Do methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1 polymorphisms modify changes in intelligence of school-age children in areas of endemic fluorosis?

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Background: Excessive exposure to fluoride can reduce intelligence. Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1 *(MTHFD1)* polymorphisms have important roles in neurodevelopment. However, the association of *MTHFD1* polymorphisms with children's intelligence changes in endemic fluorosis areas has been rarely explored.
Methods: A cross-sectional study was conducted in four randomly selected primary schools in Tongxu County, Henan Province, from April to May in 2017. A total of 694 children aged 8 to 12 years were included in the study with the recruitment by the cluster sampling method. Urinary fluoride (UF) and urinary creatinine were separately determined using the fluoride ion-selective electrode and creatinine assay kit. Children were classified as the high fluoride group and control group according to the median of urinary creatinine-adjusted urinary fluoride (UF_{Cr}) level. Four loci of *MTHFD1* were genotyped, and the Combined Raven's Test was used to evaluate children's intelligence quotient (IQ). Generalized linear model and multinomial logistic regression model were performed to analyze the associations between children's UF_{Cr} level, *MTHFD1* polymorphisms, and intelligence. The general linear model was used to explore the effects of gene-environment and gene-gene interaction on intelligence.
Results: In the high fluoride group, children's IQ scores decreased by 2.502 when the UF_{Cr} level increased by 1.0 mg/L ($\beta = -2.502$, 95% confidence interval [CI]:—4.411, —0.593), and the possibility for having "excellent" intelligence decreased by 46.3% (odds ratio = 0.537, 95% CI: 0.290, 0.994). Children with the GG genotype showed increased IQ scores than those with the AA genotype of rs11627387 locus in the high fluoride group ($P < 0.05$). Interactions between fluoride exposure and *MTHFD1* polymorphisms on intelligence were observed (Pinteraction < 0.05).
Conclusions: Our findings suggest that excessive fluoride exposure may have adverse effects on children's intelligence, and changes in children's intelligence may be associated with the interaction between fluoride and *MTHFD1* polymorphisms.
Keywords: Fluoride; Intelligence; Interaction; *MTHFD1* gene

Introduction

Low-dose fluoride can prevent dental caries and is beneficial for bone growth.[1,2] However, the health damage caused by excessive fluoride should not be ignored.[3] Dental and skeletal fluorosis are the most specific diseases caused by chronic intake of excessive fluoride.[4,5] Besides, fluoride exposure is related to the dysfunction of the reproductive system, as well as the liver and brain damage.[6-8]

Increasing studies have suggested that excessive fluoride is associated with disorders in cognition, learning, and memory ability.[9-11] Among them, a birth cohort study conducted in Canada suggested that increased fluoride concentrations in drinking water were associated with intellectual impairment in children.[12] Another study reported a negative correlation between fluoride exposure and intelligence quotient (IQ) in children,[13] which was similar to our previous study.[14] In addition, an animal study demonstrated that fluoride exposure during development could induce cognitive deficits in mice.[15] However, there was no evidence that reductions in IQ scores were caused by excessive fluoride in a prospective study conducted in a New Zealand community.[16] Similarly, a cross-sectional study conducted in China mentioned that there was no statistical difference in IQ scores between children in different fluorosis areas and children in the control group (CG).[17] Behind the different levels of fluoride exposure and assessment methods,

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different ethnic and genetic backgrounds may also explain the inconsistent results.

Individual susceptibility (eg, gene polymorphisms) is an important factor that affects the sensitivity of body health to environmental factors.[18,19] Several studies have reported that genetic polymorphisms can modify the harmful effects of fluoride, such as dental and skeletal fluorosis.[20,21] Studies have also shown that gene polymorphisms may modulate sensitivity to the effects of fluoride exposure on intelligence. For example, polymorphisms of the catechole-o-methyltransferase gene and dopamine receptor-2 gene have been reported to modify the adverse effects of fluoride on IQ scores.[22,23] Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1 *(MTHFD1)* gene encode a nicotinamide adenine dinucleotide phosphate-dependent trifunctional enzyme, which provides the one-carbon derivatives of tetrahydrofolate in three sequential reactions and is involved in the folate pathway.[24] Comprehension of the influential factors of neural development is incomplete, but folate deficiency has been implicated consistently in neuropathological lesions.[25] Previous studies have pointed out that mouse with *MTHFD1* mutations seemingly showed impaired functions of one-carbon metabolism and higher plasma homocysteine levels,[26,27] which have been further linked to cognitive impairment and Alzheimer's disease.[28,29] In addition, certain loci polymorphisms of *MTHFD1* were associated with neurological diseases in an epidemiological study.[30] All these evidences suggest that *MTHFD1* mutations may relate to neurodevelopment. However, few studies have focused on the effects of *MTHFD1* polymorphisms on children's intelligence.

