intelligence. In our previous studies, the interactions between fluoride exposure and *ESR* alpha polymorphisms affected reproductive hormone concentrations and androgen binding protein levels (An et al., 2019; Ma et al., 2017). Here we observed that the interaction between fluoride exposure and polymorphisms of rs2855262 and rs103701 affected the risk of DF, suggesting that interactions between fluoride exposure and genetic polymorphisms were associated with the risk of DF. It might because alleles showing different sensitivities to fluoride exposure affected DF. John A. Eisman (1999) summarized previous research findings and suggested that this sensitivity might also be related to age because of the accumulation of age-related environmental exposures. Further studies are required to investigate the allelic effects of possible modifications in age-related fluoride accumulation on the risk of DF.

There are several advantages to the present study. Firstly, children recruited in this study are full-time accommodated students, with a similar dietary pattern, daily schedule and general demographic characteristics, resulting in fewer confounding factors. Secondly, The GMDR analyses were used in the present study, which can improve accuracy and reduce false-positive rate with the cross-validation test (Lou et al., 2007). However, there is also a limitation in the present study. We did not collect detailed information about fluoride exposure from food, drinking water and toothpaste, and we used urinary fluoride concentrations as the internal intake levels to deal with this issue.

5. Conclusion

In conclusion, rs4880 and rs10370 in *SOD2* might be useful genetic markers for DF, and interactions between rs10370 in *SOD2* and rs2855262 in *SOD3* and fluoride exposure might affect the risk of DF.

Notes

There are no competing financial interest among authors.

Funding

This study was supported by the National Natural Science Foundation of China (81972981 and 82003401) and Key Projects of Colleges and Universities of Henan Education Department (21A330006).

Acknowledgments

We are grateful to express our deep thanks to all of children recruited in this study, their guardian and their teachers. We thank all scientific and technological worker in the investigation for their hard work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113879.

References

Abanto Alvarez, J., Rezende, K.M., Marocho, S.M., Alves, F.B., Celiberti, P., Ciamponi, A. L., 2009. Dental fluorosis: exposure, prevention and management. Med. Oral, Patol. Oral Cirugía Bucal 14, E103–E107.

Abbasoglu, Z., Dalledone, M., Wambier, L.M., Pecharki, G., Baratto-Filho, F., Andrades, K.M.R., Scariot, R., Trevilatto, P.C., Brancher, J.A., Kuchler, E.C., 2020. Single nucleotide polymorphism rs4284505 in microRNA17 and risk of dental fluorosis. Acta Odontol. Scand. 78, 463–466.

Adelakun, S.A., Akintunde, O.W., Ogunlade, B., 2021. Fluoride-induced testicular degeneration and sperm quality deteriorations: salutary role of Cyperus esculentus tubers (tiger nut) extract in animal model. Rev. Int. Androl. 19 (3), 201–212. https://doi.org/10.1016/j.androl.2020.01.003.

An, N., Zhu, J., Ren, L., Liu, X., Zhou, T., Huang, H., Sun, L., Ding, Z., Li, Z., Cheng, X., Ba, Y., 2019. Trends of SHBG and ABP levels in male farmers: influences of environmental fluoride exposure and ESR alpha gene polymorphisms. Ecotoxicol. Environ. Saf. 172, 40–44.

Ba, Y., Huang, H., Yang, Y., Cui, L., Zhu, J., Zhu, C., Liu, J., Zhang, Y., 2009. The association between osteocalcin gene polymorphism and dental fluorosis among children exposed to fluoride in People's Republic of China. Ecotoxicol. Environ. Saf. 72, 2158–2161.

Ba, Y., Zhang, H., Wang, G., Wen, S., Yang, Y., Zhu, J., Ren, L., Yang, R., Zhu, C., Li, H., Cheng, X., Cui, L., 2011. Association of dental fluorosis with polymorphisms of estrogen receptor gene in Chinese children. Biol. Trace Elem. Res. 143, 87–96.

