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# Fluoride exposure and children's intelligence: Gene-environment interaction based on SNP-set, gene and pathway analysis, using a case-control design based on a cross-sectional study

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ABSTRACT

*Background:* Excessive fluoride exposure has been associated with intelligence loss, but little is known about gene-fluoride interactions on intelligence at SNP-set, gene and pathway level.
*Objectives:* Here we conducted a population-based study in Chinese school-aged children to estimate the associations of fluoride from internal and external exposures with intelligence as well as to explore the gene-fluoride interactions on intelligence at SNP-set, gene and neurodevelopmental pathway level.
*Methods:* A total of 952 resident children aged 7 to 13 were included in the current study. The fluoride contents in drinking water, urine, hair and nail were measured using the ion-selective electrode method. LASSO Binomial regression was conducted to screen the intelligence-related SNP-set. The gene-fluoride interactions at gene and pathway levels were detected by the Adaptive Rank Truncated Product method.
*Results:* The probability of high intelligence was inversely correlated with fluoride contents in water, urine, hair and nail (all $P < 0.001$). The SNP-set based on rs3788319, rs1879417, rs57377675, rs11556505 and rs7187776 was related to high intelligence ($P = 0.001$) alone and by interaction with water, urinary and hair fluoride ($P = 0.030$, 0.040, 0.010), separately. In gene level, *CLU* and *TOMM40* interacted with hair fluoride (both $P = 0.017$) on intelligence. In pathway level, Alzheimer disease pathway, metabolic pathway, signal transduction pathway, sphingolipid signaling pathway and PI3K-AKT signaling pathway interacted with fluoride on intelligence in men.
*Conclusions:* Our study suggests that fluoride is inversely associated with intelligence. Moreover, the interactions of fluoride with mitochondrial function-related SNP-set, genes and pathways may also be involved in high intelligence loss.

## 1. Introduction

Fluoride distributes in nature widely. Although it is beneficial for dental caries prevention, prolonged exposure to excessive fluoride results in adverse effects, such as skeletal, dental and neurological fluorosis. More than 200 million people consume fluoride-enriched drinking water worldwide (Su et al., 2020), and the number exceeds 87 million in China (Lei et al., 2014). Fluoride has been classified as one of the top ten chemicals of major public health concern (Mumtaz et al., 2015). It is becoming increasingly evident that numerous factors are associated with intelligence. Pesticides, cadmium, arsenic, lead and mercury are acknowledged as environmental risk factors of intelligence loss (Saeed

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et al., 2020). Besides, the overall nutritional status and the intake of essential nutrients also have potential impacts on intelligence, especially micronutrients including vitamins and minerals, which play important roles in environmental toxicants absorption, distribution and metabolism (Guth et al., 2020). Furthermore, the impaired intelligence caused by some brain diseases, for example, Down's syndrome and cerebral trauma, could not be ignored. Recently, fluoride-related neurotoxicity has aroused extensive attention. Animal studies suggested that high fluoride exposure was associated with learning and memory impairment as well as structural and functional damage of the brain in rats (Zhu et al., 2017). Consistently, a great deal of epidemiological evidence revealed positive association between excessive fluoride exposure and poor performance of children's neurodevelopment, such as lower intelligence, and deficits in memory and cognition (Green et al., 2019). In our previous study, excessive exposure to fluoride was found to be associated with loss of excellent intelligence in children, even at low levels (Yu et al., 2018).

Fluoride-induced neurotoxicity involves a series of physiological processes at the cellular and molecular levels. Excessive fluoride exposure can change the expression of energy metabolism-related proteins, and prevent the oxidative phosphorylation process by interfering with the activity of metabolic enzymes, therefore inhibiting the use of glucose in brain tissue and affecting normal neuron activity (Chen et al., 2015; Lima Leite et al., 2014). Animal studies suggested that the abnormalities of neurotransmitters and their receptors induced by fluoride resulted in impaired learning and memory ability (Pereira et al., 2011; Shan et al., 2004). Besides, fluoride could affect the fluidity and function of synaptic membranes by down-regulating PSD-95 and up-regulating VAMP-2, therefore resulting in cognitive impairment (Liang et al., 2020; Zhu et al., 2011).

Mitochondria is the energy center and essential for many biological processes in neurodevelopment, such as neurogenesis, synaptic formation, neurotransmitter transmission, metabolic activity and enzyme catalysis (Devine and Kittler, 2018). Growing evidence has suggested that fluoride could result in mitochondrial dysfunction in neurons both *in vivo* and *in vitro* (Araujo et al., 2019; Zhao et al., 2019). Fluoride could cause the abnormality of mitochondrial morphology and intervene the electron transport in the mitochondrial respiratory chain, consequently resulting in dysfunction of energy generation and utilization. Meanwhile, the excessive reactive oxygen and increased oxidative stress induced by energy metabolism dysfunction could cause lipid peroxidation of mitochondrial membrane and DNA damage in return (Song et al., 2017; Zhao et al., 2019). In these processes, mitochondrial function-related genes may play important roles, including *MFN1*, *MFN2*, *FIS1*, *DRP1* and *OPA1* (Chen et al., 2019; Yu et al., 2019) by intervening fusion, split, autophagy and other mitochondrial dynamic activities. However, whether these genes are involved in the fluoride-induced neurotoxicity in children has barely been investigated. Though individual SNPs of two mitochondrial function-related genes *COMT* and *DRD2* may contribute to modifying the relationship of high fluoride exposure with children's intelligence (Cui et al., 2018; Zhang et al., 2015), the combined effects of multiple SNPs or genes have rarely been explored yet.

In the present study, we detected the associations of fluoride from internal and external exposures with intelligence. Besides, the combined effects of genetic variants involved in both neurodevelopment and mitochondrial biological processes on intelligence were explored at SNP-set level, gene level and neurodevelopmental pathway level, and then the interactions of these variants with fluoride exposure were further detected in the current study with Chinese school-aged children.

## 2. Materials and methods

### 2.1. Study design and population

The current study was conducted in 2015 in the rural areas of Baodi District (117°30′N, 39°72′E), Tianjin, China. As the water fluoride concentrations in rural villages kept stable over the past decade according to the annual surveillance data from the local Center for Disease Control and Prevention (CDC), the study areas were divided into historical high fluoride areas and normal fluoride areas. None of the study sites was exposed to excessive neurotoxins including lead, arsenic and mercury, or in the endemic areas of iodine deficiency based on the surveillance data from the local CDC (Yu et al., 2018). The biological samples were collected from about one-third of the total participants, using a case-control design based on a cross-sectional study. The details of participant recruitment are displayed in Fig. 1. The stratified and multi-stage random sampling method was used to select children aged 7–13 years who were permanent residents since birth from each area (Fig. 1). All the participants and their parents/guardians provided written informed consent before study enrollment, as the subjects were minors. This work was approval for research ethics from the Review Board of Tongji Medical College, Huazhong University of Science and Technology.

### 2.2. General data collection

Trained investigators conducted face-to-face interviews with the recruited children and their parents to collect demographic data, including age, sex, maternal and paternal education level, family incomes, maternal exposures (smoking, drinking, passive smoking and anemia) during pregnancy, maternal delivery conditions (hypoxia, dystocia, premature birth and post-term birth) and history of cerebral trauma. Height (1 mm precision) was measured using a standard calibrated scale. Weight (0.1 kg precision) was measured without heavy clothing and shoes. All the measurements were conducted based on the recommended standard methods (Ward et al., 2017) by nurses. The development statuses of the recruited children were further evaluated by the calculation of body mass index (BMI), derived from height and weight.

### 2.3. Sample collection

Water samples were collected from each public supply in the villages. A spot (early-morning) urine sample from each subject was also collected. Both water and urine samples were collected into sterilized, labeled polythene tubes (50 mL) and transported to the laboratory within two hours in iceboxes, then stored at −80 °C until analysis. A total of 96 drinking water samples and 1020 urine samples were collected.

Hair samples were collected from the occipital zone of the scalp using stainless steel scissors. Children who had their hair permed or dyed, or with hair samples less than 0.2 g (n = 250) were excluded from the corresponding analyses due to the potential contamination or lowest need for testing. Nail (fingernail/toenail) samples were collected with standard nail clippers. Children with dyed nails or with nails samples less than 0.2 g (n = 340) were excluded from the corresponding analyses. Both hair and nail samples were put into sterilized, labeled transparent plastic bags with zip-locks that can be sealed to prevent pollution, transported to the laboratory, and stored at 4 °C until analysis. A total of 770 hair samples and 680 nail samples were collected.

About 5 mL fasting peripheral blood sample was drawn from each subject into a polypropylene Na-EDTA tube. Lymphocytes were separated within 2–4 h after sample collection by centrifugation at 3000 r/min for 15 min, then transferred to 1.5 mL EP tubes and stored at −80 °C for subsequent analysis. A total of 1020 blood samples were collected.

### 2.4. Measurement of fluoride concentration

The hair sample was immersed in 75% ethanol solution for 0.5 h, stirred for 10–15 min twice, washed three times with deionized water, then placed in the oven for 5 h at 80 °C. After drying, the hair sample

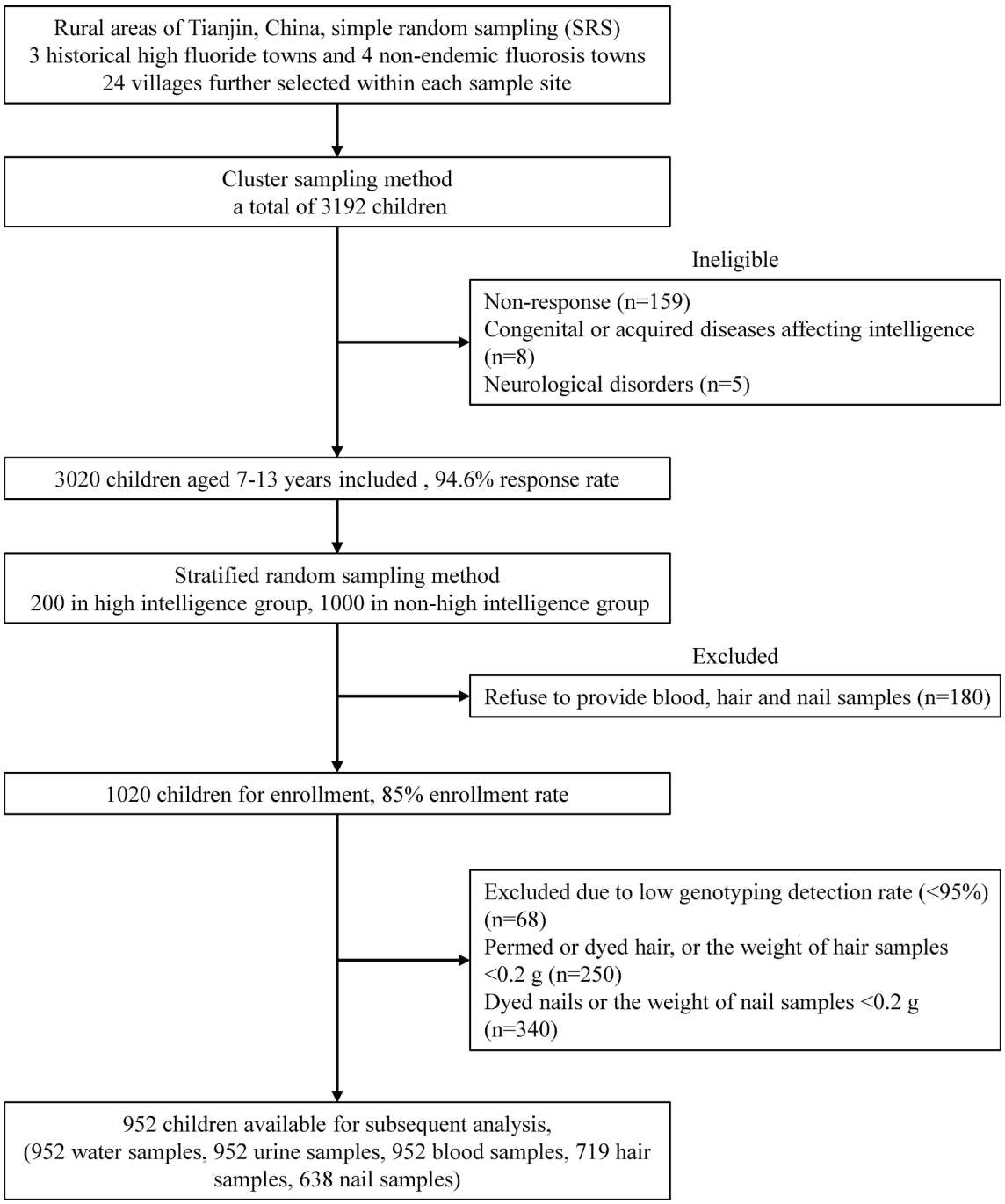


**Fig. 1.** Flow chart of recruitment process.

was cut into small sections of 0.5 cm. About 0.2–0.5 g samples were accurately weighed into a 10 mL tube, immersed in 10.0 mL of 2 mol/ L NaOH solution, and heated 2 h in a water bath at 85 °C, until the hair sample dissolved. After cooling, the sample solutions were filtered and adjusted to weak acidity (pH 5.0–5.5) with a dilute HCl solution, then the volume was set to 15 mL with deionized water, and 25 mL of total ionic strength adjusted buffered (TISAB) was subsequently added for measurement.

The nail specimen was cleaned with alcohol swab firstly, then placed into a 15 mL polypropylene plastic tube, and washed twice with 1% Triton X100 under ultrasound. After rinsing with deionized water and ethanol, the samples were placed in the oven at 50 °C overnight. After drying, about 0.2 g nail samples were weighted into the tube, and 1.0 mL mixed solution of 70% concentrated nitric acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and 30% hydrogen peroxide (Guanghua Sci-Tech Co., Ltd., Guangdong, China) (volume ration 1:1) were added, then heated 2 h in a water bath at 80 °C, until the nail samples dissolved. After cooling, the sample solutions were filtered and adjusted to weak acidity (pH 5.0–5.5) with a dilute NaOH solution, then the volume was set to 15 mL with deionized water, and 25 mL TISAB solution was added further for measurement. Different from pretreatment of hair and nail samples, the drinking water and urine samples were directly diluted with an equal volume of TISAB of pH 5–5.5 for optimal analysis of fluoride ion.

The concentration detection of $F^-$ [mg/L] was conducted by the national standardized ion-selective electrode method in China (Wu et al., 2015). For the $F^-$ selected electrode (PF-202-CF, INESA, Shanghai), the detection limit was 0.01 mg/L. The measurement

accuracy was between 97.3% and 101.9%, and the measurement error was less than ±5%. All the chemicals used were guaranteed reagents.

### 2.5. Assessment of intelligence quotient (IQ)

IQ scores were measured by the second edition of Combined Raven's Test – The Rural in China (CRT-RC2) (Liu et al., 2009) for children aged 7 to 13 years. The CRT-RC2 comprises 72 nonverbal items in six sets, including A, AB and B sets (the Raven's colored progressive matrix), and C, D and E sets (the Raven's standard progressive matrix). Each set stands for different domains: A for perceptual awareness, B for comparative ability of similarity, C for reasoning by analogy, D for serial reasoning ability, E for abstract reasoning, while AB is a comprehensive set suitable for children and retarded intelligence population (Xu et al., 2014). The CRT-RC2 scale is an effective test for basic cognition, which is less affected by the difference in language, culture and ethnicity.

The CRT-RC2 was completed by each child based on the instruction manual within 40 min. In each test, 40 children were randomly assigned to a classroom to finish the test independently, under four professionals' supervision. The children's IQ scores were categorized into seven degrees as follow: ≥130 (excellent), 120–129 (superior), 110–119 (high normal), 90–109 (normal), 80–89 (dull normal), 70–79 (marginal), and ≤69 (retarded) according to the norm of rural children in China (Ding et al., 2011; Wang et al., 2007).

### 2.6. SNP selection, and DNA isolation and genotyping

Literature review and bioinformatic analysis were conducted to select genes related to mitochondrial function and involved in nervous system development. Bioinformatic tools were utilized to screen potential functional tag-SNPs of the selected genes in the Chinese population. The specific steps were as follow: (1) 547 functional genes related to human intelligence and cognition (intelligence, intellectual disabilities, cognition, cognition disorder, learning disorders, and neurodevelopmental related diseases such as Autistic disorder and schizophrenia) were initially selected by literature review based on Genome-wide Association Studies (GWAS), large-scale studies and meta-analysis. (2) Based on gene ontology (GO) analysis results, 60 genes involved in both nervous system development and mitochondrial biological process were further selected. (3) The genes and SNPs data of the Chinese Han Beijing (CHB) population were downloaded from the 1000 Genomes Project website (http://phase3browser.1000genomes.org/index.html), and then imported into Haploview software version 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA). A total of 279 tag-SNPs were kept with a minor allele frequency (MAF) ≥ 0.05 in the CHB population. (4) Functional effects prediction (transcription factor binding site, splicing, miRNA, nonsynonymous SNP, stop codon, etc.) was conducted by SNPinfo Web Server online software (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). The predicted non-functional tag-SNPs were replaced with their pairwise linkage disequilibrium sites (set as $r^2 \geq 0.8$). A total of 110 functional tag-SNPs were further selected. (5) Considering the MAF of SNPs locus, Hardy-Weinberg Equilibrium (HWE), published researches, and the predicted success rate of locus typing, 60 functional tag-SNPs from 19 candidate genes were finally identified for subsequent analysis (Table S1).

DNA was isolated from lymphocytes using a commercial DNA extraction Kit (Generay Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions, and quality assessment was performed with Nanodrop ND1000 (Thermo scientific, Wilmington, DE, USA). DNA was diluted to a final concentration of 200 ng/μL. Primers were designed using Primer 3 online version 0.4.0 (http://frodo.wi.mit.edu/), then synthesized in the Shanghai Genomics Institute (Bioligo Co., LTD, Shanghai, China). The details of the primers are given in Table S2. An efficient multiple gene region enrichment/ next generation sequencing-based SNPseq assay was designed for SNP genotyping (Novogene Co., Ltd., Beijing, China). The SNPs were genotyped by a three-round multiplex PCR and next generation sequencing method (Xiong et al., 2016). The amplification procedures and reaction conditions for PCR are shown in Table S3.

Among the 60 SNPs included, 1 (rs4680) fell through the assay design, 2 (rs17554825 and rs9658258) were excluded as the genotyping call rate below 92%, and 4 (rs12098908, rs7928, rs1800844 and rs6356) did not meet Hardy-Weinberg equilibrium ($P < 0.05$). Sixty-eight samples were excluded as the individual call rate less than 95%. Finally, 53 SNPs from 17 genes were available for the 952 subjects. Additionally, 5% of the samples were randomly selected as validation duplicates to be re-genotyped, and the concordance rate was 100%.

### 2.7. Statistical analysis

The whole population was firstly divided into high (IQ ≥ 120) and non-high intelligence group (70 ≤ IQ < 120). Data were presented as mean (with standard deviation) or median ($P_{25}$-$P_{75}$) for continuous variables, and number (percentage/proportion) for categorical variables. The baseline characteristics and fluoride exposure levels were compared by a student's *t*-test or Wilcoxon test for continuous variables, whereas a Chi-square test was used for the comparisons of the discrete data between the two groups. We calculated correlation coefficients among the four fluoride exposure indexes (water, urinary, hair and nail fluoride) by Spearman's rank correlation analysis. Multivariable piecewise linear regression and logistic regression were utilized to examine the effects of fluoride exposure on IQ scores and the prevalence of high intelligence. In the piecewise linear regression model, the turning point was chosen according to the maximum likelihood model using the trial-and-error method, along with a log likelihood ratio test to examine the statistical significance (Yu et al., 2013). Linear trends across increasing tertiles of fluoride exposures on high intelligence were performed by treating fluoride indicators as ordinal variables. The selection of covariates was based on the characteristics of the study population and previous literatures.

The genotype distribution of SNPs between high and non-high intelligence group was compared by Chi-square test, and the Cochran-Armitage trend test was further conducted with the wild homozygous genotype as reference group. To develop the high intelligence-related SNP-set, Least Absolute Shrinkage and Selection Operator (LASSO) binomial regression was applied to identify the most correlated SNPs, with the genotype coded as 0, 1, and 2 for protective homozygote, heterozygote and risk homozygote, respectively. Multivariate logistic regression was further conducted to evaluate the associations of the SNPs selected by LASSO regression with intelligence by calculating the odds ratios (ORs) and 95% confidence intervals (95% CIs). The SNPs selected were weighted by the corresponding regression coefficients and then summed to constitute the SNP-set score. In addition, the association between SNP-set and high intelligence was assessed by calculating the ORs for SNP-set score quartiles with the highest quartile as reference, and the trend test was performed with the quartiles of SNP-set score as an ordinal variable. We also tested the gene-environment interactions between SNP-set and fluoride exposures on high intelligence by calculating the association between fluoride exposures and the probability of high intelligence stratified by the median of SNP-set score. Furthermore, to quantify the interaction effects, we included interaction terms in the multivariate logistic regression model, in which the SNP-set score and four fluoride indexes were set as binary variables according to the corresponding median levels or permissible limits (only for water fluoride).