Present study focused on four loci of *MTHFD1* which are related to neurodevelopment: rs11627387, rs1076991, rs2236224, and rs2236225.[31-33] Studies have suggested that variations of these four loci can modify the impact of certain factors on health.[34-36] However, whether *MTHFD*1 polymorphisms are involved in the effect of fluoride exposure on children's intelligence remains unclear.

Based on the above analyses, we conducted a cross-sectional study in an endemic drinking water-borne fluorosis area in Tongxu County, Kaifeng (Henan Province, China). We aimed at evaluating the effects of polymorphisms of *MTHFD1* loci (rs11627387, rs1076991, rs2236224, and rs2236225) and excessive exposure to fluoride on children's intelligence and explore the role of *MTHFD1* polymorphisms in relationship between fluoride exposure and changes in children's intelligence to provide a novel clue for the study of neurotoxicological mechanisms of fluoride.

Methods

Ethical approval

The study protocol (No. ZZUIRB2017-018) was approved by the Ethics Review Board of Zhengzhou University (Zhengzhou, China). Children and their guardians were fully aware of the aim and process of our research and provided written informed consent.

Study design and population

As described thoroughly in our previous study,[14] we conducted a cross-sectional study in Tongxu County, Henan Province, from April to May in 2017. Four primary schools were randomly selected. We excluded children who were non-local residents, on calcium supplements, had diseases based on calcium or phosphorus metabolism, had digestive diseases, and had thyroid diseases. Subsequently, 694 school-age children aged 8 to 12 years from grades 2 to 6 in four selected schools were recruited by cluster sampling. All children lived on campus and they had similar living conditions, living habits, and dietary structure.

A questionnaire was designed in advance and included information on sociodemographic data, medical history, maternal pregnancy, information on birth, and other information (eg, exercise). The height and weight of children were measured twice and the mean value was taken, which were accurate to 0.1 cm and 0.1 kg, respectively. Then, the body mass index (BMI) was calculated. In addition, fluoride-free containers were used to collect mid-flow morning urine and whole blood from the cubital vein after an overnight fasting. Samples of urine and blood were stored, respectively, at —20°C and —80°C for subsequent measurements.

Exposure assessment

Exposure assessment has been described in detail in our previous study.[14] In accordance with the standard detailed by the health industry of China (WS/T 892015), a method based on a fluoride ion-selective electrode (Shanghai Exactitude Instruments, Shanghai, China) was conducted to determine the urinary fluoride (UF) level of children. A creatinine assay kit (Jiancheng Bioengineering Institute, Nanjing, China) was used to determine the concentration of urinary creatinine (U_{Cr}). Each determination of levels of UF and U_{Cr} was undertaken twice and averaged for data analyses. We calculated the urinary creatinine-adjusted urinary fluoride (UF_{Cr}) level to correct the influence of urine dilution on the UF level using the following equation: $(UF_{Cr}[mg/L] = UF[mg/L]/UCr[mg/L]) \times U_{Cr-}(mg/L)$, where $U_{Cr\text{-}mean}$ denotes the mean U_{Cr} concentration of the total population.[37] Then, according to the median value of UF_{Cr}, children were separated into the high fluoride group (HFG, $UF_{Cr} > 1.33$ mg/L) and CG, $UF_{Cr} \leq 1.33$ mg/L.

Intelligence assessment

The IQ was assessed using the second revision of the Combined Raven's Test - the Rural in China (CRTRC2).[38] Each student completed the paper independently with the supervision of trained investigators. Answer sheets were scored in accordance with the standard of the Combined Raven's Test. The intelligence levels were defined by the IQ scores and classified as "retarded" (≤69); "marginal" (70–79); "dull normal" (80–89); "normal" (90–109); "highnormal" (110–119);

"superior" (120–129) and "excellent" (≥130). Only 11 children had retarded, marginal, ordullnormalintelligence (IQscore<90); so, 683 children were finally included and were further separated into four groups (normal, high normal, superior, and excellent) according to their IQ scores in this study.

Genotyping of gene polymorphisms

Genomic DNA was extracted from whole-blood samples by a genomic DNA miniprep kit (LifeFeng Biotechnology, Shanghai, China). Four single-nucleotide polymorphism (SNP) loci with minor allele frequency >0.1 were retrieved from Haploview (www.broadinstitute.org/haploview/haploview). All of these loci polymorphisms have been reported to be related to neurodevelopmental defects.[31-33] rs11627387, rs1076991, rs2236224, and rs2236225 loci were located in intron 26, 2 KB upstream, intron 21, and exon 20, respectively, in *MTHFD1*. All polymorphisms of the four loci were genotyped through a custom-by-design 48-Plex SNPscan™ Kit (catalog number, G0104; Genesky Biotechnologies, Shanghai, China). The kit was developed according to the patented SNP genotyping technology of Genesky Biotechnologies, which was based on double ligation and multiplex fluorescence polymerase chain reaction (PCR).[39] Briefly, DNA samples (100–200 ng) were denatured at 98°C for 5 min and then mixed with the premix containing ligase and the probe. The ligation reaction was carried out in a thermal cycler (ABI2720; Applied Biosystems, Foster City, CA, USA). Then, two fluorescent PCRs were undertaken for each ligation product. PCR products were separated and detected by capillary electrophoresis in a sequencer (ABI3730XL; Applied Biosystems). Genotyping was completed according to the obtained information for the labelingdye color and fragment size of allele-specific ligation PCR products. About 4% of the genotyping was done repeatedly, and the consistency rate was >96%.

Statistical analyses

A database was set up by Epidata 3.0 (Epidata Association, Odense, Denmark) in which two operators independently imported all data. Mean ± standard deviation and number (%) are presented for continuous and categorical variables, respectively.

Differences in continuous data between the two groups were compared using the Student's *t*-test or Mann–Whitney U-test. The distribution of categorical variables was compared using the Chi-squared test. Potential confounders (children's age, gender, BMI, age at which pregnancy occurred, gestational weeks, birth weight, birth modes, paternal and maternal education level) were chosen as adjustment variables based on existing literature and population characteristics of this study.[13] The generalized linear model (GLM) was used to analyze the association between children'sUF$_{Cr}$ level, *MTHFD1* polymorphisms, and IQ scores. The multinomial logistic regression model was applied to analyze the relationship between children'sUFCr level, *MTHFD1* polymorphisms, and intelligence levels. And normal intelligence children were reference in the analyses of the intelligence levels. For exploration of the relationship between the UFCr level and children's intelligence, the UF$_{Cr}$ level was separated into categorical variables according to tertiles, and the median of each segment was regarded as a continuous variable to estimate the linear trend of children's intelligence. The general linear model was used to explore models of possible gene-environment and genegene interaction on intelligence. Data were processed using SPSS 21.0 (IBM, Armonk, NY, USA). Plots were drawn by GraphPad Prism 8.0.1. $P < 0.05$ was considered significant.

Results

General characteristics of participants

Since 11 children had an IQ score < 90, a total of 683 eligible children aged 8 to 12 years were included in this study and were further classified as the CG ($n = 342$) and HFG ($n = 341$) according to the median of children's UF$_{Cr}$ level (1.33 mg/L). The distribution of children's age was consistent in the CG and HFG (10.05 ± 1.24 and 10.08 ± 1.23 years, respectively). The concentration of UFCr in the HFG (2.15 ± 0.91 mg/L) was significantly higher than that in the CG (0.83 ± 0.30 mg/L) (P < 0.001), whereas the distribution of other sociodemographic characteristics (except U$_{Cr}$ and UF levels) presented no significant differences between the two groups (P > 0.05 for all) [Table 1].

Association between children's UF$_{Cr}$ level and intelligence

The GLM and multinomial logistic regression model were employed to evaluate if there were associations between the UFCr level and children's IQ scores or intelligence levels, respectively [Figure 1]. For each increase of 1.0 mg/L in the UFCr level, children's IQ scores decreased by 2.502 ($\beta = -2.502$, 95% confidence interval [CI]: −4.411, −0.593, $P = 0.010$), and the possibility of developing " excellent" intelligence decreased by 46.3% with reference to the normal intelligence children in the HFG (odds ratio [OR] = 0.537, 95% CI: 0.290, 0.994, $P = 0.048$). After stratifying children according to the tertiles of children's UFCr concentration in different groups, the trend test showed no significance ($P_{trend} > 0.05$ for all).

Association between MTHFD1 polymorphisms and intelligence

The genotype distributions of rs11627387, rs1076991, rs2236224, and rs2236225 loci in *MTHFD1* were in accordance with the Hardy–Weinberg equilibrium (P > 0.05 for all loci) [Supplementary Table 1, http://links.lww.com/CM9/A987], which indicated that the investigated participants were representative of the population. We also estimated the difference in the distribution of genotypes/allele of rs11627387, rs1076991, rs2236224, and rs2236225 between the CG and HFG, but significant differences were not found (P > 0.05 for all) [Supplementary Table 2, http://links.lww.com/CM9/A987].

Table 1: Demographic data of the study population.

Variables	Total ($n = 683$)	CG ($n = 342$)	HFG ($n = 341$)	Statistics	*P* values
Age (years)	10.07 ± 1.24	10.05 ± 1.24	10.08 ± 1.23	0.375*	0.708
Gender				0.333†	0.564
Boys	324 (47.44)	166 (48.54)	158 (46.33)		
Girls	359 (52.56)	176 (51.46)	183 (53.67)		
BMI (kg/m^2)	17.50 ± 2.96	17.68 ± 2.96	17.33 ± 2.95	–1.618*	0.106
U_{Cr} (mg/L)	1089 ± 607	1277 ± 606	901 ± 548	–8.261*	<0.001
UF (mg/L)	1.27 ± 0.79	0.98 ± 0.62	1.56 ± 0.82	9.811*	<0.001
UF_{Cr} (mg/L)	1.49 ± 0.95	0.83 ± 0.30	2.15 ± 0.91	22.616*	<0.001
Age at which pregnancy occurred (years)	25.82 ± 4.28	25.95 ± 4.52	25.70 ± 4.01	–0.750*	0.453
Gestational weeks (weeks)	36.87 ± 4.84	36.53 ± 5.31	37.22 ± 4.30	1.742*	0.081
Birth weight (kg)	3.34 ± 0.52	3.31 ± 0.55	3.36 ± 0.48	0.987*	0.324
Birth modes				0.280†	0.869
Natural birth	467 (68.37)	232 (67.84)	235 (68.91)		
Cesarean delivery	209 (30.60)	107 (31.29)	102 (29.91)		
Rest	7 (1.02)	3 (0.88)	4 (1.17)		
Paternal education				0.497†	0.780
Primary school and below	57 (8.35)	31 (9.06)	26 (7.62)		
Middle school	463 (67.79)	229 (66.96)	234 (68.62)		
High school and above	163 (23.87)	82 (23.98)	81 (23.75)		
Maternal education				0.457†	0.796
Primary school and below	87 (12.74)	46 (13.45)	41 (12.02)		
Middle school	453 (66.33)	223 (65.20)	230 (67.45)		
High school and above	143 (20.94)	73 (21.35)	70 (20.53)		
IQ scores	122.05 ± 11.88	121.50 ± 12.14	122.61 ± 11.61	1.059*	0.290
Intelligence levels				2.162†	0.539
90–109	112 (16.40)	60 (17.54)	52 (15.25)		
110–119	173 (25.33)	85 (24.85)	88 (25.81)		
120–129	216 (31.63)	113 (33.04)	103 (30.21)		
≥130	182 (26.65)	84 (24.56)	98 (28.74)		

Data are presented as mean ± SD or *n* (%). * Mann–Whitney *U*-test. † χ^2 test. BMI: Body mass index; CG: Control group; HFG: High fluoride group; IQ: Intelligence quotient; SD: Standard deviation; U_{Cr}: Urinary creatinine; UF: Urinary fluoride; UF_{Cr}: Urinary creatinine-adjusted urinary fluoride.

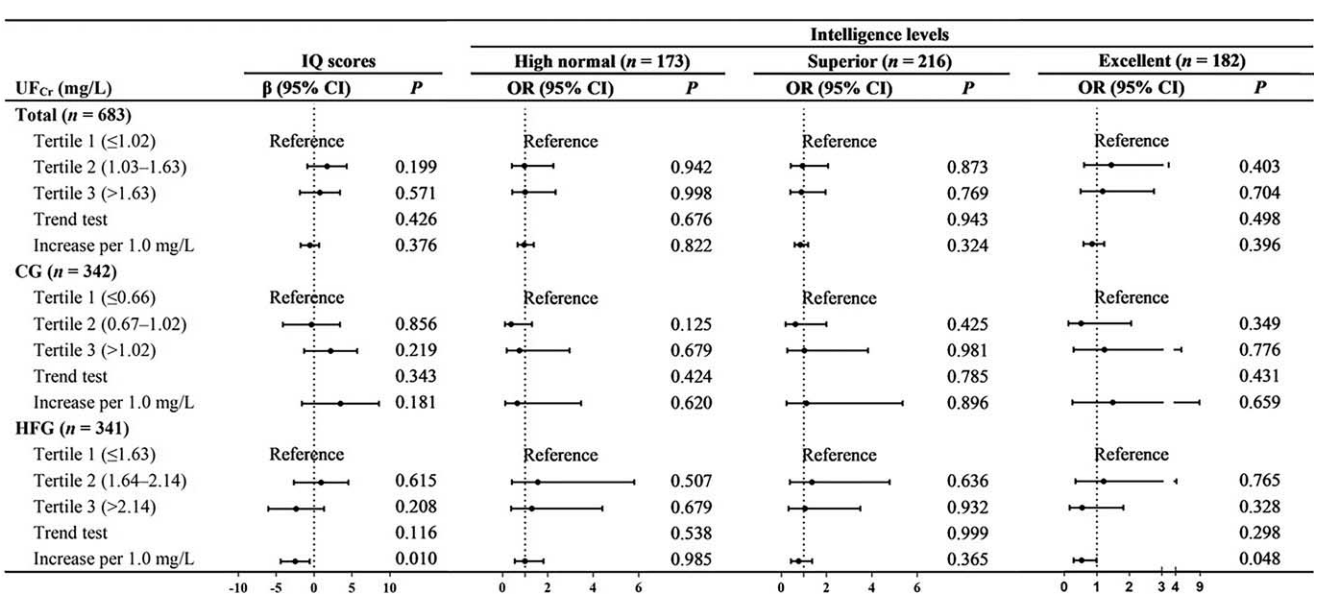

Figure 1: Relationship between fluoride exposure and children's intelligence. Analyses were adjusted for age, gender, BMI, age at which pregnancy occurred, gestational weeks, birth modes, birth weight, and paternal and maternal education level. Normal intelligence children were reference to the analyses of the intelligence levels. BMI: Body mass index; CG: Control group; CI: Confidence interval; HFG: High fluoride group; IQ: Intelligence quotient; OR: Odds ratio; UF_{Cr}: Urinary creatinine-adjusted urinary fluoride.

Furthermore, the relationship between *MTHFD1* polymorphisms and intelligence was assessed by the GLM and multinomial logistic regression model. In the general population and HFG, children with the GG genotype of rs11627387 showed increased IQ score relative to those with the AA genotype of rs11627387 (β = 3.574, 95% CI: 0.274, 6.874, $P = 0.034$ for the general population, β = 4.723, 95% CI: 0.277, 9.168, $P = 0.037$ for the HFG) [Figure 2]. There was an increment in IQ score in children carrying the *G allele* than in those with the A allele of rs11627387 in the HFG, and the association was borderline significant ($P = 0.059$) [Figure 2]. In addition, the possibility of developing "high normal" intelligence was lower in the HFG in children with the AG genotype when compared with thosecarryingtheAAgenotypeofrs11627387 (OR = 0.212, 95% CI: 0.045,0.997, $P = 0.049$) [Figure 3]. With respect to rs2236225 locus, participants with the AA genotype seemingly showed a lower possibility of developing "high normal" intelligence when compared with children with the GG genotype in the HFG, and the association was borderline significant ($P = 0.056$) [Figure 3]. However, a statistical significance was not found in the association between *MTHFD1* polymorphism and children's intelligence levels in the total group and CG ($P > 0.050$) [Supplementary Tables 3 and 4, http://links.lww.com/CM9/A987].

Effects of gene-gene and gene-environment interaction on children's intelligence

The general linear model was used to explore geneenvironment and gene-gene interactions. Loci rs11627387, rs1076991, and rs2236225 may have interactive effects on the IQ scores according to analyses of gene-gene interaction [Figure 4], where the association showed marginal significance ($F = 1.726$, $P = 0.059$). In addition, the interaction between rs11627387, rs1076991, rs2236224, and the UF_{Cr} level might affect children's IQ scores ($F = 1.669$, $P = 0.021$), as well as the interactive effects of loci rs11627387, rs1076991, rs2236225, and the UFCr level ($F = 1.764$, $P = 0.012$). An effect of the interaction between these four loci and the UF_{Cr} level on the IQ scores of children was also found ($F = 1.614$, $P = 0.012$). However, statistical significances were not found in other models [Supplementary Table 5, http://links.lww.com/CM9/A987].

Discussion

We evaluated the interaction between fluoride exposure and *MTHFD1* polymorphisms on the intelligence of children living in areas of endemic fluorosis. We found that the decline in the IQ scores in children was associated with excessive exposure to fluoride and that changes in

Loci	Genotypes /Allele	*n*	Total (*n* = 683) β (95% CI)	*P*	CG (*n* = 342) β (95% CI)	*P*	HFG (*n* = 341) β (95% CI)	*P*
rs11627387	AA	93	Reference		Reference		Reference	
	AG	316		0.247		0.824		0.339
	GG	252		0.034		0.705		0.037
	AG+GG	568		0.096		0.722		0.174
	A	502	Reference		Reference		Reference	
	G	820		0.068		0.903		0.059
rs1076991	TT	368	Reference		Reference		Reference	
	TC	253		0.778		0.080		0.307
	CC	39		0.091		0.801		0.103
	TC+CC	292		0.819		0.145		0.156
	T	989	Reference		Reference		Reference	
	C	331		0.438		0.303		0.098
rs2236224	GG	326	Reference		Reference		Reference	
	GA	260		0.303		0.860		0.219
	AA	74		0.849		0.851		0.932
	GA+AA	334		0.453		0.775		0.409
	G	912	Reference		Reference		Reference	
	A	408		0.726		0.719		0.620
rs2236225	GG	375	Reference		Reference		Reference	
	GA	241		0.215		0.601		0.435
	AA	45		0.925		0.969		0.804
	GA+AA	286		0.257		0.702		0.455
	G	991	Reference		Reference		Reference	
	A	331		0.377		0.819		0.473

Figure 2: Regression analyses of *MTHFD1* polymorphisms and the IQ scores. Analyses were adjusted for age, gender, BMI, age at which pregnancy occurred, gestational weeks, birth modes, birth weight, and paternal and maternal education level. Twenty-two participants were not genotyped successfully for rs11627387 and rs2236225 respectively, and 23 participants were not genotyped successfully for rs1076991 and rs2236224 respectively. BMI: Body mass index; CG: Control group; CI: Confidence interval; HFG: High fluoride group; IQ: Intelligence quotient; *MTHFD1:* Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1.

Loci	Genotypes /Allele	Intelligence levels: High normal (n = 88) OR (95% CI)	P	Superior (n = 103) OR (95% CI)	P	Excellent (n = 98) OR (95% CI)	P
HFG (n = 341)							
rs11627387	AA	Reference		Reference		Reference	
	AG		0.049		0.357		0.697
	GG		0.449		0.848		0.573
	AG+GG		0.109		0.543		0.949
	A	Reference		Reference		Reference	
	G		0.666		0.524		0.328
rs1076991	TT	Reference		Reference		Reference	
	TC		0.500		0.792		0.814
	CC		0.850		0.832		0.233
	TC+CC		0.711		0.799		0.460
	T	Reference		Reference		Reference	
	C		0.835		0.858		0.277
rs2236224	GG	Reference		Reference		Reference	
	GA		0.140		0.457		1.000
	AA		0.164		0.610		0.549
	GA+AA		0.069		0.276		0.771
	G	Reference		Reference		Reference	
	A		0.063		0.247		0.687
rs2236225	GG	Reference		Reference		Reference	
	GA		0.721		0.559		0.926
	AA		0.056		0.266		0.531
	GA+AA		0.312		0.325		0.961
	G	Reference		Reference		Reference	
	A		0.100		0.167		0.820

Figure 3: Regression analyses of *MTHFD1* polymorphisms and intelligence levels in the HFG. Analyses were adjusted for age, gender, BMI, age at which pregnancy occurred, gestational weeks, birth modes, birth weight, and paternal and maternal education level. Normal intelligence children were referenced (n = 52). BMI: Body mass index; CI: Confidence interval; HFG: High fluoride group; *MTHFD1:* Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1; OR: Odds ratio.

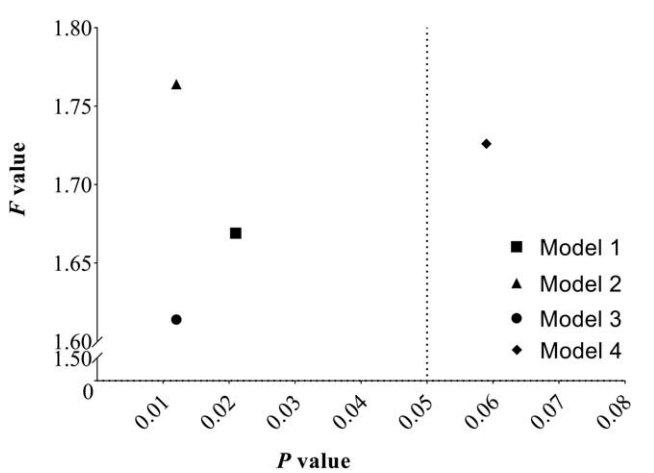

Figure 4: Interaction analyses of fluoride exposure and *MTHFD1* polymorphisms. Analyses were adjusted for age, gender, BMI, age at which pregnancy occurred, gestational weeks, birth modes, birth weight, and paternal and maternal education level. Dashed line denotes the P value of 0.05 for significance. BMI: Body mass index; Locus 1: rs11627387; Locus 2: rs1076991; Locus 3: rs2236224; Locus 4: rs2236225; *MTHFD1:* Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1; UF_{Cr}: Urinary creatinine-adjusted urinary fluoride.

children's intelligence might be modified by rs11627387 locus polymorphisms of *MTHFD1* to some extent. Moreover, the interactive effects of the four loci of *MTHFD1* and fluoride exposure on children's IQ scores showed different models in our study.

The health and function of the nervous system can be affected by exposure to various environmental factors.[40,41]

In terms of fluoride, drinking water containing a high concentration of fluoride is the main way to the exposure of superabundant fluoride.[42] Fluoride entering the body can be distributed widely throughout the body after absorption, and most of it is deposited in bone and teeth.[43] The absorption rate of fluoride in children is about 80% to 90%, much higher than that in adults.[44] In addition, fluoride can penetrate the blood-brain barrier into brain tissue and seems to accumulate in the areas of the brain responsible for learning and memory functions.[45,46] But the evidence regarding a link between fluoride exposure and intelligence impairment is not definitive. Most of the scholars conceive that fluoride in brain tissue can damage nerve functions and even lead to

intellectual loss.[47-49] For example, animal studies found that, upon exposure to increasing concentrations of sodium fluoride in drinking water, the learning abilities of mice were impaired.[10,50] However, the results of population-based epidemiological studies are not completely consistent. A cohort study from Canada revealed a positive association between fluoride exposure during pregnancy and intelligence decline in offspring.[51] Our current study support this finding, that is, an inverse association between excessive exposure to fluoride and children's IQ scores in the HFG, which recollects that excessive fluoride exposure may have negative influence on normal development of children's intelligence. Whereas, a prospective study conducted in New Zealand did not find a positive association between lower intelligence and a higher fluoride level.[16] A cross-sectional study conducted in China did not observe the significant difference in IQ scores between children in fluorosis areas and children in the CG, either.[17] Differences in study design, levels of fluoride exposure, exposure patterns, ethnicity, and assessment methods can explain these inconsistences to a certain extent. On the other hand, genetic susceptibility (eg, genetic polymorphisms) is also one of the important reasons that is worthy of further discussion.

We further discussed the association between *MTHFD1* polymorphisms and children's intelligence according the study design. We found that, compared with children carrying the AA genotype of the rs11627387 locus, children carrying the GG genotype might have increased IQ score in the HFG, whereas the possibility for having "high normal" intelligence was lower for children carrying the AG genotype. It can be suggested that polymorphisms of the rs11627387 locus of *MTHFD1* may have effects on changes in children's intelligence. Specifically, the GG genotype of rs11627387 may retard the intelligence decline caused by excessive fluoride exposure in schoolage children, as carrying the GG genotype of rs11627387 may exert a positive effect on IQ scores compared to carrying the AA genotype when UFCr >1.33 mg/L in children. Although few studies have focused on the relationship between *MTHFD1* polymorphisms and intelligence, others are still evaluating the effects of *MTHFD1* polymorphisms on neural development based on animals and humans. An animal study reported that mice with loss of one allele of *MTHFD1 via a* gene-trap mutation showed impaired learning ability.[52] Another study revealed that reduced gene expression of the alpha seven nicotinic cholinergic receptor was observed in a mouse model which simulated polymorphisms at the rs2236225 locus of *MTHFD1* in humans.[53] Also, agonists of the alpha seven nicotinic cholinergic receptor gene could be used to treat neurocognitive dysfunction in schizophrenia.[54] In addition, population-based studies also pointed out that polymorphisms of rs11627387, rs1076991, rs2236224, and rs2236225 loci are involved in neural tube defects.[31,33] These studies demonstrate that *MTHFD1* polymorphisms can have effects on normal neurocognitive functions and may modify the susceptibility of neurological diseases. Overall, our results support the correlation between *MTHFD1* polymorphisms and intellectual changes in school-age children, which is worthy of confirmation in further studies.

Intelligence is not only modified by genetic factors such as gene polymorphisms but also related to gene-environment interaction. For example, Zhao *et al*[55] conducted a crosssectional study in Tianjin and reported that ankyrin repeat and kinase domain 1 (ANKK1), catechol-O-methyltransferase (COMT), monoamine oxidase A (MAOA) gene polymorphisms may have interactive effects with UF on children's intelligence. In our study, the effects of gene-environment and gene-gene interaction on children's IQ were explored. We did not observe an interaction between a single locus of *MTHFD1* and fluoride exposure on children's intelligence but found the different models of interaction between multiple loci of *MTHFD1* and fluoride exposure. These results suggest that phenotypes can be affected by the interaction of environmental factors with multiple loci of one gene and even multiple genes[56]; the modification effect of one locus polymorphism is minor. All these findings suggest that *MTHFD1* polymorphisms may be involved in the effects of fluoride exposure on intelligence in school-age children, and we further provide a novel clue for the study of neurotoxicological mechanisms of fluoride.

There are several advantages in this study. First, the study was a population-based epidemiological study which explored the impact of fluoride and gene polymorphisms on children's intelligence, where the results suggest that people living in fluorosis areas should pay attention to the harmful effects of excessive fluoride intake on school-age children's intelligence. Second, this study was conducted in the middle of the semester which avoided the impact of students' psychological stress caused by the beginning of the semester or the final exam on intelligence tests. Third, as described in the study design in our previous publication,[14] all the children lived on campus and they had similar living conditions, living habits, and dietary structure, which minimized the bias. Finally, the investigated areas in Tongxu County, Henan Province, are relatively underdeveloped, with no industrial fluorine and other pollutants such as lead and mercury, etc, that may affect intelligence.

Our study also had some limitations. First, we did not adjust the children's diet, although children included in the study were all boarding school students, and the dietary structure was relatively consistent; the differences in dietary habits and physical fitness of children may lead to differences in nutrients intake, which may further affect mental development. Therefore, children's diet will be considered in our subsequent studies. Second, the study is a cross-sectional study with only one sampling; so, the causal relationship is weak. So, long-term large-scale epidemiological or cohort studies should be conducted to provide more evidence. Third, although we adjusted for confounding factors such as children's age, gender, BMI, maternal age at which pregnancy occurred, gestational weeks, birth weight, birth modes, and paternal and maternal education level, there may still be some other confounding factors (such as folic acid). However, bounded to the eugenics policy, almost all the mothers

of the investigated students had taken folic acid in the early stage of pregnancy [57]; so, the bias may be reduced. Studies involving larger populations and more questionnaire information are in progress.

Conclusions

We evaluated the effects of polymorphisms of *MTHFD1* and fluoride exposure on children's intelligence in endemic fluorosis areas. Excessive fluoride exposure may have adverse effects on children's intelligence, and changes in children's intelligence may be associated with the interaction between fluoride and *MTHFD1* polymorphisms.

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Conflicts of interest

None.

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