Bashash, M., Thomas, D., Hu, H., Martinez-Mier, E.A., Sanchez, B.N., Basu, N., Peterson, K.E., Ettinger, A.S., Wright, R., Zhang, Z., Liu, Y., Schnaas, L., Mercado-Garcia, A., Tellez-Rojo, M.M., Hernandez-Avila, M., 2017. Prenatal fluoride exposure

and cognitive outcomes in children at 4 and 6-12 Years of age in Mexico. Environ. Health Perspect. 125, 097017.
Bresciani, G., Cruz, I., González-Gallego, J., 2015. Manganese superoxide dismutase and oxidative stress modulation. Adv. Clin. Chem. 68, 87–130.
Cao, K., Xiang, J., Dong, Y.T., Xu, Y., Li, Y., Song, H., Zeng, X.X., Ran, L.Y., Hong, W., Guan, Z.Z., 2019. Exposure to fluoride aggravates the impairment in learning and memory and neuropathological lesions in mice carrying the APP/PS1 double-transgenic mutation. Alzheimer's Res. Ther. 11, 35.
Ceballos, D.M., Young, A.S., Allen, J.G., Specht, A.J., Nguyen, V.T., Craig, J.A., Miller, M., Webster, T.F., 2021. Exposures in nail salons to trace elements in nail polish from impurities or pigment ingredients - a pilot study. Int. J. Hyg Environ. Health 232, 113687.
Charone, S., Kuchler, E.C., Leite, A.L., Silva Fernandes, M., Taioqui Pela, V., Martini, T., Brondino, B.M., Magalhaes, A.C., Dionisio, T.J., C, F.S., Buzalaf, M.A.R., 2019. Analysis of polymorphisms in genes differentially expressed in the enamel of mice with different genetic susceptibilities to dental fluorosis. Caries Res. 53, 228–233.
Chen, X., Li, S., Yang, Y., Yang, X., Liu, Y., Liu, Y., Hu, W., Jin, L., Wang, X., 2012. Genome-wide association study validation identifies novel loci for atherosclerotic cardiovascular disease. J. Thromb. Haemostasis : JTH 10, 1508–1514.
Chouhan, S., Lomash, V., Flora, S.J., 2010. Fluoride-induced changes in haem biosynthesis pathway, neurological variables and tissue histopathology of rats. J. Appl. Toxicol. : JAT 30, 63–73.
Dalledone, M., Cunha, A.S., Ramazzotto, L.A., Pecharki, G.D., Nelson-Filho, P., Scariot, R., Trevilatto, P.C., Vieira, A.R., Kuchler, E.C., Brancher, J.A., 2019. Estrogen receptor gene is associated with dental fluorosis in Brazilian children. Clin. Oral Invest. 23, 3565–3570.
DenBesten, P.K., Thariani, H., 1992. Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. J. Dent. Res. 71, 1238–1243.
Eisman, J.A., 1999. Genetics of osteoporosis. Endocr. Rev. 20, 788–804.
Escobar-Garcia, D., Mejia-Saavedra, J., Jarquin-Yanez, L., Molina-Frechero, N., Pozos-Guillen, A., 2016. Collagenase 1A2 (COL1A2) gene A/C polymorphism in relation to severity of dental fluorosis. Community Dent. Oral Epidemiol. 44, 162–168.
Everett, E.T., 2011. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J. Dent. Res. 90, 552–560.
Folz, R.J., Peno-Green, L., Crapo, J.D., 1994. Identification of a homozygous missense mutation (Arg to Gly) in the critical binding region of the human EC-SOD gene (SOD3) and its association with dramatically increased serum enzyme levels. Hum. Mol. Genet. 3, 2251–2254.
Garcia-Montalvo, E.A., Reyes-Perez, H., Del Razo, L.M., 2009. Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. Toxicology 263, 75–83.
Huang, H., Ba, Y., Cui, L., Cheng, X., Zhu, J., Zhang, Y., Yan, P., Zhu, C., Kilfoy, B., Zhang, Y., 2008. COL1A2 gene polymorphisms (Pvu II and Rsa I), serum calciotropic hormone levels, and dental fluorosis. Community Dent. Oral Epidemiol. 36, 517–522.
Jarquin-Yneza, L., Alegria-Torres, J.A., Castillo, C.G., de Jesus Mejia-Saavedra, J., 2018. Dental fluorosis and a polymorphism in the COL1A2 gene in Mexican children. Arch. Oral Biol. 96, 21–25.
Jha, S.K., Mishra, V.K., Sharma, D.K., Damodaran, T., 2011. Fluoride in the environment and its metabolism in humans. Rev. Environ. Contam. Toxicol. 211, 121–142.
Kuchler, E.C., Dea Bruzamolin, C., Ayumi Omori, M., Costa, M.C., Antunes, L.S., Pecharki, G.D., Trevilatto, P.C., Vieira, A.R., Brancher, J.A., 2018. Polymorphisms in nonamelogenin enamel matrix genes are associated with dental fluorosis. Caries Res. 52, 1–6.
Kuchler, E.C., Tannure, P.N., Oliveira, D.S., Charone, S., Nelson-Filho, P., Silva, R.A., Costa, M.C., Antunes, L.S., Calasans Maia, M.D., Antunes, L.A., 2017. Polymorphisms in genes involved in enamel development are associated with dental fluorosis. Arch. Oral Biol. 76, 66–69.
Lewandowski, L., Kepinska, M., Milnerowicz, H., 2020. Alterations in concentration/activity of superoxide dismutases in context of obesity and selected single nucleotide polymorphisms in genes: SOD1, SOD2, SOD3. Int. J. Mol. Sci. 21.
Li, P., Xue, Y., Zhang, W., Teng, F., Sun, Y., Qu, T., Chen, X., Cheng, X., Song, B., Luo, W., Yu, Q., 2013. Sodium fluoride induces apoptosis in odontoblasts via a JNK-dependent mechanism. Toxicology 308, 138–145.
Li, W., Jiang, B., Cao, X., Xie, Y., Huang, T., 2017. Protective effect of lycopene on fluoride-induced ameloblasts apoptosis and dental fluorosis through oxidative stress-mediated Caspase pathways. Chem. Biol. Interact. 261, 27–34.
Li, X., Han, Y., Yuan, G., Liang, Z., Bai, C., Li, Y., 2012. Aluminum induces osteoblast apoptosis through the oxidative stress-mediated JNK signaling pathway. Biol. Trace Elem. Res. 150, 502–508.
Liu, J., Tao, N., Luo, M., Zhao, X., Yang, S., Zeng, R., Zhang, Y., Wang, K., 2018. Association between paraoxonase 1 rs662 polymorphism and dental fluorosis. Chin J Ctrl Endem Dis 33, 247–249.
Liu, J., Yang, S., Luo, M., Tao, N., Zhao, X., Zhang, Y., 2019. Association between catalase single nucleotide polymorphism and dental fluorosis. Journal of Fujian Medical University 53, 85–89.
Lou, X.Y., Chen, G.B., Yan, L., Ma, J.Z., Zhu, J., Elston, R.C., Li, M.D., 2007. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. Am. J. Hum. Genet. 80, 1125–1137.
Lu, Y., Luo, Q., Cui, H., Deng, H., Kuang, P., Liu, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2017. Sodium fluoride causes oxidative stress and apoptosis in the mouse liver. Aging 9, 1623–1639.
Lyaruu, D.M., Bervoets, T.J., Bronckers, A.L., 2006. Short exposure to high levels of fluoride induces stage-dependent structural changes in ameloblasts and enamel mineralization. Eur. J. Oral Sci. 114 (Suppl. 1), 111–115 discussion 127-119, 380.
Ma, Q., Huang, H., Sun, L., Zhou, T., Zhu, J., Cheng, X., Duan, L., Li, Z., Cui, L., Ba, Y., 2017. Gene-environment interaction: does fluoride influence the reproductive hormones in male farmers modified by ERalpha gene polymorphisms? Chemosphere 188, 525–531.
Molina-Frechero, N., Nevarez-Rascon, M., Nevarez-Rascon, A., Gonzalez-Gonzalez, R., Irigoyen-Camacho, M.E., Sanchez-Perez, L., Lopez-Verdin, S., Bologna-Molina, R., 2017. Impact of dental fluorosis, socioeconomic status and self-perception in adolescents exposed to a high level of fluoride in water. Int. J. Environ. Res. Publ. Health 14.
Moller, I.J., 1982. Fluorides and dental fluorosis. Int. Dent. J. 32, 135–147.
Narwaria, Y.S., Saksena, D.N., 2013. Prevalence of dental fluorosis among primary school children in rural areas of karera block, Madhya Pradesh. Indian J. Pediatr. 80, 718–720.
Ni, J., Li, Y., Zhang, W., Shu, R., Zhong, Z., 2018. Sodium fluoride causes oxidative stress and apoptosis in cementoblasts. Chem. Biol. Interact. 294, 34–39.
Pendrys, D.G., Stamm, J.W., 1990. Relationship of total fluoride intake to beneficial effects and enamel fluorosis. J. Dent. Res. 69, 529–538 discussion 556-527.
Rahila, C., Aswath Narayanan, M.B., Ramesh Kumar, S.G., Leena Selvamary, A., Sujatha, A., John Kirubaharan, J., 2019. Association of COL1A2 (PvuII) gene polymorphism with risk and severity of dental fluorosis - a case control study. The Saudi dental journal 31, 463–468.
Robinson, C., Kirkham, J., 1990. The effect of fluoride on the developing mineralized tissues. J. Dent. Res. 69, 685–691 discussion 721.
Romualdo, P.C., Pucinelli, C.M., Tannure, P.N., Nelson-Filho, P., Segato, R.A.B., Brancher, J.A., Magalhaes, N.L., Costa, M.C., Antunes, L.A.A., Antunes, L.S., Buzalaf, M.A.R., Charone, S., Kuchler, E.C., 2019. Evaluation of genetic polymorphisms in MMP2, MMP9 and MMP20 in Brazilian children with dental fluorosis. Environ. Toxicol. Pharmacol. 66, 104–108.
Saha, D., Goswami, R., Majumdar, K.K., Sikdar, N., Pramanik, S., 2021. Evaluating the association between dental fluorosis and polymorphisms in bone development and mineralization genes among population from a fluoride endemic region of Eastern India. Biol. Trace Elem. Res. 199, 1–8.
Soler-Blasco, R., Murcia, M., Lozano, M., Gonzalez-Safont, L., Amoros, R., Ibarluzea, J., Broberg, K., Irizar, A., Lopez-Espinosa, M.J., Lertxundi, N., Marina, L.S., Ballester, F., Llop, S., 2020. Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int. J. Hyg Environ. Health 224, 113443.
Wan, H., Chen, S., Cai, Y., Chen, Y., Wang, Y., Zhang, W., Chen, C., Wang, N., Guo, Y., Lu, Y., 2021. Lead exposure and its association with cardiovascular disease and diabetic kidney disease in middle-aged and elderly diabetic patients. Int. J. Hyg Environ. Health 231, 113663.
Wang, A., Ma, Q., Gong, B., Sun, L., Afrim, F.K., Sun, R., He, T., Huang, H., Zhu, J., Zhou, G., Ba, Y., 2021. DNA methylation and fluoride exposure in school-age children: Epigenome-wide screening and population-based validation. Ecotoxicol. Environ. Saf. 223, 112612.
Wang, L., Zhu, Y., Wang, D., 2016. High-fluoride activates the FasL signalling pathway and leads to damage of ameloblast ultrastructure. Arch. Oral Biol. 71, 31–37.
Wang, Y., Branicky, R., Noe, A., Hekimi, S., 2018. Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. J. Cell Biol. 217, 1915–1928.
Wen, S., Li, A., Cui, L., Huang, Q., Chen, H., Guo, X., Luo, Y., Hao, Q., Hou, J., Ba, Y., 2012. The relationship of PTH Bst BI polymorphism, calciotropic hormone levels, and dental fluorosis of children in China. Biol. Trace Elem. Res. 147, 84–90.
Xu, H.M., Xu, L.F., Hou, T.T., Luo, L.F., Chen, G.B., Sun, X.W., Lou, X.Y., 2016. GMDR: versatile software for detecting gene-gene and gene-Environ- ment interactions underlying complex traits. Curr. Genom. 17, 396–402.
Yu, X., Chen, J., Li, Y., Liu, H., Hou, C., Zeng, Q., Cui, Y., Zhao, L., Li, P., Zhou, Z., Pang, S., Tang, S., Tian, K., Zhao, Q., Dong, L., Xu, C., Zhang, X., Zhang, S., Liu, L., Wang, A., 2018. Threshold effects of moderately excessive fluoride exposure on children's health: a potential association between dental fluorosis and loss of excellent intelligence. Environ. Int. 118, 116–124.
Zelko, I.N., Mariani, T.J., Folz, R.J., 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic. Biol. Med. 33, 337–349.
Zhang, M., Wang, A., He, W., He, P., Xu, B., Xia, T., Chen, X., Yang, K., 2007. Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. Toxicology 236, 208–216.
Zhang, R., Li, S., Pan, L., Yue, L., Yu, C., 2010. Relationship between the polymorphisms of CDX2 binding site in vitamin D receptor gene and the children's dental fluorosis. Acta Univ. Med. Anhui 45, 241–243.
Zhao, L., Yu, C., Lv, J., Cui, Y., Wang, Y., Hou, C., Yu, J., Guo, B., Liu, H., Li, L., 2021. Fluoride exposure, dopamine relative gene polymorphism and intelligence: a cross-sectional study in China. Ecotoxicol. Environ. Saf. 209, 111826.
Zhou, G., Yang, L., Luo, C., Liu, H., Li, P., Cui, Y., Liu, L., Yu, X., Zeng, Q., Chen, J., Zhao, Q., Dong, L., Niu, Q., Zhang, S., Wang, A., 2019a. Low-to-moderate fluoride exposure, relative mitochondrial DNA levels, and dental fluorosis in Chinese children. Environ. Int. 127, 70–77.
Zhou, X., Chen, Z., Zhong, W., Yu, R., He, L., 2019b. Effect of fluoride on PERK-Nrf2 signaling pathway in mouse ameloblasts. Hum. Exp. Toxicol. 38, 833–845.