We also investigated the associations of intelligence with genetic variations at the gene or pathway level, using the Adaptive Rank Truncated Product (ARTP) method that combines association signals from the SNPs in a given gene or from the genes in a special pathway to provide a *P* value at the gene or pathway level, respectively (Broc et al., 2018). The ARTP method was further utilized to detect the interactions between genetic variations at the gene or pathway level and fluoride exposures, stratified by sex. The relationships among SNPs, genes and

pathways are shown in Table S4. All *P* values of the associations or interactions with fluoride exposures at gene or pathway level were corrected for multiple testing by false discovery rate method (FDR) (Huang et al., 2018), and adjusted for age, sex, maternal and paternal education.

Epidata (version 3.0, Epidata Association, Odense, Denmark) was used for database construction. Statistical analyses were performed with SAS software package (version 9.4, SAS Institute Inc., Cary, NC, USA), and Empower (R) (www.empowerstats.com, X&Y solutions, inc. Boston MA) and R (version 3.5.3, http://www.R-project.org). The LASSO Binomial regression and ARTP method were performed using R package glmnet (version 2.0–18) and PIGE (version 1.1), respectively. All *P* values were two-sided with a significance level of less than 0.05.

## 3. Results

### 3.1. Characteristics of the participants

Out of 1020 participants, 68 individuals were excluded due to lower genotyping detection rate (<95%), leaving 952 subjects available for subsequent analysis. Based on the intelligence level, the subjects were divided into the high intelligence group (IQ ≥ 120) and the non-high intelligence group (70 ≤ IQ < 120). The mean IQ level in the non-high intelligence group was lower compared to the high intelligence group (105.47 ± 9.57 vs 126.34 ± 5.60). Except for maternal and paternal education levels, the distributions of age, sex, body mass index, family incomes, history of cerebral trauma, maternal exposures (smoking, drinking, passive smoking and anemia) during pregnancy, and delivery condition (hypoxia, dystocia, premature birth and post-term birth) were comparable between the two groups (Table 1). Besides, the comparisons of the characteristics between the included and excluded participants from the 3020 eligible children were conducted. Most characteristics were comparable between the included and excluded children, including age, sex, body mass index, intelligence quotient scores, family incomes, maternal and paternal education, maternal exposures during pregnancy, delivery conditions, history of cerebral trauma and water fluoride exposure (Table S5).

The mean levels of fluoride concentrations in drinking water, urine, hair and nail in the high intelligence group were 0.70 mg/L, 0.33 mg/L, 8.26 μg/g and 11.71 μg/g, respectively, compared with 1.00 mg/L, 0.60 mg/L, 14.39 μg/g and 19.76 μg/g in the non-high intelligence group (Table 1). As shown in Fig. 2, the hair fluoride and nail fluoride were positively correlated, with a spearman's correlation coefficient ($r_s$) of 0.77, and they both were correlated with urinary fluoride ($r_s$ = 0.52, 0.33, respectively). Hair fluoride, nail fluoride and urinary fluoride were all positively related to water fluoride ($r_s$ = 0.45, 0.33, 0.71, respectively) (all $P < 0.001$).

**Table 1**
The characteristics of participants by intelligence levels.

| Variables | Intelligence | | *P*[d] |
|---|---|---|---|
| | High (IQ ≥ 120) | Non-high (70 ≤ IQ <120) | |
| Participants, No. | 173 | 779 | |
| Age[a] (years) | 9.8 ± 1.1 | 9.9 ± 1.1 | 0.707 |
| Sex[b] | | | 0.663 |
| Male | 83 (48.0%) | 388 (49.8%) | |
| Female | 90 (52.0%) | 391 (50.2%) | |
| Height (cm)[a] | 142.5 ± 8.9 | 142.5 ± 8.9 | 0.976 |
| Weight (kg)[a] | 35.4 ± 9.6 | 36.7 ± 10.6 | 0.143 |
| Body mass index (kg/m$^2$)[a] | 17.73 ± 3.46 | 17.71 ± 3.64 | 0.496 |
| Intelligence quotient score[a] | 126.34 ± 5.60 | 105.47 ± 9.57 | <0.001 |
| Family income (RMB/year)[b] | | | 0.538 |
| <10,000 | 9 (5.2%) | 58 (7.5%) | |
| 10,000–30,000 | 78 (45.1%) | 355 (45.5%) | |
| >30,000 | 86 (49.7%) | 366 (47.0%) | |
| Maternal education[b] | | | 0.053 |
| Middle school or lower | 19 (11.0%) | 132 (16.9%) | |
| High school | 126 (72.8%) | 541 (69.5%) | |
| Junior college or above | 28 (16.2%) | 106 (13.6%) | |
| Paternal education[b] | | | 0.046 |
| Middle school or lower | 9 (5.2%) | 90 (11.5%) | |
| High school | 135 (78.0%) | 570 (73.2%) | |
| Junior college or above | 29 (16.8%) | 119 (15.3%) | |
| Maternal exposure during pregnancy[b] | | | |
| Smoking | 1 (0.6%) | 5 (0.6%) | 0.999 |
| Drinking | 0 (0.0%) | 3 (0.4%) | / |
| Passive smoking | 14 (8.1%) | 67 (8.6%) | 0.828 |
| Anemia | 4 (2.4%) | 29 (3.8%) | 0.375 |
| Delivery conditions[b] | | | |
| Hypoxia | 5 (2.9%) | 14 (1.8%) | 0.352 |
| Dystocia | 6 (3.5%) | 21 (2.7%) | 0.580 |
| Premature birth | 5 (2.9%) | 25 (3.2%) | 0.828 |
| Post-term birth | 3 (1.7%) | 33 (4.2%) | 0.119 |
| History of cerebral trauma[b] | 1 (0.6%) | 4 (0.5%) | 0.999 |
| Water fluoride (mg/L)[c] | 0.70 (0.40–1.00) | 1.00 (0.50–1.90) | <0.001 |
| Urinary fluoride (mg/L)[c] | 0.33 (0.13–0.81) | 0.60 (0.16–2.22) | <0.001 |
| Hair fluoride (μg/g)[c] | 8.26 (5.72–10.48) | 14.39 (10.25–20.56) | <0.001 |
| Nail fluoride (μg/g)[c] | 11.71 (8.53–14.64) | 19.76 (14.16–27.32) | <0.001 |

Abbreviation: IQ, intelligence quotient, RMB, renminbi, the official currency of the People's Republic of China, and its basic unit is yuan.

[a] Data were presented as mean ± SD for continuous variables.

[b] Number (percentage/proportion) for categorical variables.

[c] Data were presented as median ($P_{25}$-$P_{75}$) for continuous variables.

[d] Student's *t*-test or Wilcoxon test was applied to compare the difference of continuous variables, and Chi-square test or Fisher exact test was used to compare the difference of categorical variables.

### 3.2. Association between fluoride exposure and intelligence

Higher fluoride concentrations in drinking water, urine, hair and nail were associated with lower possibility of high intelligence (all $P_{trend}$ < 0.001 across tertiles) (Table 2). Specifically, the adjusted ORs were 0.39 (95% CI: 0.25, 0.61) for children exposed to >1.40 mg/L water fluoride compared to those exposed to ≤0.60 mg/L water fluoride, and 0.41 (95% CI: 0.26, 0.66) for children exposed to >1.80 mg/L urinary fluoride compared to those exposed to ≤0.22 mg/L urinary fluoride. For hair fluoride, the adjusted ORs were 0.16 (95% CI: 0.09, 0.29) and 0.08 (95% CI: 0.04, 0.16) when exposed to 10.41–17.02 μg/g and >17.02 μg/g, respectively, compared with ≤10.40 μg/g. Similarly, the adjusted ORs were 0.15 (95% CI: 0.08, 0.29) and 0.09 (95% CI: 0.04, 0.19) when exposed to 14.65–23.41 μg/g and >23.41 μg/g nail fluoride, respectively, compared with ≤14.64 μg/g (tertile 1).

The dose-response relationships between fluoride exposures and intelligence were non-linear (Fig. 3). Piecewise linear results showed that IQ decreased by 4.21 for every 0.50 mg/L increase in water fluoride when exceeding 3.40 mg/L. IQ decreased by 5.23 for every 0.50 mg/L increase in urinary fluoride from 1.60 mg/L, and reached stabilized up to 2.50 mg/L. For every 1.00 μg/g increase in hair and nail fluoride, IQ decreased by 2.34 and 1.10, and tended to be stable at 10.50 μg/g and 14.50 μg/g, respectively (Fig. 3, Table S6).

### 3.3. Association of SNP-set - fluoride interaction with high intelligence

Out of 53 SNPs, rs3788319, rs1879417, rs57377675, rs11556505 and rs7187776 were associated with intelligence (Table S7), and were main contributors to the SNP-set (Fig. 4, Table 3). The performance was not improved significantly when there were more than five SNPs included in the model. The SNP-set score was inversely associated with high intelligence, with a *P*-trend of 0.001 (Table S8). Stratification analysis based on the median of SNP-set score showed different effects of

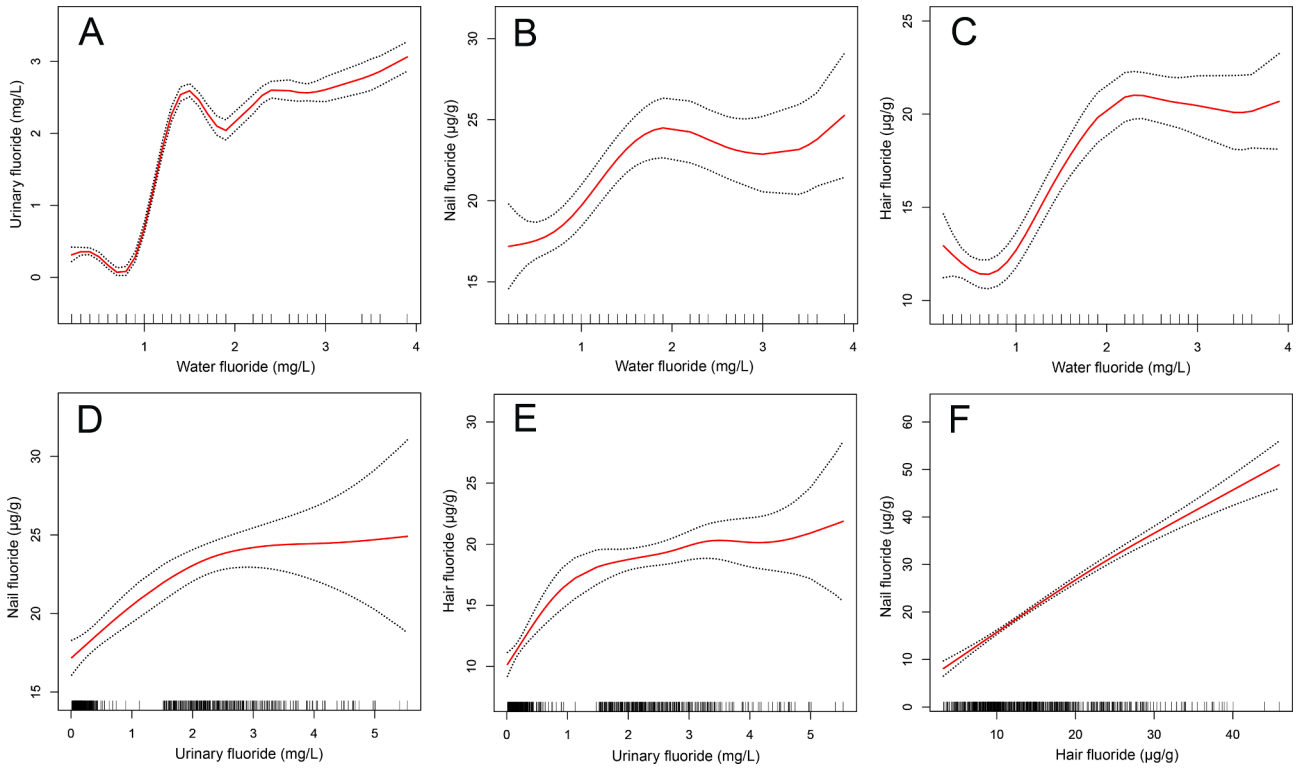


**Fig. 2.** Relationships among fluoride concentrations in different exposure indicators of (A) urinary fluoride and water fluoride (B) nail fluoride and water fluoride (C) hair fluoride and water fluoride (D) nail fluoride and urinary fluoride (E) hair fluoride and urinary fluoride (F) nail fluoride and hair fluoride. The adjusted factors were age, sex, maternal education and paternal education. The solid line and the dashed line represent the estimated values and their corresponding 95% confidence intervals.

**Table 2**
Relationships between fluoride exposure and high intelligence.

| Fluoride exposure | Crude OR (95% CI) | Adjusted OR (95% CI)[a] | *P*-trend[b] |
|---|---|---|---|
| Water fluoride (mg/L) | | | <0.001 |
| Tertile 1 (≤0.60) | Reference | Reference | |
| Tertile 2 (0.61–1.40) | 0.95 (0.65, 1.38) | 0.94 (0.64, 1.37) | |
| Tertile 3 (>1.40) | 0.38 (0.24, 0.59) | 0.39 (0.25, 0.61) | |
| Urinary fluoride (mg/L) | | | <0.001 |
| Tertile 1 (≤0.22) | Reference | Reference | |
| Tertile 2 (0.23–1.80) | 1.26 (0.87, 1.83) | 1.26 (0.87, 1.84) | |
| Tertile 3 (>1.80) | 0.41 (0.26, 0.65) | 0.41 (0.26, 0.66) | |
| Hair fluoride (μg/g) | | | <0.001 |
| Tertile 1 (≤10.40) | Reference | Reference | |
| Tertile 2 (10.41–17.02) | 0.16 (0.10, 0.29) | 0.16 (0.09, 0.29) | |
| Tertile 3 (>17.02) | 0.08 (0.04, 0.16) | 0.08 (0.04, 0.16) | |
| Nail fluoride (μg/g) | | | <0.001 |
| Tertile 1 (≤14.64) | Reference | Reference | |
| Tertile 2 (14.65–23.41) | 0.15 (0.08, 0.29) | 0.15 (0.08, 0.29) | |
| Tertile 3 (>23.41) | 0.09 (0.04, 0.18) | 0.09 (0.04, 0.19) | |

Abbreviation: OR, odds ratio, the risk of intelligence loss; CI, confidence interval.

[a] Adjustment: age, sex, maternal education and paternal education.

[b] The *P*-value for trend with the fluoride contents as a categorical variable adjusted for covariates in footnote a.

fluoride exposures on high intelligence (Fig. 5). Based on the above findings, the participants were further dichotomized based on the median levels or permissible limits of fluoride exposures. As shown in Table S9, in the high score group, the possibility of developing high intelligence for children exposed to higher water fluoride (>1.00 mg/L), urine fluoride (>1.60 mg/L), hair fluoride (>14.00 μg/g) and nail fluoride (>19.60 μg/g) decreased by 67% (OR = 0.33, 95% CI: 0.20, 0.55), 63% (OR = 0.37, 95% CI: 0.22, 0.62), 83% (OR = 0.17, 95% CI: 0.08, 0.34) and 87% (OR = 0.13, 95% CI: 0.06, 0.31), respectively, compared to those exposed to lower levels of fluoride. Correspondingly, in the low score group, the adjusted ORs for higher exposures of fluoride in drinking water, urine, hair and nail were 0.27 (95% CI: 0.14, 0.54), 0.32 (95% CI: 0.16, 0.63), 0.12 (95% CI: 0.04, 0.35) and 0.12 (95% CI: 0.04, 0.37), respectively. Specially, SNP-sets were interacted with water fluoride ($P = 0.03$), urinary fluoride ($P = 0.04$) and hair fluoride ($P = 0.01$) on intelligence, respectively.

Further analyses indicated that the components of the SNP-set generally increased the probability of normal intelligence ($90 \leq IQ < 120$) and low intelligence ($70 \leq IQ < 90$) when taking the high intelligence ($IQ \geq 120$) as control group. The wide range of 95% CIs indicated insufficient statistical power in low intelligence group (Table S10). Heterogeneity across the groups was assessed by $I^2$ statistic (ranging from 0 to 100%) with a small value indicating less heterogeneity. The heterogeneity was generally null or low in the between-group differences, as $I^2$ for heterogeneity between groups were 0%, 0%, 0%, 0% and 30%, respectively. Hence, it might be suitable to dichotomize intelligence into high ($IQ \geq 120$) and non-high ($70 \leq IQ < 120$) groups for the main analysis.

### *3.4. Association between gene/pathway- fluoride interaction and high intelligence*

As shown in Table 4, no single gene was associated with high intelligence after FDR correction. An interaction between *CLU* and hair fluoride was found in females (FDR corrected $P = 0.017$). Besides, *TOMM40* was interacted with hair fluoride for the intelligence level (FDR correlated $P = 0.051$), especially in females (FDR correlated $P = 0.017$). In pathway level, Alzheimer disease pathway presented

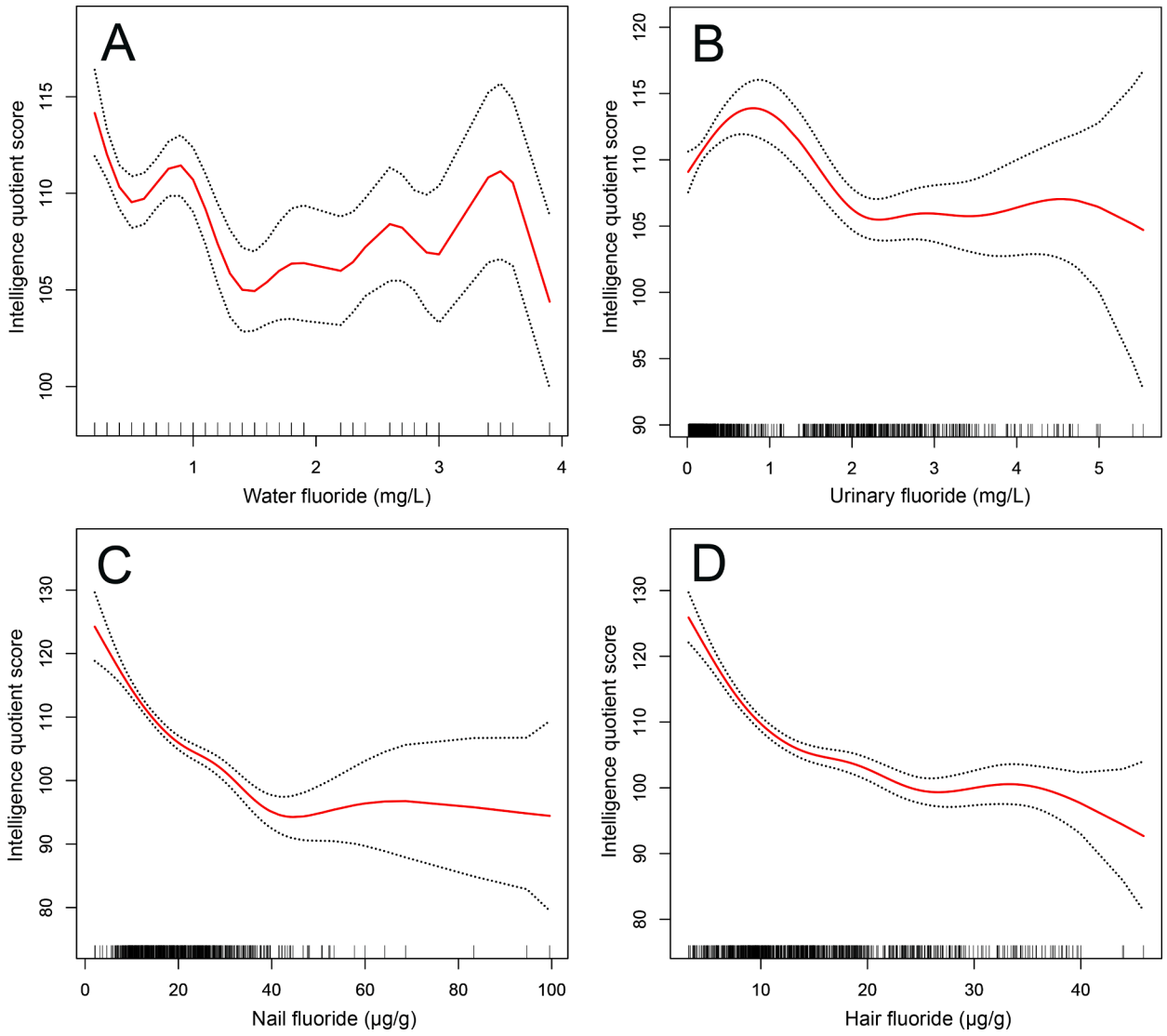


**Fig. 3.** Does-response relationships of the intelligence quotient scores with the fluoride in drinking water (A), urine (B), nail (C), and hair (D). The adjusted factors were age, sex, maternal education and paternal education. The solid line and the dashed line represent the estimated values and their corresponding 95% confidence intervals.

interaction with water fluoride (FDR corrected $P = 0.049$) on intelligence in males. Besides, metabolic pathway, signal transduction pathway, sphingolipid signaling pathway and PI3K-AKT signaling pathway presented suggestive interactions with water fluoride and urinary fluoride on intelligence in males (all FDR correlated $P = 0.074$) (Table 5).

## 4. Discussion

In this population-based study, we explored gene-fluoride interactions on intelligence systematically and comprehensively. Fluoride concentrations in drinking water, urine, hair and nail were found inversely associated with intelligence scores and the probability of high intelligence. Furthermore, we found the interactions of fluoride with a SNP-set based on 5 SNPs (rs3788319, rs1879417, rs57377675, rs11556505 and rs7187776) involved in neurodevelopment and mitochondrial biological process on intelligence levels. Besides, the current study also revealed interactions of fluoride with *CLU* and *TOMM40* on intelligence in females, and that with Alzheimer disease pathway, metabolic pathway, signal transduction pathway, sphingolipid signaling pathway and PI3K-AKT signaling pathway in males. The interactions of fluoride with the SNP panel, genes and the neurodevelopmental pathway brought new sights to potential mechanisms of fluoride-induced neurotoxicity.

Fluoride is able to cross the blood-brain barrier. Excessive accumulation of fluoride in the brain contributes to neurological damage. Population-based studies suggested adverse effects of high fluoride exposure on children's neurodevelopment, including intelligence loss and cognitive decline (Till et al., 2020). Similarly, animal evidence indicated that excessive exposure to fluoride might induce deficits in attention, memory and cognition (Zhu et al., 2017). Although numerous studies have uncovered the harmful effects of fluoride on intelligence, the exposure estimation was mainly based on water or urine samples. As relatively long-term internal exposure indicators, fluoride contents in hair and nail are crucial to reflect the chronic exposure levels (Rango et al., 2017). In our study, hair and nail fluoride were both positively correlated with water and urinary fluoride. Besides, increased fluoride contents in hair and nail were both associated with reduced IQ scores in non-linear model and lower possibility of developing high intelligence, which were consistent with the findings from water and urinary fluoride. Based on multiple fluoride exposures, our study suggested the potential intellectual damage caused by excessive fluoride exposure.

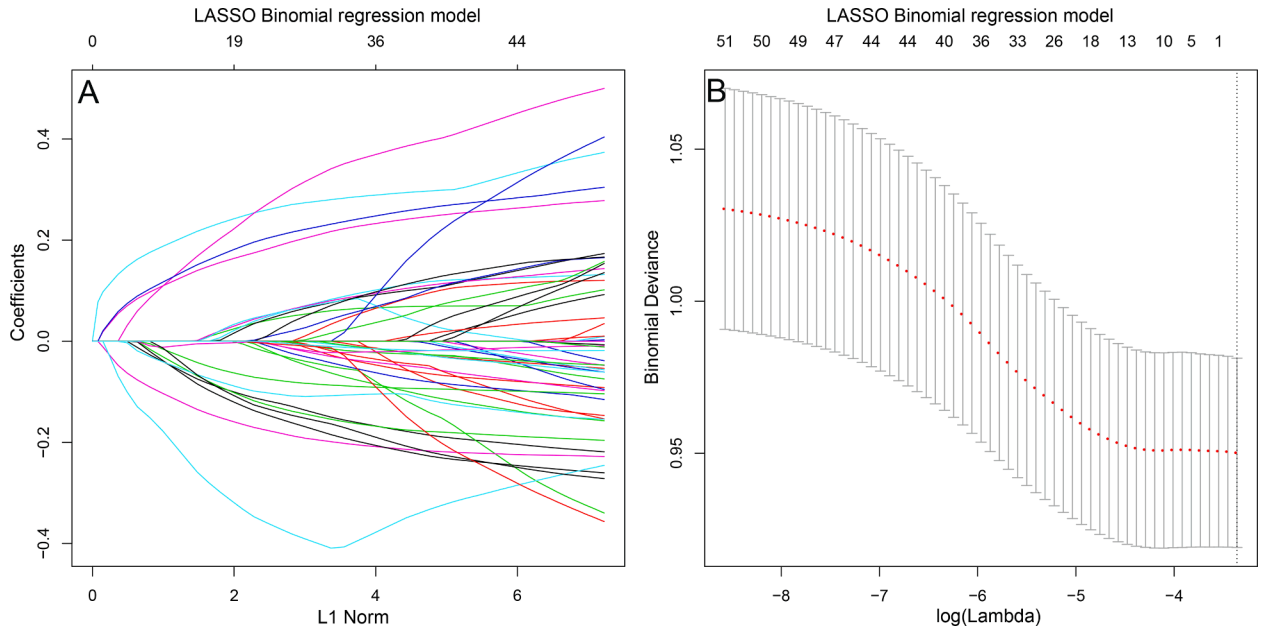


**Fig. 4.** Changes of parameters in LASSO Binomial regression model for SNP-set selection. (A) The trajectory of each independent coefficient changes with L1 Norm (the sum of the absolute values of non-zero coefficients in the model). Each curve with different colors represents a SNP. The upper abscissa represents the number of non-zero coefficients in LASSO Binomial regression model. The number of non-zero coefficients decreases along with the decline of the L1 Norm, indicating the reduced number of variables. (B) The variation of binomial deviance with log (Lambda) value. The adjusted factors were age, sex, maternal education and paternal education.

**Table 3**
Components of the SNP-set and their relationships with high intelligence.

| SNP | $\beta_{LASSO}$[a] | Weight[b,c] | OR (95% CI)[c] | $P$[c] |
|---|---|---|---|---|
| rs3788319 | 0.1321831 | −0.281 | 0.76 (0.60, 0.96) | 0.019 |
| rs1879417 | −0.04731035 | −0.273 | 0.76 (0.60, 0.97) | 0.029 |
| rs57377675 | 0.05476791 | −0.241 | 0.79 (0.61, 1.01) | 0.060 |
| rs11556505 | −0.06857724 | −0.518 | 0.60 (0.36, 0.99) | 0.043 |
| rs7187776 | 0.05844264 | −0.257 | 0.77 (0.60, 1.00) | 0.051 |

Abbreviation: OR, odds ratio, the risk of intelligence loss, CI, confidence interval.

[a] Regression coefficient for LASSO binomial regression.

[b] Regression coefficient for multivariable logistic regression.

[c] Adjustment: age, sex, maternal education and paternal education.

Genetic background has been reported to play important roles in fluoride-induced adverse health effects. Polymorphisms in *COL1A2* and *CTR* were found to alter the risk of dental fluorosis (Huang et al., 2008; Jiang et al., 2015), and genetic variants in *MMP-2* and *VDR* were significantly correlated with the severity of skeletal fluorosis (Pei et al., 2017; Yang et al., 2016). However, these studies predominantly focused on dental and skeletal fluorosis, neurotoxicity related to fluorine was rarely studied. Besides, previous studies mainly focused on single SNP analysis. However, this approach is not powerful enough to illustrate the association of candidate genes with fluorosis, especially with intelligence, which is a polygenic complex trait. A panel of SNP (SNP-set) is an effective strategy to assess the combined effects of genetic polymorphisms weakly associated with outcomes or may not be detected in single-SNP analyses, which has been widely applied in the fields of cancer and metabolic disease research (Shieh et al., 2019). To address this issue, we performed LASSO regression to develop a SNP-set associated with intelligence. Out of the 53 SNPs in our research, five (rs3788319, rs1879417, rs11556505, rs1879417 and rs11556505) were selected, and significant association was found between the SNP-set score and high intelligence loss. Moreover, the SNP-set score presented interactions with various fluoride exposure indicators on high intelligence, although we could only roughly quantify the interaction effects stratified by the median levels or permissible limits of four fluoride indexes and the median of the SNP-set score due to the limitation of sample size. These SNPs have been reported to be associated with mental and cognitive disorders possibly by regulating genes and proteins involved in neural development (Davies et al., 2014; Ferrari et al., 2017; Wigner et al., 2018). To our best knowledge, this is the first study to explore gene-fluoride interactions on intelligence at SNP-set level, which brings new insights into interpreting the potential mechanisms underlying susceptibility to fluoride-induced neurotoxicity. It is therefore meaningful if our findings could be validated or similar studies could be conducted across different populations in the future.

Recently, several studies have revealed the potential roles of genetic factors in the neurotoxicity caused by excessive fluoride *in vivo* and *in vitro*. For rats, *COX1*, *COX2* and ERK/CREB signaling pathway may affect the impacts of fluoride on brain features and the memory ability by altering gene and protein expression (Dec et al., 2019), while *NCAM* and *NF-kappaB* were found related to fluoride-induced apoptosis of hippocampal neurons, partially through DNA damage and oxidative stress (Zhang et al., 2007; Zhang et al., 2008). However, to date, limited population-based studies have been performed to uncover the genetic association with fluoride exposure on intelligence. One study reported that *DRD2* altered the relationship between fluoride exposure and IQ (Cui et al., 2018), and another study (Zhang et al., 2015) suggested that *COMT* might modify the susceptibility to the intelligence loss due to fluoride exposure. In our study, ARTP was performed to evaluate the gene-fluoride interactions on intelligence at gene level, by combining SNPs in a given gene. The results showed that *CLU* and *TOMM40* interacted with hair fluoride on intelligence, especially in females. According to the previous reports, these two genes were mainly involved in mitochondrial dynamics (Herring et al., 2019; Puertas-Frias et al., 2019), and may affect the nervous system by altering mitochondrial function. The stronger interactions in females imply that the specific gender may be more vulnerable to fluoride neurotoxicity (Green et al., 2019). Since the limited mechanism studies of fluoride-induced neurotoxicity and most animal studies have focused on fluoride exposure in

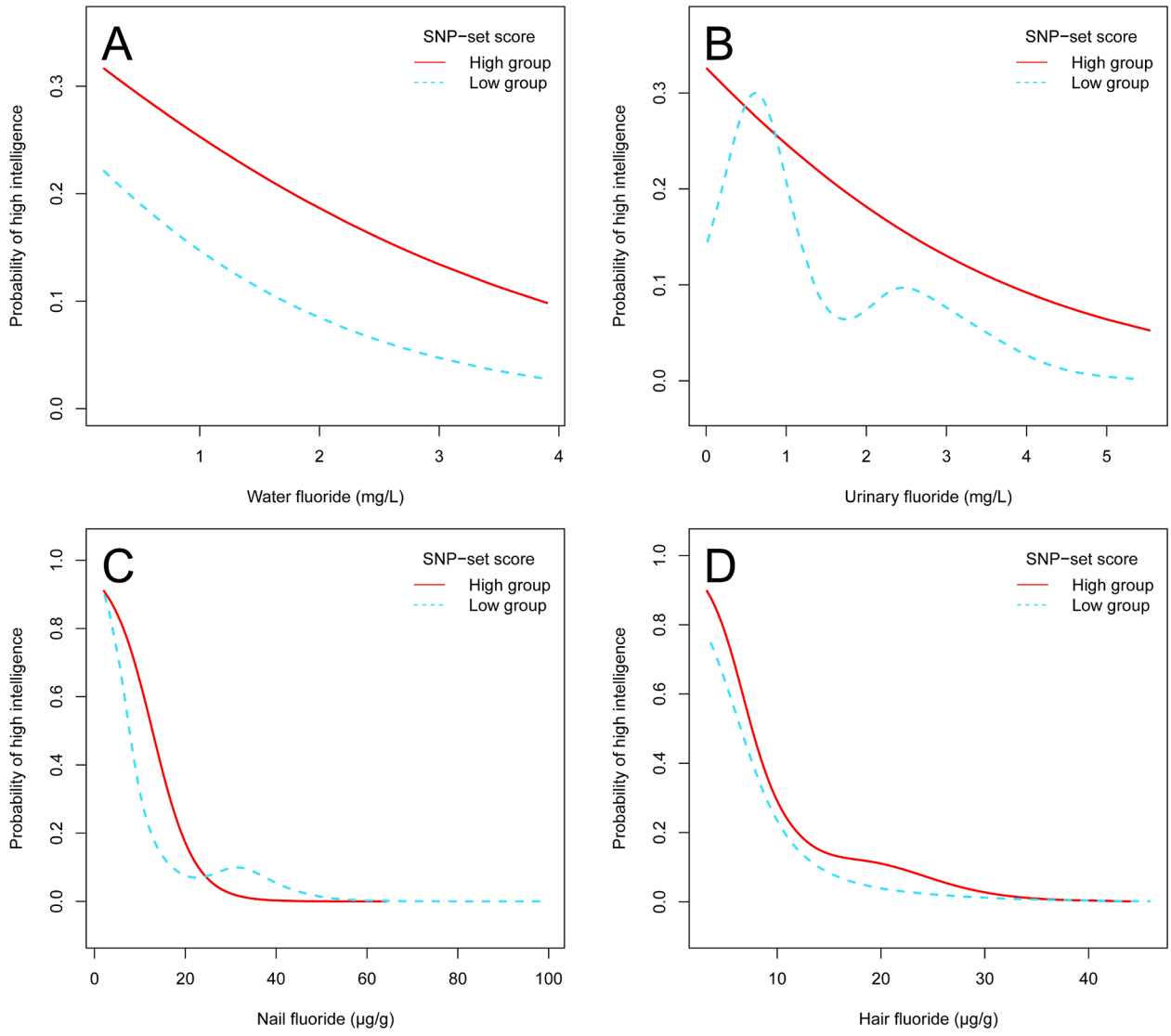


**Fig. 5.** Relationships of the probability of high intelligence with different fluoride exposures of (A) water fluoride (B) urinary fluoride (C) nail fluoride (D) hair fluoride stratified by the median of SNP-set score.

male rats (McPherson et al., 2018), the exact explanations need to be explored. Taken together, our findings suggested the potential role of mitochondrial function-related genes in the development of neurotoxicity induced by fluoride, with gender difference. Considering the genetically diverse nature of populations worldwide, these interactions are required to be identified across different populations and areas in the future.

Pathway analysis provides the opportunity to combine association evidence from multiple genetic variants, thus potentially better identifying the relationships between pathways and health outcomes. In the current study, we found that Alzheimer disease pathway, metabolic pathway, signal transduction pathway, sphingolipid signaling pathway and PI3K-AKT signaling pathway interacted with fluoride in male children. Although we found interactions between hair fluoride with *CLU* and *TOMM40*, which were not enriched in any of the seven biological pathways in our study, no interaction between fluoride exposure with *TOMM40*-related pathway or *CLU*-related pathway was observed. Based on the published researches, these five pathways play important roles in neurogenesis, neuron growth, differentiation and survival (He et al., 2018), energy metabolism (Cheon et al., 2019), oxidative stress and signal transduction (Lo Vasco, 2018). These neurodevelopmental physiological processes are closely related to mitochondria (Iannielli et al., 2019), and their dysfunction may contribute to the risk of cognitive disability, neurodevelopment disorders and degenerative diseases. Considering the synergistic effects of genes, it might be reasonable to observe the interaction of fluoride exposure with the above-mentioned pathways but not single genes in these pathways. However, due to the limited research till now, further studies focusing on both male and female animals with different exposure levels and periods of fluoride are still required to deeply explore the gene-fluoride interaction on intelligence, by collecting various biological samples and detecting the expression levels of key genes and proteins. Besides, the current findings need to be verified *in vitro* to illustrate the biological mechanisms of fluoride-induced neurotoxicity, especially at SNP-set, gene and pathway levels. In general, our findings revealed the pathways which might be involved in fluoride-induced neurotoxicity, and provided new insights for future studies to clarify the mechanisms that influence fluorosis by gene pathways on intelligence development. Notably, this is the first study to explore the neurotoxicity of fluoride at pathway level.

Our study has several strengths. Using four fluoride exposure indicators including water fluoride, urinary fluoride, hair fluoride and nail fluoride, which reflect the external and internal, and short-term and long-term exposures, makes the evaluation of fluoride exposures more comprehensive and reliable. Besides, due to the relatively rare studies on

**Table 4**
Association of intelligence with individual gene, and their interactions with fluoride exposure on high intelligence among all children and stratified by sex, by top 4 genes.

| Gene | All children | | Male | | Female | |
|---|---|---|---|---|---|---|
| | $P^{a}$ | $P_{FDR}{}^{a}$ | $P^{b}$ | $P_{FDR}{}^{b,c}$ | $P^{b}$ | $P_{FDR}{}^{b,c}$ |
| **Individual gene** | | | | | | |
| *COMT* | 0.049 | 0.496 | 0.023 | 0.317 | 0.560 | 0.949 |
| *SLC25A12* | 0.065 | 0.496 | 0.457 | 0.670 | 0.039 | 0.663 |
| *TH* | 0.162 | 0.496 | 0.056 | 0.317 | 0.940 | 0.949 |
| *TUFM* | 0.122 | 0.496 | 0.049 | 0.317 | 0.627 | 0.949 |
| **Interaction** | | | | | | |
| Water fluoride | | | | | | |
| *BDNF* | 0.268 | 0.523 | 0.012 | 0.102 | 0.355 | 0.802 |
| *GSK3B* | 0.141 | 0.523 | 0.936 | 0.936 | 0.044 | 0.459 |
| *NOS3* | 0.229 | 0.523 | 0.006 | 0.102 | 0.425 | 0.802 |
| *SLC25A12* | 0.656 | 0.797 | 0.620 | 0.730 | 0.070 | 0.459 |
| Urinary fluoride | | | | | | |
| *BCL2L10* | 0.035 | 0.595 | 0.217 | 0.187 | 0.114 | 0.638 |
| *GSK3B* | 0.135 | 0.595 | 0.722 | 0.925 | 0.061 | 0.519 |
| *NOS3* | 0.140 | 0.595 | 0.011 | 0.187 | 0.940 | 0.955 |
| *TH* | 0.258 | 0.690 | 0.029 | 0.247 | 0.737 | 0.895 |
| Hair fluoride | | | | | | |
| *CLU* | 0.153 | 0.650 | 0.862 | 0.997 | 0.001 | 0.017 |
| *FOXO3* | 0.552 | 0.845 | 0.010 | 0.170 | 0.184 | 0.626 |
| *FYN* | 0.077 | 0.650 | 0.589 | 0.902 | 0.075 | 0.425 |
| *TOMM40* | 0.003 | 0.051 | 0.233 | 0.899 | 0.002 | 0.017 |
| Nail fluoride | | | | | | |
| *CLU* | 0.329 | 0.917 | 0.273 | 0.597 | 0.048 | 0.408 |
| *FYN* | 0.048 | 0.845 | 0.656 | 0.777 | 0.007 | 0.119 |
| *GSK3B* | 0.246 | 0.845 | 0.009 | 0.153 | 0.960 | 0.987 |
| *TOMM40* | 0.343 | 0.816 | 0.540 | 0.765 | 0.078 | 0.442 |

[a] Adjustment: age, sex, maternal education and paternal education.

[b] Adjustment: age, maternal education and paternal education.

[c] The *P*-value was adjusted using false discovery rate method for multiple testing.

**Table 5**
Association of intelligence with pathways, and their interactions with fluoride exposure on high intelligence among all children and stratified by sex.

| Pathway | All children | | Male | | Female | |
|---|---|---|---|---|---|---|
| | $P^{a}$ | $P_{FDR}{}^{a}$ | $P^{b}$ | $P_{FDR}{}^{b,c}$ | $P^{b}$ | $P_{FDR}{}^{b,c}$ |
| **Individual pathway** | | | | | | |
| Dopaminergic synapse | 0.118 | 0.434 | 0.044 | 0.249 | 0.935 | 0.994 |
| Alzheimer disease | 0.618 | 0.802 | 0.592 | 0.753 | 0.658 | 0.994 |
| Neurotrophin | 0.924 | 0.924 | 0.747 | 0.753 | 0.994 | 0.994 |
| Metabolic | 0.124 | 0.434 | 0.071 | 0.249 | 0.355 | 0.994 |
| Signal transduction | 0.687 | 0.802 | 0.709 | 0.753 | 0.737 | 0.994 |
| Sphingolipid | 0.517 | 0.802 | 0.597 | 0.753 | 0.510 | 0.994 |
| PI3K-AKT | 0.599 | 0.802 | 0.753 | 0.753 | 0.642 | 0.994 |
| **Interaction** | | | | | | |
| Water fluoride | | | | | | |
| Dopaminergic synapse | 0.280 | 0.362 | 0.494 | 0.494 | 0.182 | 0.501 |
| Alzheimer disease | 0.028 | 0.196 | 0.007 | 0.049 | 0.262 | 0.501 |
| Neurotrophin | 0.269 | 0.362 | 0.259 | 0.302 | 0.197 | 0.501 |
| Metabolic | 0.517 | 0.517 | 0.032 | 0.074 | 0.700 | 0.700 |
| Signal transduction | 0.278 | 0.362 | 0.053 | 0.074 | 0.358 | 0.501 |
| Sphingolipid | 0.310 | 0.362 | 0.036 | 0.074 | 0.616 | 0.700 |
| PI3K-AKT | 0.204 | 0.362 | 0.051 | 0.074 | 0.295 | 0.501 |
| Urinary fluoride | | | | | | |
| Dopaminergic synapse | 0.190 | 0.286 | 0.520 | 0.520 | 0.138 | 0.402 |
| Alzheimer disease | 0.044 | 0.286 | 0.024 | 0.074 | 0.243 | 0.402 |
| Neurotrophin | 0.183 | 0.286 | 0.197 | 0.230 | 0.154 | 0.402 |
| Metabolic | 0.485 | 0.485 | 0.051 | 0.074 | 0.850 | 0.921 |
| Signal transduction | 0.204 | 0.286 | 0.030 | 0.074 | 0.287 | 0.402 |
| Sphingolipid | 0.305 | 0.356 | 0.041 | 0.074 | 0.921 | 0.921 |
| PI3K-AKT | 0.139 | 0.286 | 0.053 | 0.074 | 0.242 | 0.402 |
| Hair fluoride | | | | | | |
| Dopaminergic synapse | 0.992 | 0.992 | 0.980 | 0.980 | 0.969 | 0.988 |
| Alzheimer disease | 0.960 | 0.992 | 0.926 | 0.980 | 0.988 | 0.988 |
| Neurotrophin | 0.435 | 0.992 | 0.039 | 0.217 | 0.075 | 0.264 |
| Metabolic | 0.897 | 0.992 | 0.647 | 0.906 | 0.670 | 0.938 |
| Signal transduction | 0.311 | 0.992 | 0.585 | 0.906 | 0.151 | 0.264 |
| Sphingolipid | 0.261 | 0.992 | 0.433 | 0.906 | 0.089 | 0.264 |
| PI3K-AKT | 0.796 | 0.992 | 0.062 | 0.217 | 0.120 | 0.264 |
| Nail fluoride | | | | | | |
| Dopaminergic synapse | 0.130 | 0.624 | 0.049 | 0.676 | 0.160 | 0.373 |
| Alzheimer disease | 0.446 | 0.624 | 0.078 | 0.179 | 0.778 | 0.996 |
| Neurotrophin | 0.568 | 0.663 | 0.041 | 0.179 | 0.996 | 0.996 |
| Metabolic | 0.361 | 0.624 | 0.579 | 0.676 | 0.361 | 0.632 |
| Signal transduction | 0.274 | 0.624 | 0.102 | 0.179 | 0.053 | 0.186 |
| Sphingolipid | 0.185 | 0.624 | 0.997 | 0.997 | 0.019 | 0.133 |
| PI3K-AKT | 0.753 | 0.753 | 0.079 | 0.179 | 0.975 | 0.996 |

Abbreviation: Dopaminergic synapse, Dopaminergic synapse pathway; Alzheimer disease, Alzheimer disease pathway, Neurotrophin, Neurotrophin signaling pathway, Metabolic, Metabolic pathway, Signal transduction, Signal transduction pathway, Sphingolipid, Sphingolipid signaling pathway, PI3K-AKT, PI3K-AKT signaling pathway.

[a] Adjustment: age, sex, maternal education and paternal education.

[b] Adjustment: age, maternal education and paternal education.

[c] The *P*-value was adjusted using false discovery rate method for multiple testing.

low-to-moderate level fluoride exposure in hair and nail, our results also enrich the epidemiological evidence across different fluoride indicators and levels. Compared to previous studies which mainly focused on the effect of single SNP or gene on intelligence and its interaction with fluoride exposures, our study is the first one to explore the interactions between SNP-set and fluoride on intelligence loss. Besides, this is the first study that evaluated the gene-fluoride interactions at gene and pathway levels by using the ARTP method. Furthermore, selection bias in this study is relatively small given the comparable characteristics between the included and excluded children, along with 94.6% response rate in the multistage random sampling step.

Our study also has limitations. Due to the case-control design based on a cross-sectional study, our study has limited power in identifying the causal relationship between fluoride exposures and intelligence. However, based on the annual surveillance data from the local CDC, the fluoride contents kept stable in the study area, which makes the observed associations relatively reliable. But further studies with prospective design and repeated measures are still essential to validate our findings. Secondly, the components of the SNP-set may not be uniform across ethnicity, which limits its application in a standardized manner. However, it provides a novel approach to detect gene-environment interactions for fluorosis in other populations, and contributes primary data for future pooled analyses or multicenter studies. Due to the limited sample size, we could not draw a solid conclusion on the association between low intelligence and the five components of the SNP-set. Future studies with larger sample sizes are required to increase the statistical efficiency and precision. A limitation can't be ignored is that some environmental risk factors such as pesticides, which are frequently used in rural areas, may contribute to children's intelligence loss (Saeed et al., 2020). Although these environmental risk factors might not be largely influenced by the distribution of fluoride in nature, the co-exposures to fluoride with other environmental pollutants may also exist. Therefore, the effect of fluoride on intelligence might be modified by these environmental risk factors partly. Furthermore, other potential confounders such as breastfeeding and nutritional status for the observed associations between fluoride and intelligence were not available in our study. Inadequate adjustment for these potential confounders may affect the precision of the evaluated association. Further studies with more comprehensive assessments of environmental exposures are needed to minimize the bias caused by the potential confounders.

In summary, our study revealed inverse associations of fluoride exposures with intelligence. Furthermore, we found the interactions of fluoride with genetic variations involved in nervous system development and mitochondrial biological process at SNP-set, gene and

pathway level. The current findings suggest that mitochondrial function-related genes as *CLU* and *TOMM40*, and multiple neurodevelopmental biological pathways related to metabolism might be involved in the fluoride-induced neurotoxicity. Our study provides a novel insight into a better understanding of differential susceptibility of fluoride-induced neurotoxicity, which is meaningful for public prevention of fluorosis for children.

## CRediT authorship contribution statement

**Xingchen Yu:** Methodology, Software, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Lu Xia:** Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Shun Zhang:** Validation, Formal analysis, Investigation, Writing - review & editing, Project administration. **Guoyu Zhou:** Validation, Investigation, Data curation. **Yonggang Li:** Investigation, Resources. **Hongliang Liu:** Investigation, Resources. **Changchun Hou:** Investigation, Resources. **Qian Zhao:** Investigation. **Lixin Dong:** Investigation. **Yushan Cui:** Investigation. **Qiang Zeng:** Investigation. **Aiguo Wang:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Li Liu:** Conceptualization, Methodology, Investigation, Writing - review & editing, Project administration.

## 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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106681.

## References

Araujo, T.T., Barbosa Silva Pereira, H.A., Dionizio, A., Sanchez, C.D.C., de Souza Carvalho, T., da Silva Fernandes, M., Rabelo Buzalaf, M.A., 2019. Changes in energy metabolism induced by fluoride: Insights from inside the mitochondria. Chemosphere. 236, 124357. https://doi.org/10.1016/j.chemosphere.2019.124357.

Broc, C., Evangelou, M., Guenel, P., Truong, T., Liquet, B., 2018. Investigating Gene- and Pathway-environment Interaction analysis approaches. J. Sfds. 159, 56–83.

Chen, L., Chen, H., Yao, C., Chang, C., Xia, H., Zhang, C., Zhou, Y., Yao, Q., Chen, K., 2015. The toxicity of NaF on BmN cells and a comparative proteomics approach to identify protein expression changes in cells under NaF-stress: impact of NaF on BmN cells. J. Hazard. Mater. 286, 624–631. https://doi.org/10.1016/j.jhazmat.2014.12.056.

Chen, Y., Yan, L., Zhang, Y., Yang, X., 2019. The role of DRP1 in ropivacaine-induced mitochondrial dysfunction and neurotoxicity. Artif. Cells Nanomed. Biotechnol. 47, 1788–1796. https://doi.org/10.1080/21691401.2019.1594858.

Cheon, S., Kaur, K., Nijem, N., Tuncay, I.O., Kumar, P., Dean, M., Juusola, J., Guillen-Sacoto, M.J., Bedoukian, E., Ierardi-Curto, L., Kaplan, P., Schaefer, G.B., Mishra, P., Chahrour, M.H., 2019. The ubiquitin ligase UBE3B, disrupted in intellectual disability and absent speech, regulates metabolic pathways by targeting BCKDK. Proc. Natl. Acad. Sci. U. S. A. 116, 3662–3667. https://doi.org/10.1073/pnas.1818751116.

Cui, Y., Zhang, B., Ma, J., Wang, Y., Zhao, L., Hou, C., Yu, J., Zhao, Y., Zhang, Z., Nie, J., Gao, T., Zhou, G., Liu, H., 2018. Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: a school-based cross-sectional study. Ecotoxicol. Environ. Saf. 165, 270–277. https://doi.org/10.1016/j.ecoenv.2018.09.018.

Davies, G., Harris, S.E., Reynolds, C.A., Payton, A., Knight, H.M., Liewald, D.C., Lopez, L. M., Luciano, M., Gow, A.J., Corley, J., Henderson, R., Murray, C., Pattie, A., Fox, H. C., Redmond, P., Lutz, M.W., Chiba-Falek, O., Linnertz, C., Saith, S., Haggarty, P., McNeill, G., Ke, X., Ollier, W., Horan, M., Roses, A.D., Ponting, C.P., Porteous, D.J., Tenesa, A., Pickles, A., Starr, J.M., Whalley, L.J., Pedersen, N.L., Pendleton, N., Visscher, P.M., Deary, I.J., 2014. A genome-wide association study implicates the APOE locus in nonpathological cognitive ageing. Mol. Psychiatry. 19, 76–87. https://doi.org/10.1038/mp.2012.159.

Dec, K., Lukomska, A., Skonieczna-Zydecka, K., Kolasa-Wolosiuk, A., Tarnowski, M., Baranowska-Bosiacka, I., Gutowska, I., 2019. Long-term exposure to fluoride as a factor promoting changes in the expression and activity of cyclooxygenases (COX1 and COX2) in various rat brain structures. Neurotoxicology 74, 81–90. https://doi.org/10.1016/j.neuro.2019.06.001.

Devine, M.J., Kittler, J.T., 2018. Mitochondria at the neuronal presynapse in health and disease. Nat. Rev. Neurosci. 19, 63–80. https://doi.org/10.1038/nrn.2017.170.

Ding, Y., Gao, Y., Sun, H., Han, H., Wang, W., Ji, X., Liu, X., Sun, D., 2011. The relationships between low levels of urine fluoride on children's intelligence, dental fluorosis in endemic fluorosis areas in Hulunbuir, Inner Mongolia, China. J. Hazard. Mater. 186, 1942–1946. https://doi.org/10.1016/j.jhazmat.2010.12.097.

Ferrari, R., Wang, Y., Vandrovcova, J., Guelfi, S., Witeolar, A., Karch, C.M., Schork, A.J., Fan, C.C., Brewer, J.B., Momeni, P., Schellenberg, G.D., Dillon, W.P., Sugrue, L.P., Hess, C.P., Yokoyama, J.S., Bonham, L.W., Rabinovici, G.D., Miller, B.L., Andreassen, O.A., Dale, A.M., Hardy, J., Desikan, R.S., 2017. Genetic architecture of sporadic frontotemporal dementia and overlap with Alzheimer's and Parkinson's diseases. J. Neurol. Neurosurg. Psychiatry. 88, 152–164. https://doi.org/10.1136/jnnp-2016-314411.

Green, R., Lanphear, B., Hornung, R., Flora, D., Martinez-Mier, E.A., Neufeld, R., Ayotte, P., Muckle, G., Till, C., 2019. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. JAMA Pediatr. 173, 940–948. https://doi.org/10.1001/jamapediatrics.2019.1729.

Guth, S., Hüser, S., Roth, A., Degen, G., Diel, P., Edlund, K., Eisenbrand, G., Engel, K.H., Epe, B., Grune, T., Heinz, V., Henle, T., Humpf, H.U., Jäger, H., Joost, H.G., Kulling, S.E., Lampen, A., Mally, A., Marchan, R., Marko, D., Mühle, E., Nitsche, M. A., Röhrdanz, E., Stadler, R., van Thriel, C., Vieths, S., Vogel, R.F., Wascher, E., Watzl, C., Nöthlings, U., Hengstler, J.G., 2020. Toxicity of fluoride: critical evaluation of evidence for human developmental neurotoxicity in epidemiological studies, animal experiments and in vitro analyses. Arch. Toxicol. 94, 1375–1415. https://doi.org/10.1007/s00204-020-02725-2.

He, H., Liu, W., Zhou, Y., Liu, Y., Weng, P., Li, Y., Fu, H., 2018. Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway. Drug Des. Devel. Ther. 12, 629–638. https://doi.org/10.2147/dddt.s158313.

Herring, S.K., Moon, H.J., Rawal, P., Chhibber, A., Zhao, L., 2019. Brain clusterin protein isoforms and mitochondrial localization. Elife. 8, e48255. https://doi.org/10.7554/eLife.48255.

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. https://doi.org/10.1111/j.1600-0528.2007.00424.x.

Huang, Q.Q., Ritchie, S.C., Brozynska, M., Inouye, M., 2018. Power, false discovery rate and Winner's Curse in eQTL studies. Nucleic Acids Res. 46, e133. https://doi.org/10.1093/nar/gky780.

Iannielli, A., Ugolini, G.S., Cordiglieri, C., Bido, S., Rubio, A., Colasante, G., Valtorta, M., Cabassi, T., Rasponi, M., Broccoli, V., 2019. Reconstitution of the Human Nigro-striatal Pathway on-a-Chip Reveals OPA1-Dependent Mitochondrial Defects and Loss of Dopaminergic Synapses. Cell Rep. 29, 4646–4656.e4644. https://doi.org/10.1016/j.celrep.2019.11.111.

Jiang, M., Mu, L., Wang, Y., Yan, W., Jiao, Y., 2015. The relationship between Alu I polymorphisms in the calcitonin receptor gene and fluorosis endemic to Chongqing, China. Med. Princ. Pract. 24, 80–83. https://doi.org/10.1159/000368435.

Lei, Z.L., Yan, J., Zhang, S.B., Shen, H.M., Gao, Y.H., Sun, D.J., 2014. The national condition of control of priority endemic diseases and main tasks (in Chinese). Chinese J. Endemiol. 33, 475–478. https://doi.org/10.3760/cma.j.issn.2095-4255.2014.05.002.

Liang, C., Gao, Y., He, Y., Han, Y., Manthari, R.K., Tikka, C., Chen, C., Wang, J., Zhang, J., 2020. Fluoride induced mitochondrial impairment and PINK1-mediated mitophagy in Leydig cells of mice: In vivo and in vitro studies. Environ. Pollut. 256, 113438. https://doi.org/10.1016/j.envpol.2019.113438.

Lima Leite, A., Gualiume Vaz Madureira Lobo, J., Barbosa da Silva Pereira, H.A., Silva Fernandes, M., Martini, T., Zucki, F., Sumida, D.H., Rigalli, A., Buzalaf, M.A., 2014. Proteomic analysis of gastrocnemius muscle in rats with streptozotocin-induced diabetes and chronically exposed to fluoride. PLoS ONE. 9, e106646. https://doi.org/10.1371/journal.pone.0106646.

Liu, H.L., Lam, L.T., Zeng, Q., Han, S.Q., Fu, G., Hou, C.C., 2009. Effects of drinking water with high iodine concentration on the intelligence of children in Tianjin, China. J. Public Health (Oxf) 31, 32–38. https://doi.org/10.1093/pubmed/fdn097.

Lo Vasco, V.R., 2018. The Phosphoinositide Signal Transduction Pathway in the Pathogenesis of Alzheimer's Disease. Curr. Alzheimer Res. 15, 355–362. https://doi.org/10.2174/1567205014666170829100230.

McPherson, C.A., Zhang, G., Gilliam, R., Brar, S.S., Wilson, R., Brix, A., Picut, C., Harry, G.J., 2018. An Evaluation of Neurotoxicity Following Fluoride Exposure from Gestational Through Adult Ages in Long-Evans Hooded Rats. Neurotox. Res. 34, 781–798. https://doi.org/10.1007/s12640-018-9870-x.

Mumtaz, N., Pandey, G., Labhasetwar, P.K., 2015. Global Fluoride Occurrence, Available Technologies for Fluoride Removal, and Electrolytic Defluoridation: A Review. Crit. Rev. Environ. Sci. Technol. 45, 2357–2389. https://doi.org/10.1080/10643389.2015.1025638.

Pei, J., Li, B., Liu, Y., Liu, X., Li, M., Chu, Y., Yang, Q., Jiang, W., Chen, F., Darko, G.M., Yang, Y., Gao, Y., 2017. Matrix Metallopeptidase-2 Gene rs2287074 Polymorphism is Associated with Brick Tea Skeletal Fluorosis in Tibetans and Kazaks, China. Sci. Rep. 7, 40086. https://doi.org/10.1038/srep40086.

Pereira, M., Dombrowski, P.A., Losso, E.M., Chioca, L.R., Da Cunha, C., Andreatini, R., 2011. Memory impairment induced by sodium fluoride is associated with changes in brain monoamine levels. Neurotox. Res. 19, 55–62. https://doi.org/10.1007/s12640-009-9139-5.

Puertas-Frias, G., Del Arco, A., Pardo, B., Satrustegui, J., Contreras, L., 2019. Mitochondrial movement in Aralar/Slc25a12/AGC1 deficient cortical neurons. Neurochem. Int. 131, 104541. https://doi.org/10.1016/j.neuint.2019.104541.

Rango, T., Vengosh, A., Jeuland, M., Whitford, G.M., Tekle-Haimanot, R., 2017. Biomarkers of chronic fluoride exposure in groundwater in a highly exposed population. Sci. Total Environ. 596–597, 1–11. https://doi.org/10.1016/j.scitotenv.2017.04.021.

Saeed, M., Malik, R.N., Kamal, A., 2020. Fluorosis and cognitive development among children (6–14 years of age) in the endemic areas of the world: a review and critical analysis. Environ. Sci. Pollut. Res. Int. 27, 2566–2579. https://doi.org/10.1007/s11356-019-06938-6.

Shan, K.R., Qi, X.L., Long, Y.G., Nordberg, A., Guan, Z.Z., 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity–a mechanism relating to a damage at the level in post-transcription of the receptor genes. Toxicology 200, 169–177. https://doi.org/10.1016/j.tox.2004.03.013.

Shieh, Y., Fejerman, L., Lott, P.C., Marker, K., Sawyer, S.D., Hu, D., Huntsman, S., Torres, J., Echeverry, M., Bohorquez, M.E., Martinez-Chequer, J.C., Polanco-Echeverry, G., Estrada-Florez, A.P., Haiman, C.A., John, E.M., Kushi, L.H., Torres-Mejia, G., Vidaurre, T., Weitzel, J.N., Zambrano, S.C., Carvajal-Carmona, L.G., Ziv, E., Neuhausen, S.L., 2019. A polygenic risk score for breast cancer in U.S. Latinas and Latin-American women. J. Natl. Cancer Inst. 112, 590–598. https://doi.org/10.1093/jnci/djz174.

Song, C., Zhao, J., Fu, B., Li, D., Mao, T., Peng, W., Wu, H., Zhang, Y., 2017. Melatonin-mediated upregulation of Sirt3 attenuates sodium fluoride-induced hepatotoxicity by activating the MT1-PI3K/AKT-PGC-1α signaling pathway. Free Radic. Biol. Med. 112, 616–630. https://doi.org/10.1016/j.freeradbiomed.2017.09.005.

Su, J.F., Zhang, H., Huang, T.L., Hu, X.F., Chen, C.L., Liu, J.R., 2020. The performance and mechanism of simultaneous removal of fluoride, calcium, and nitrate by calcium precipitating strain Acinetobacter sp. H12. Ecotoxicol. Environ. Saf. 187, 109855. https://doi.org/10.1016/j.ecoenv.2019.109855.

Till, C., Green, R., Flora, D., Hornung, R., Martinez-Mier, E.A., Blazer, M., Farmus, L., Ayotte, P., Muckle, G., Lanphear, B., 2020. Fluoride exposure from infant formula and child IQ in a Canadian birth cohort. Environ. Int. 134, 105315. https://doi.org/10.1016/j.envint.2019.105315.

Wang, D., Di, M., Qian, M., 2007. A Report on the third revision of Combined Raven's Test (CRT-C3) for children in China. Chinese J. Clin. Psychol. 15, 559–561.

Ward, Z.J., Long, M.W., Resch, S.C., Giles, C.M., Cradock, A.L., Gortmaker, S.L., 2017. Simulation of Growth Trajectories of Childhood Obesity into Adulthood. N. Engl. J. Med. 377, 2145–2153. https://doi.org/10.1056/NEJMoa1703860.

Wigner, P., Czarny, P., Synowiec, E., Bijak, M., Bialek, K., Talarowska, M., Galecki, P., Szemraj, J., Sliwinski, T., 2018. Variation of genes involved in oxidative and nitrosative stresses in depression. Eur. Psychiatry. 48, 38–48. https://doi.org/10.1016/j.eurpsy.2017.10.012.

Wu, J., Wang, W., Liu, Y., Sun, J., Ye, Y., Li, B., Liu, X., Liu, H., Sun, Z., Li, M., Cui, J., