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Fluoride exposure, dopamine relative gene polymorphism and intelligence: A cross-sectional study in China

Liang Zhao [a,b], Canqing Yu [a], Jun Lv [a,c,d], Yushan Cui [b], Yang Wang [b], Changchun Hou [b], Jingwen Yu [e], Baihui Guo [e], Hongliang Liu [e,f], Liming Li [a,*]

[a] Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, Beijing 100191, PR China
[b] Institute of Environment and Health, Tianjin Centers for Disease Control and Prevention, Tianjin 300011, PR China
[c] Key Laboratory of Molecular Cardiovascular Sciences (Peking University), Ministry of Education, Beijing 100191, PR China
[d] Peking University Institute of Environmental Medicine, Beijing 100191, PR China
[e] School of public health, Tianjin Medical University, Tianjin 300070, PR China
[f] Tianjin Municipal Bureau of Health Inspection, Tianjin 300070, PR China

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ABSTRACT

Background: Excessive fluoride exposure is related to adverse health outcomes, but whether dopamine (DA) relative genes are involved in the health effect of low-moderate fluoride exposure on children's intelligence remain unclear.
Objectives: We conducted a cross-sectional study to explore the role of DA relative genes in the health effect of low-moderate fluoride exposure in drinking water.
Methods: We recruited 567 resident children, aged 6–11 years old, randomly from endemic and non-endemic fluorosis areas in Tianjin, China. Spot urine samples were tested for urinary fluoride concentration, combined Raven`s test was used for intelligence quotient test. Fasting venous blood were collected to analyze *ANKK1 Taq1A* (rs1800497), *COMT Val158Met* (rs4680), *DAT1* 40 bp VNTR and *MAOA* uVNTR. Multivariable linear regression models were used to assess associations between fluoride exposure and IQ scores. We applied multiplicative and additive models to appraise single gene-environment interaction. Generalized multifactor dimensionality reduction (GMDR) was used to evaluate high-dimensional interactions of gene-gene and gene-environment.
Results: In adjusted model, fluoride exposure was inversely associated with IQ scores ($\beta = -5.957$, 95% *CI*: −9.712, −2.202). The mean IQ scores of children with high-activity *MAOA* genotype was significantly lower than IQ scores of those with low-activity ($P = 0.006$) or female heterozygote ($P = 0.016$) genotype. We detected effect modification by four DA relative genes (*ANKK1*, *COMT*, *DAT1* and *MAOA*) on the association between UF and IQ scores. We also found a high-dimensional gene-environment interaction among UF, *ANKK1*, *COMT* and *MAOA* on the effect of IQ (testing balanced accuracy = 0.5302, CV consistency: 10/10, $P = 0.0107$).
Conclusions: Our study suggests DA relative genes may modify the association between fluoride and intelligence, and a potential interaction among fluoride exposure and DA relative genes on IQ.

1. Introduction

Fluoride is widely distributed in the crust, groundwater is the major source of fluoride intake by humans (Jha et al., 2011). Although insufficient fluoride consumption may result in dental caries (Harrison, 2005), excessive fluoride exposure is of primary concern due to its hazardous effects on human health. To protect human health, the World Health Organization (WHO) sets strict water quality standard that fluoride in drinking water should below 1.5 mg/L (World Health Organization, 2011), while China has a more stringently permissible limit of 1.0 mg/L and 1.2 mg/L in urban and rural areas respectively (2006).

Fluoride crosses the blood-brain barrier raises the possibility that fluoride can affect the structure and functions of the central nervous system (Shalini and Sharma, 2015). Accumulating epidemiological investigations displayed an essential public health issue that children living in fluoride endemic areas performed lower general cognitive

* Corresponding author.
E-mail address: lmleeph@vip.163.com (L. Li).

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capacities of intelligence quotient (IQ) (Das and Mondal, 2016; Induswe et al., 2018; Rocha-Amador et al., 2007; Seraj et al., 2012; Yu et al., 2018). Animal experiments confirmed neurotoxicity of fluoride to central nervous system (CNS), which supported the epidemiological finding (Raghu et al., 2016; Yuan et al., 2019). However, the exact mechanisms by which fluorine interferes cognitive process and intelligence were not clearly defined.

Results of animal experiments showed that fluoride exposure affects dopamine (DA) concentration in brain (Reddy et al., 2014). Excessive fluoride intake could increase DA concentration in cerebellum, hippocampus and cortex, while decrease DA concentration in striatum (Kupnicka et al., 2020; Pereira et al., 2011). DA in brain is related to intelligence including cognition, learning and memory (Clark and Noudoost, 2014; Previc, 1999; Puig et al., 2014). Recent studies found that the catechol-O-methyltransferase (*COMT*) gene Val158Met polymorphism (rs4680) has modifying effect of fluoride on IQ by variation of susceptibility (Zhang et al., 2015), and children with different *ANKK1* Taq1A (rs1800497) genotypes were heterogeneous in the association of urinary fluoride and IQ (Cui et al., 2018). Both these two genes are involved in the function and metabolism of dopamine in CNS (Dos Santos et al., 2019; Huotari et al., 2002), indicating that DA relative genes may be critical in the adverse effect of fluoride on IQ.

The DA receptor D2 (DRD2) is a G protein-coupled receptor. It is located on postsynaptic dopaminergic neurons, involved in the processes of data transformation in the central nervous system (CNS) (Kemppainen et al., 2003). The ankyrin repeat and kinase domain 1 (*ANKK1*) gene adjacented to the *DRD2* gene, is involved in DRD2 coding (Neville et al., 2004). The *ANKK1* gene has a *Taq1A* (*rs1800497*) single nucleotide polymorphism (SNP), which has been studied most widely for it is associated with neuro functions (Masiak et al., 2020; Ramos-Lopez et al., 2019). *ANKK1 Taq1A* polymorphism could affect DRD2 density and auto-receptor mediated DA synthesis inhibition in brain (Beaver et al., 2010), and influence cognition (Fagundo et al., 2014; Failla et al., 2015).

Catechol-O-methyltransferase is a key enzyme affecting catecholamine metabolism, which regulate the degradation of norepinephrine and dopamine (Huotari et al., 2002). The *COMT* gene is located on the long arm of 22th chromosome (22q11.1-q11.2) (Grossman et al., 1992). Over three hundred kinds of *COMT* gene polymorphisms have been found, among which Val 158 Met (rs4680) is the most concerned in recent years (Calati et al., 2011). The *COMT* rs4680 polymorphism affect enzyme activity (Chen et al., 2004), and is closely related to working memory and cognitive ability (Schmack et al., 2008; Yeh et al., 2010). Monoamine oxidase A (MAOA) is a key enzyme which metabolize DA into 3,4-dihydroxyphenylacetic acid (DOPAC) (Kopin, 1985). The *MAOA* gene is located on the X chromosome (Xp11.23), and has a functional polymorphism of 30-bp variable number of tandem repeats (*MAOA* uVNTR) in the promoter region upstream of the MAOA coding sequences. Alleles with 3.5 or 4 copies are transcribe more efficiently than the others (Sabol et al., 1998), and modulate dopamine turnover in the CNS (Ducci et al., 2006). The *MAOA* uVNTR polymorphism is involved in the intelligence and in behaviors males (Qian et al., 2010; Schlüter et al., 2015). Nevertheless, because *MAOA* gene is located on the X chromosome, different from males, there are heterozygous genotypes in females which may cause various modulation effect from homozygous groups (Byrd et al., 2018; Volavka et al., 2005). *MAOA* polymorphism is associated with spatial learning, memory and cognition (Barnett et al., 2011; Mueller et al., 2014).

The dopamine transporter (DAT) reuptake DA into presynaptic nerve terminals, and terminates synaptic transmission (Amara and Kuhar, 1993). The *DAT1* (also named *SLC6A3*) gene is located on the short arm of the 5th chromosome (5p15.3). There is a 40-bp VNTR polymorphism in the 3′-untranslated region (3′-UTR) of the *DAT1* gene, including 3–13 repeat alleles, in which 10-repeat and 9-repeat are most observed (Bannon et al., 2001; Fuke et al., 2001). However, researches on the association of *DAT1* VNTR and DAT expression showed inconsistent results, some studies found that the 9-repeat allele is associated with higher *DAT1* gene expression (Jelaš et al., 2018), some other studies found excessive levels of the DAT protein in homozygotes (10/10) subjects (Brookes et al., 2007), while some studies found no functional differences among *DAT1* VNTR polymorphism groups (Martinez et al., 2001). Studies found that *DAT1* VNTR polymorphism is associated with cognitive flexibility and intelligence (Fagundo et al., 2014; Qian et al., 2010).

It is estimated that over 200 million people worldwide are suffering fluoride endemic (Amini et al., 2008; Rasool et al., 2018). Over the past decades, the Chinese government has made great efforts in drinking water quality improvement by implementing defluoridation projects in rural areas. However, in some areas in China, the fluoride levels remain beyond the Chinese criteria (Zhang et al., 2017), causing excessive fluoride exposure in daily life constantly. Although numerous studies have uncovered the adverse effect of fluoride on intelligence (Choi et al., 2012; Duan et al., 2018), few studies provided epidemiological evidence on the role of MAOA uVNTR and *DAT1* VNTR gene polymorphism in the effect of fluoride on intelligence. To address the issues, we performed a comprehensive and systematic study in a population of Chinese school-age children with low-moderate excessive fluoride exposure, in purpose of identifying (1) the effect modification of DA relative genes polymorphism in the effect of fluoride exposure on intelligence, and (2) whether fluoride exposure and DA relative genes polymorphism have interaction effects on intelligence.

2. Materials and methods

2.1. Study design and population

A school based cross-sectional study was performed in rural areas in 2018 to obtain the baseline population data in Tianjin City, China. According to the monitoring data of Tianjin CDC, we divided all the rural areas into historical high fluoride areas and normal fluoride areas according to Chinese Standards for drinking water quality GB (5749-2006). Over the past ten years, the fluoride level of drinking water maintained stable in these areas. None of these study sites were endemic areas of iodine deficiency disorders, and the concentration of some potential neurotoxic heavy metals (e.g., lead and arsenic) in drinking water met the standard as well. Stratified and multistage random strategy was used for study participants sampling. Briefly, five towns were selected using the simple random sampling (SRS) method at first. There are four historical high fluoride areas (fluoride concentration in drinking water (WF): 1.53–2.84 mg/L) and one non-endemic fluorosis area (WF: 0.15–0.37 mg/L) among the five selected towns. Next, we selected one primary school within each town with SRS. Finally, we recruit children of 6–11 years among the second to fifth grades from each chosen school using cluster sampling method.

A total of 616 children were enrolled. The recruitment process (Fig. 1) was: (1) 12 children were excluded because of negative long-term residents. (2) 3 children were excluded because there was immediate family member mentally retarded. (3) 20 children were excluded because of missing IQ test, questionnaire or physical examination. (4) 14 children were excluded because no results of genotyping measurement, leaving 567 children for the study. Informed consent was provided by all participants and their parents/guardians. Our research protocol was approved by the Ethical Committee of Tianjin Centers for Disease Control and Prevention.

2.2. Data collection

Participants received assessments and medical examinations by a team of trained staff with medical backgrounds, under supervision of their teachers. IQ test and urine sample collection were performed by public health doctors from Tianjin CDC, physical examination and venous blood samples collection were conducted by medical doctors

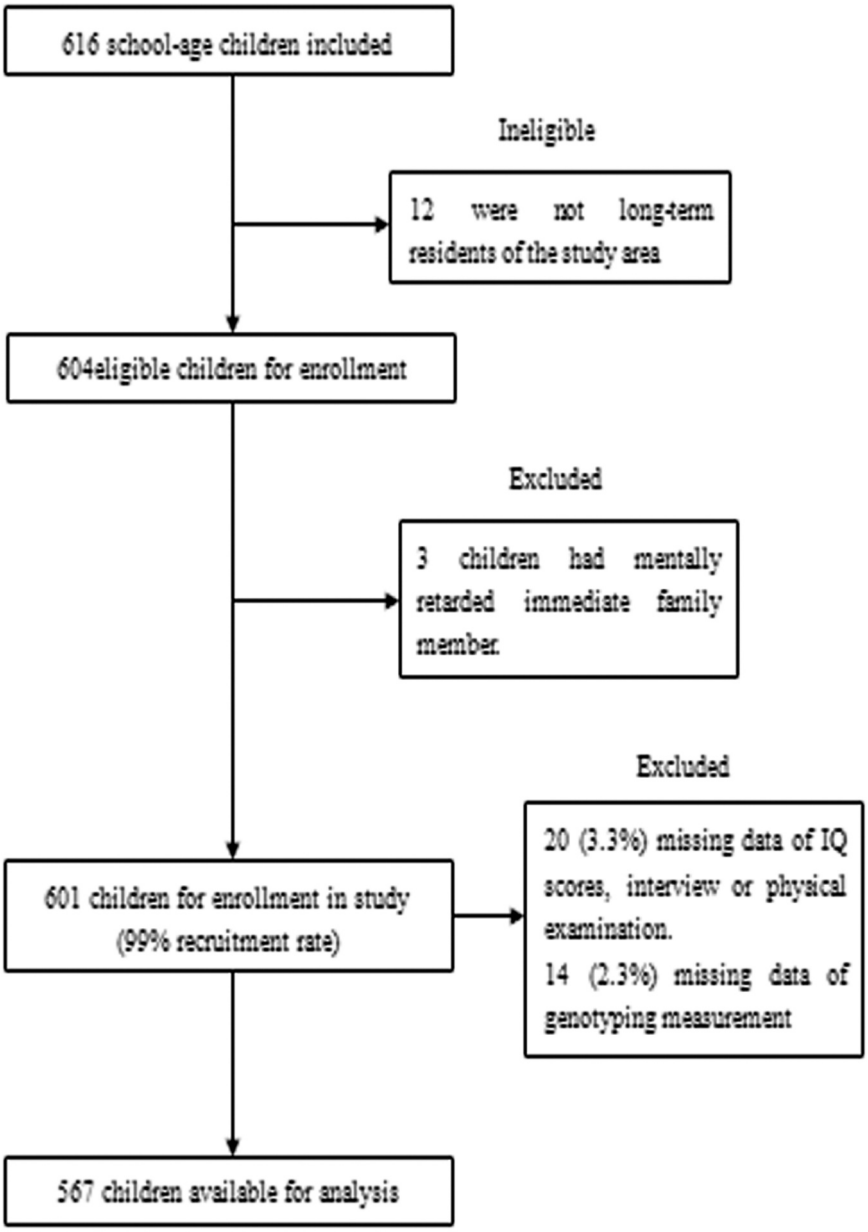

Fig 1. Flow chart of recruitment process.

Fig. 1. Flow chart of recruitment process.

from local hospitals. Demographic information data (e.g., age, gender, parental education level, physical residence, parental occupation, household income, et. al) was obtained by questionnaire with detailed filling instructions filled by the parents of participants enrolled in the study. The development status of participants was assessed by body mass index (BMI) which was further calculated from their weight and height measured during physical examination.

2.3. Bio-sample collection

Venipuncture was conducted to collect 5 ml of fasting venous blood sample into an anticoagulant vacuum tube and kept at − 80 ℃ for subsequent genotyping. Spot urine samples were collected at the first urine in the morning before breakfast and stored at − 20 ℃ for later urinary fluoride concentration analysis.

2.4. Urinary fluoride concentrations measurement

According to the national standardized method (Wu et al., 2015), ion analyzer EA940 with F-ion selective electrode (Shanghai constant magnetic electronic technology Co, Ltd, China) was used for Urinary fluoride (UF) concentrations measurement. Samples were tested twice, and the means of two results were adopted as final results. The quantitation limit was 0.05 mg/l, and the recovery rate was 93.4%− 108.3%.

2.5. Gene polymorphism measurement

We isolated Genomic DNA from venous blood using a commercial DNA extraction Kit (Tiangen Biotech, Beijing, China). Primers were designed and synthesized by Shanghai Sangon Bioengineering Co., Ltd., China (Table S1). The gel photographs were interpreted by two

independent readers. We randomly retested 10% of the samples, and the results were identical.

(1) ***ANKK1* Taq1A SNP and *COMT* Val158Met SNP measurement**

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods were used to identified the genotype of *ANKK1* Taq1A SNP (rs1800497) and COMT Val158Met SNP (rs4680). The PCR products were digested with *Taq*I or *Nla*III restriction enzyme (New England Biolabs, MA, USA), respectively. The digestion products were separated on a 4% agarose gel and stained by GoldView I (Solarbio, Beijing, China).

After electrophoresed and compared with DNA Markers (Tiangen Biotech, Beijing, China), the *ANKK1* gene was divided into three genotypes: wild type CC (117, 87 bp), hybrid type CT (204, 117, and 87 bp), and variant type TT (204 bp). The *COMT* gene was also divided into three genotypes: wild type GG (114 bp, 20 bp), hybrid type GA (114 bp, 96 bp, 20 bp), and variant type AA (96 bp, 20 bp, 18 bp) (Cui et al., 2018; Zhang et al., 2015).

(2) ***DAT1* 40 bp VNTR and MAOA uVNTR measurement**

The polymorphism of *DAT1* 40 bp VNTR and MAOA uVNTR were identified by conventional PCR. The PCR products were determined by electrophoresis on a 2% agarose gel and stained by GoldView I (Solarbio, Beijing, China) (Byrd et al., 2018; Dos Santos et al., 2019).

Compared to DNA Markers (Tiangen Biotech, Beijing, China), we observed 6 R (320 bp), 7 R (360 bp), 8 R (400 bp), 9 R (440 bp), 10 R (480 bp), 11 R (520 bp) and 12 R (560 bp) of *DAT1* 40 bp VNTR; We observed 2 R (294 bp), 3 R (324 bp), 4 R (354 bp), 5 R (384 bp) and 6 R (414 bp) of *MAOA* uVNTR. The *DAT1* gene was categorized into two genotypes: homozygous 10 R/10 R (10/10) and the others (non-10/10) (Brookes et al., 2007). The MAOA gene was categorized into three genotypes: (1) High-activity (MAOA_H), 4 R in males and homozygous 4 R/4 R in females; (2) Low-activity (MAOA_L), other repeats in males and other homozygous repeats in females expect 4 R; and (3) Female Heterozygote (FH), heterozygote repeats in females (Byrd et al., 2018).

2.6. Intelligence quotient assessment

The Combined Raven's Test (modified in China) was used for IQ assessment. The test is validated for basic cognitive abilities. With the advantages of non-verbal and less affected by language and ethnic differences, this test has been widely used in China, especially for school-age children (Sun et al., 2015; Yu et al., 2018). It comprises 72 questions in six sets: A, AB, B, C, D and E. According to the instruction manual, the tests were conducted in a quiet environment within 40 min and the administers were blinded to participants' drinking water fluoride levels. For students $\geq$8 years old, group test was used; for students $<$8 years old, we did one on one test by trained staff. The test results were inverted into IQ scores according to the norm for children in rural areas in China (CRT-RC_2) (Ding et al., 2011).

2.7. Statistical analysis

Descriptive statistics for demographic characteristics and health status were performed using mean $\pm$ standard deviance (SD) or frequency (proportion) for continuous and categorical variables, respectively. Student's *t*-test or one-way ANOVA was applied to compare the differences of IQ score among genotype subgroups, and bonferrroni correction was used for post-hoc analysis.

We performed multivariate analysis to estimate the association of urinary fluoride level and IQ score. Multivariate linear regression models were established to estimate the linear relationship. Two models were analyzed, model 1: crude model with no covariates; and model 2: adjusted model with all potential confounding covariates. We used Q-Q charts of standardized residuals to verify the normality of the regression, and the relational diagrams of studentized residuals and fitted values to verify the homogeneity of variances. A bootstrap procedure with 1000 substitutions was performed for more robust estimations.

Interactions between urinary fluoride level and single gene polymorphism on IQ were evaluated by multiplicative and addictive interaction models. We included two risk factors and their interaction terms into logistic regression model adjusted for potential confounders to calculate multiplicative OR for multiplicative interaction appraisement. We evaluated additive interactions by three indexes: relative excess risk due to interaction (RERI), attributable proportion of interaction (AP) and synergy index (SI) (Li et al., 2020). Generalized multifactor dimensionality reduction (GMDR) was used to analyze the high-dimensional interaction among 4 genes and UF on IQ. Sign test with $p < 0.05$ and simplest model with maximum cross validation consistency (CVC) was considered the best interaction model (Xu et al., 2016).

For interaction analysis, IQ was transformed into bicategorical variable: Lower group (IQ score $<$ 110; Including retarded, marginal, dull normal and normal of CRT-RC_2) and Higher group (IQ score $\geq$ 110; including high normal, superior and excellent of CRT-RC_2) (Ding et al., 2011); UF was also transformed into bicategorical variable: Lower group (urinary fluoride concentration $<$ median) and Higher group (urinary fluoride concentration $\geq$ median).

We selected potential confounders based on current literature of covariates that could influence both UF levels and IQ scores in children (Cui et al., 2018; Yu et al., 2018). And we finally kept age, gender, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery as the covariates. For the analysis that MAOA is involved, gender was detached from covariates as MAOA categorization was gender dependent.

We used R software 3.6.1 and SPSS 24.0 (IBM, Chicago, IL, USA) for general data analysis, Andersson's-excel for addictive interaction calculation of single gene-UF interaction analysis (Andersson et al., 2005). We used GMDR software (version 0.7) for Gene-Gene and Gene-Environment interactions underlying complex traits, and ten-fold cross-validation was set. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of the participants

Descriptive data of all 567 subjects are listed in Table 1. Among them, there are 50.1% boys and 49.9% girls. Their mean ($\pm$SD) age and BMI was 9.15 ($\pm$1.02) years old and 17.76 ($\pm$3.55) kg/m^2, respectively. Most of their parents had educational background as high school or above, and most of their family had a household income $\geq$30,000 RMB/year (76.2%). When they were born, 64.4% of the mothers were $\leq$30 years old. 18.7% of the subjects had abnormal birth situation (including premature or postmature delivery, dystocia, fetal distress, birth asphyxia or hypoxia and low birth weight). Their mean ($\pm$SD) IQ score was 112.17 ($\pm$11.75). The urinary fluoride concentration was not normally distributed, and the median (quantile 1, quantile 3) was 1.03 (0.72, 1.47) mg/L. After logarithmic transformed, the mean ($\pm$SD) Log_UF was 0.015 ($\pm$0.252).

As shown in Table 2, for *ANKK1* gene, 55.4% of subjects had the CT genotype, 16.8% had the TT genotype, with variant allele frequency of 0.45. For *COMT* gene, 35.8% had GA genotype, 5.5% had AA genotype, with variant allele frequency of 0.23. For *MAOA* gene, 55.0% had genotype of low-activity, 23.3% had genotype of high-activity, and 21.7% had genotype of female heterozygote. Most of the subjects (81.0%) had 10 R/10 R genotype of *DAT1* gene. The results of post-hoc analysis showed that the mean IQ scores of children with high-activity *MAOA* genotype was significantly lower than low-activity ($P = 0.006$) or female heterozygote ($P = 0.016$) genotype.

Table 1
Basic characteristics of study population.

Characteristics	Mean ± SD or n (%)
Sample size	567
Age (years old)	9.15 ± 1.02
Gender	
Boys	284 (50.1)
Girls	283 (49.9)
Body mass index (kg/m^2)	17.76 ± 3.55
Paternal education level	
<high school	162 (28.6)
≥high school	405 (71.4)
Maternal education level	
< high school	177 (31.2)
≥ high school	390 (68.8)
Household income (RMB/year)	
≤ 30,000	135 (23.8)
30,000–100,000	303 (53.4)
> 100,000	129 (22.8)
Maternal age at delivery (years old)	
≤ 30	365 (64.4)
> 30	202 (35.6)
Abnormal birth	
no	461 (81.3)
yes	106 (18.7)
Log_UF	0.015 (0.252)
IQ scores	112.17 (11.75)

Table 2
Gene polymorphism characteristics and IQ scores of study population.

Gene polymorphism	N (%)	IQ score		Post-hoc analysis
		Mean (SD)	*P*	
ANKK1 Taq1A				
CC	158 (27.9)	112.09 (11.90)	0.805[a]	
CT	314 (55.4)	112.40 (11.70)		
TT	95 (16.8)	111.51 (11.77)		
COMT Val158Met				
GG	333 (58.7)	112.28 (10.77)	0.170[a]	
GA	203 (35.8)	111.45 (13.18)		
AA	31 (5.5)	115.68 (11.77)		
DAT1 40 bp VNTR				
10 R/10 R	459 (81.0)	112.00 (11.75)	0.496[b]	
Non-10R/10 R	108 (19.0)	112.86 (11.81)		
MAOA uVNTR				
Low-activity	312 (55.0)	112.97 (11.53)	0.004[a,*]	L-level >H-level, $\Phi = 3.76$, $P = 0.006$;
High-activity	132 (23.3)	109.21 (12.79)		Female Heterozygote >H-level, $\Phi = 4.10$,
Female Heterozygote	123 (21.7)	113.31 (10.69)		$P = 0.016$;

Note: Bonferrroni correction was used for post-hoc analysis.
[*] $P < 0.05$ was considered as statistically significant.
[a] ANOVA analysis was used to compare the difference of more than two variables.
[b] Student's *t*-test was used to compare the difference of two variables.

3.2. Associations between UF and IQ scores

In the overall participants, the Log_UF were inversely linear associated with IQ score ($P < 0.05$) in both crude model and adjusted model, the coefficient (95% *CI*) were −5.159 (−8.996, −1.321) and −5.957 (−9.712, −2.202), respectively. Bootstrapped estimation of the variance had a similar result (95% *CI*: −10.356, −1.834; $P = 0.006$) (Table 3).

3.3. Associations between UF and IQ scores according to genotypes

The linear regression results of Log_UF and IQ stratified by different genotypes are presented in Table 3. For *ANKK1* gene, children with CT or TT genotypes had inverse linear associations between Log_UF and IQ, the adjusted coefficient (β) and 95% *CI* were −7.615 (−12.543, −2.688) and −11.445 (−21.056, −1.834), respectively; In children with CC genotype, Log_UF was not significantly associated with IQ. For *COMT* gene, Log_UF was inversely associated with IQ ($\beta = -9.779$; 95% *CI*: −16.414, −3.143) in GA subgroup, while the associations were not significant in GG and AA subgroups. For *DAT1* gene, the IQ score decreased for 6.085 (95% *CI*: −10.245, −1.924) with every 1 increase of Log_UF in 10 R/10 R subgroup, but was not significantly decreased in non-10R/10 R subgroup. For *MAOA* gene, children with low-activity genotype had inverse linear associations between Log_UF and IQ ($\beta = -6.254$; 95% *CI*: −11.283, −1.225); In high-activity subgroup, Log_UF and IQ were not significantly associated; Nevertheless, in girls with heterozygote genotype, Log_UF and IQ were not significantly associated in crude model, but was significantly associated in the adjusted model ($\beta = -7.521$; 95% *CI*: −14.831, −0.210). For all the analysis stratified by genotypes, bootstrapped estimations of the variance were consistent with corresponding adjusted models (Table 3).

3.4. Interactions between UF and single gene polymorphism on IQ

The results of crossover analysis and Interactions between UF and single gene polymorphism on IQ are shown in Table 4. We found that $OR_{G\times E}$ of all four genes are above 1, but none of their 95% *CI* excluded 1, indicating their multiplicative interaction were not statistically significant. Evaluated according to the 95% *CI* of RERI, AP and SI, the addictive interaction of all four pairs were not statistically significant either.

3.5. GMDR analyses for Gene-Gene and Gene-Environment interaction

Table 5 presents the Gene-Gene and Gene-Environment interaction model by GMDR. For Gene-Gene interaction, we did not find any approving multi-dimensional model. For Gene-fluoride interaction, one four locus model (UF×*ANKK1*×*COMT*×*MAOA*) contributed to the best model with the smallest prediction error (1- testing balanced accuracy [0.5302] = 0.4698), and the greatest CVC (10/10; sign test $P = 0.0107$). Fig. 2 shows the score distributions in the best model. The scores between IQ groups in different cells various, indicating that patterns of risk differ across multi-locus dimensions.

4. Discussion

In the present study, we found that fluoride exposure is inversely related to children's IQ scores, while DA related genes polymorphism (*ANKK1* Taq1A, *COMT* Val 158 Met, *DAT1* 40 bp VNTR and *MAOA* uVNTR) may show modifying effects on the association between urinary fluoride and IQ scores. UF, *ANKK1* Taq1A, *COMT* Val 158 Met and *MAOA* uVNTR showed a high-dimensional interaction on IQ.

As the annual surveillance data from the local CDC revealed that the water fluoride concentration (WF) is within or close to the WHO standard upper limit (1.5 mg/L) (World Health Organization, 2011) and maintained stable in our study sample sites, indicating that our study data could represent long-term external fluoride exposure at low-moderate level. Notably, kidney is the major organ for active fluoride metabolism and excretion (Jha et al., 2011), and it has been proved that urinary fluoride level is positive correlated with water fluoride concentration (Wang et al., 2020; Yu et al., 2018). Therefore, we selected urinary fluoride as the internal measure index of fluoride

Table 3
Linear regression models for Log_UF on IQ scores in overall and different gene polymorphism subgroups.

	Unadjusted model			Adjusted model				
		Linearized Estimation			Linearized Estimation		Bootstrapped Estimation	
	β	95% *CI*	*P*	*β*	95% *CI*	*P*	95% *CI*	*P*
Overall (N = 567)[a]	−5.159	−8.996, −1.321	0.009*	−5.957	−9.712, −2.202	0.002*	−10.356, −1.834	0.006*
ANKK1[a]								
CC (n = 158)	0.158	−7.354, 7.670	0.967	0.199	−7.391, 7.790	0.959	−7.589, 7.432	0.964
CT (n = 314)	−5.845	−10.954, −0.736	0.025*	−7.615	−12.543, −2.688	0.003*	−13.141, −2.548	0.007*
TT (n = 95)	−12.404	−21.960, −2.848	0.012*	−11.445	−21.056, −1.834	0.020*	-21.342, −0.376	0.019*
COMT[a]								
GG (n = 333)	−1.809	−6.510, 2.892	0.449	−3.644	−8.334, 1.047	0.127	−8.303, 0.903	0.133
GA (n = 203)	−11.034	−17.815, −4.253	0.002*	−9.779	−16.414, −3.143	0.004*	−16.960, −2.976	0.010*
AA (n = 31)	−1.619	−23.678, 20.441	0.882	17.103	−9.899, 44.104	0.202	−11.893, 47.876	0.223
DAT1[a]								
10/10 (n = 459)	−5.349	−9.561, −1.136	0.013*	−6.085	−10.245, −1.924	0.004*	−10.265, −1.829	0.004*
Non-10/10 (n = 108)	−4.475	−13.970, 5.020	0.352	−5.870	−15.159, 3.418	0.213	−15.673, 3.041	0.207
MAOA[b]								
Low-activity (n = 312)	−5.278	−10.414, −0.143	0.044*	−6.254	−11.283, −1.225	0.015*	−12.404, −0.533	0.040*
High-activity (n = 132)	−2.409	−11.244, 6.425	0.590	−3.001	−11.961, 5.959	0.509	−12.129, 5.362	0.512
Female Heterozygote (n = 123)	−6.311	−13.667, 1.046	0.092	−7.521	−14.831, −0.210	0.044*	−14.667, −0.065	0.044*

* $P < 0.05$ was considered as statistically significant;
[a] Adjustment: age, gender, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.
[b] Adjustment: age, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.

Table 4
Interaction between urinary fluoride and single gene polymorphism on IQ.

Interaction items	N	IQ < 110, n (%)	adjusted OR (95% CI)	Multiplicative interaction	Additive interaction
UF - ANKK1[a]					
UF_L / ANKK1_CC	72	29 (40.3)	1 (ref)	UF×ANKK1 OR (95% CI): 1.40 (0.65, 3.00)	RERI (95% CI): 1.14 (−6.90, 9.17);
UF_H / ANKK1_CC	84	37 (43.0)	1.10 (0.58, 2.10)		AP (95% CI): 0.28 (−1.49, 2.05);
UF_L / ANKK1_CT+TT	214	74 (34.6)	0.75 (0.43, 1.32)		SI (95% CI): 1.60 (0.04, 58.49)
UF_H / ANKK1_CT+TT	195	87 (44.6)	1.16 (0.66, 2.04)		
UF - COMT[a]					
UF_L / COMT_GG	169	63 (37.3)	1 (ref)	UF×COMT OR (95% CI): 1.55 (0.77, 3.15)	RERI (95% CI): 1.80 (−5.67, 9.26);
UF_H / COMT_GG	164	66 (40.2)	1.17 (0.74, 1.85)		AP (95% CI): 0.38 (−0.93, 1.69);
UF_L / COMT_GA+AA	117	40 (34.2)	0.92 (0.55, 1.54)		SI (95% CI): 1.93 (0.09, 40.28)
UF_H / COMT_GA+AA	117	58 (49.6)	1.68 (1.02, 2.74)		
UF - DAT1[a]					
UF_L / DAT1_non-10/10	48	18 (37.5)	1 (ref)	UF×DAT1 OR (95% CI): 1.12 (0.46, 2.69)	RERI (95% CI): −1.39 (−12.34, 9.56);
UF_H / DAT1_non-10/10	60	26 (43.3)	1.28 (0.58, 2.84)		AP (95% CI): −0.45 (−4.42, 3.51);
UF_L / DAT1_10/10	238	85 (35.7)	0.94 (0.49, 1.81)		SI (95% CI): 0.60 (0.02, 23.82)
UF_H / DAT1_10/10	221	98 (44.3)	1.36 (0.70, 2.61)		
UF - MAOA[b]					
UF_L / MAOA_L+FH	227	77 (33.9)	1 (ref)	UF×MAOA OR (95% CI): 1.03 (0.46, 2.30)	RERI (95% CI): −3.88 (−14.94, 7.17);
UF_H / MAOA_L+FH	208	87 (41.8)	1.44 (0.93, 2.05)		AP (95% CI): −1.38 (−6.42, 3.65);
UF L / MAOA_H	59	26 (44.1)	1.01 (0.79, 2.61)		SI (95% CI): 0.32 (0.02, 5.14)
UF H / MAOA_H	73	37 (50.7)	2.06 (1.18, 3.59)		

Note: UF_L urinary fluoride concentration ≤ median; UF_H urinary fluoride concentration > median; MAOA_L MAOA low-activity; MAOA_H+FH MAOA high-activity and female heterozygote; RERI relative excess risk due to interaction; AP attributable proportion of interaction; SI synergy index.
[a] Adjustment: age, gender, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.
[b] Adjustment: age, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.

Table 5
GMDR analysis for the best gene–environment interaction models.

Locus No.	Model	Training Bal. Acc.	Testing Bal. Acc.	Sign Test (*P*)	CV Consistency
	Gene-gene interactions[a]				
2	COMT × MAOA	0.5452	0.4581	2 (0.9893)	5/10
3	ANKK1 × COMT × MAOA	0.5665	0.4604	1 (0.9990)	7/10
4	ANKK1 × DAT1 × COMT × MAOA	0.5862	0.4477	0 (1.0000)	10/10
	Gene-fluoride interactions[b]				
2	UF×COMT	0.5572	0.5170	7 (0.1719)	10/10
3	UF×ANKK1 × COMT	0.5766	0.4637	2 (0.9893)	4/10
4	UF×ANKK1 × COMT × MAOA	0.6178	0.5302	9 (0.0107)	10/10
5	UF×DAT1 × ANKK1 × COMT × MAOA	0.6430	0.5178	7 (0.1719)	10/10

Notes: Training Bal. Acc. training balanced accuracy, Testing Bal. Acc. testing balanced accuracy, CV Consistency cross validation consistency.
[a] Adjustment: UF, age, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.
[b] Adjustment: age, BMI, paternal education level, maternal education level, household income, abnormal birth and maternal age at delivery.

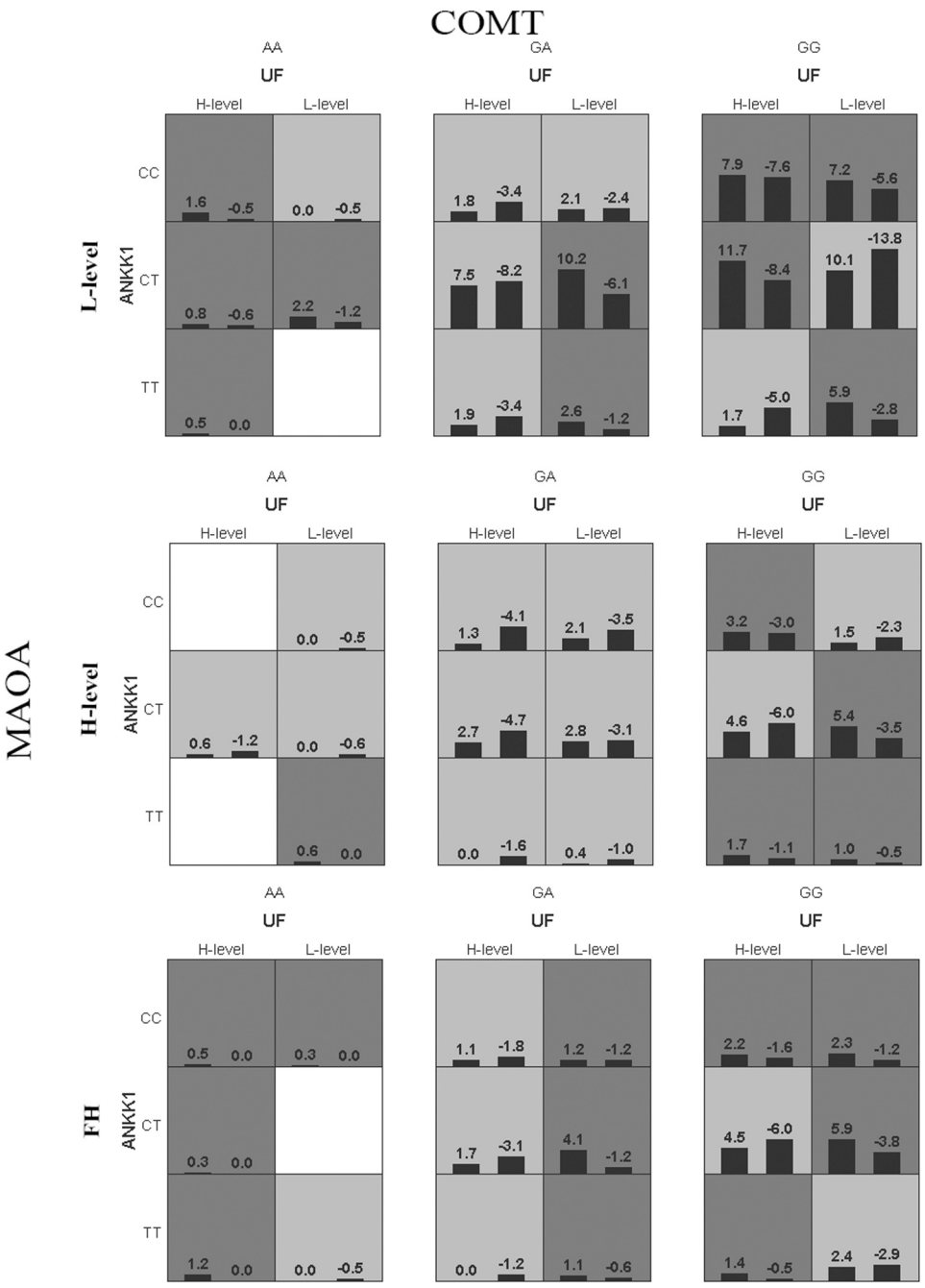

Fig. 2. The best adjusted GMDR model for gene-fluoride interaction. The adjusted covariates included age, paternal education level, household income, abnormal birth and maternal age at delivery. The best model is composed of UF, *ANKK1*, *COMT* and *MAOA*. In each cell, the left bar represents a positive score, and the right bar a negative score. High-risk cells are indicated by dark shading, low-risk cells by light shading, and empty cells by no shading. The patterns of high-risk and low-risk cells differ across each of the different multi locus dimensions, presenting evidence of epistasis.

exposure.

Early studies observed IQ decrease in areas with excessive fluoride concentration in drinking water globally, and in most of which, the water fluoride concentrations were extremely high (e.g. WF > 5 mg/L) (Seraj et al., 2012; Trivedi et al., 2007). In recent years, more studies focused on the relationship between low-moderate fluoride exposure and IQ, and the results had public health implications for population more widely. In the current study, our results demonstrated that urinary fluoride levels were adversely associated with IQ scores in children with low-moderate fluoride exposure. These results are consistent with a systematic review and dose-response meta-analysis between water fluoride and the level of children's intelligence of 26 observational studies including 7258 children (mainly from China) (Duan et al., 2018), especially with the findings reported by Wang, that water fluoride exposure at low-moderate levels (WF: 0.20–2.84 mg/L, UF: 1.28 ± 1.30 mg/L) is inversely related to children's IQ scores (Wang et al., 2020).

We further examined the roles of DA related genes in the relationship between fluoride exposure and found that the linear association performed differently in *COMT* and *ANKK1* genotype subgroups. These results confirm the findings reported by Zhang and Cui (Cui et al., 2018; Zhang et al., 2015). In addition, we found linear association similar with all participants in the *DAT1*_10 R/10 R, *MAOA*_low-activity and *MAOA*_female-heterozygote subgroup, while the association were not statistically significant in *DAT1*_non-10R/10R or *MAOA*_high-activity genotype subgroups, indicating effect modification of *DAT1* and *MAOA* gene polymorphism in the association of fluoride exposure and IQ. Laboratory experiments found that DA plays and important role in cognition, learning and memory (Backman et al., 2011; Jenkins et al., 2010; Mohebi et al., 2019). In DA neurons, DA is taken up and stored in

storage vesicles, and released as a neurotransmitter in human brain. DA is bound by D2 receptor, re-uptake by DAT, and extra DA are deamination into homovanillic acid (HVA) by MAO or COMT, or sulfated by phenolsulfotransferase (PST) (Fuke et al., 2001; Ritchie and Noble, 2003; Rivett et al., 1982). Studies found that gene polymorphism could affect the number and activity of DA related proteins in brain. The number of D2 receptors in *ANKK1* CT/TT population is about 30–40% lower than that in CC population, while the binding capacity was similar (Ritchie and Noble, 2003). The activity of COMT enzyme in *COMT* AA population is 35–50% lower than that in GG population (Chen et al., 2004). The transcriptional activity of population with *MAOA* high-activity genotype is 2–10 folds higher and that of *MAOA* low-activity group (Sabol et al., 1998). *DAT1* 10/10 repeat allele is associated with higher DAT protein expression (Brookes et al., 2007). As the four genes are closely related to brain function, it could suggest biological bases for the modification effect of their polymorphisms in the association of fluoride exposure and IQ.

We did not find interaction between UF and single gene polymorphism. However, we found a high-dimensional interaction of UF×*ANKK1*×*COMT*×*MAOA* on IQ. Accumulating epidemiological evidences proved that excessive fluoride exposure is associated with ADHD (Bashash et al., 2018; Riddell et al., 2019). DA and relative genes are the key pathway of ADHD mechanism (Kim et al., 2018; Shang et al., 2018). A recent study found that fluoride affects DA concentration in striatum and cerebellum of rats' brain and causes changes in the expression of dopamine receptors (Kupnicka et al., 2020). As the four genes and fluoride are all associated with DA metabolism or function, single gene-fluoride interaction results may be interfered by other genes. Our finding of a high-dimensional interaction of UF×*ANKK1*×*COMT*×*MAOA* on IQ supported this hypothesis. These epidemiological findings suggest a mechanism of fluoride on IQ, that DA may play a critical role in the neuro-toxicity of fluoride. DA relative pathway might be one of the toxicological mechanisms of fluoride on IQ, for which more further studies are needed to confirm.

Our study has several strengths. So far as we know, this is the first epidemiological study to uncover the effect modification of *DAT1* and *MAOA* gene polymorphism in the relationship between fluoride exposure and IQ, and the first study to analyze the high-dimensional interaction among fluoride exposure and the four DA relative genes. Our findings suggest a novel clue for the neuro-toxicological mechanism of fluoride.

Our study also has some limitations. We used urinary fluoride to represent the fluoride exposure from drinking water, which ignored the bias of short-term fluoride intake from diet. However, our finding could still reveal the health effects of internal fluoride exposure. Due to the cross-sectional design, our results are not strong for causal demonstration. Small sample size also limited the statistical power. Further prospective studies with larger sample size are essential to validate our findings. Nevertheless, the effect modification of DA relative genes and interaction among UF and these genes are still noteworthy.

5. Conclusions

In summary, our analysis results indicate that low-moderate fluoride exposure is inversely related to children's IQ; DA related genes polymorphism (*ANKK1* Taq1A, *COMT* rs4680, *DAT1* 40 bp VNTR and *MAOA* uVNTR) have modifying effects of fluoride exposure on IQ; UF, *ANKK1* Taq1A, *COMT* Val 158 Met and *MAOA* uVNTR have a high-dimensional interaction on IQ. Our findings may provide epidemiological evidences for preventive policy making for water fluoride endemics residents with different susceptibility, and provide a clue for clarifying the neuro-toxicological mechanism of fluoride exposure.

CRediT authorship contribution statement

Liang Zhao: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Canqing Yu**: Methodology, Formal analysis. **Jun Lv**: Methodology, Formal analysis. **Yushan Cui**: Conceptualization, Data curation, Investigation, Visualization. **Yang Wang**: Funding acquisition, Resources, Investigation. **Changchun Hou**: Methodology, Investigation, Supervision. **Jingwen Yu**: Data Curation, Investigation. **Baihui Guo**: Investigation. **Hongliang Liu**: Funding acquisition, Supervision. **Liming Li:** Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111826.

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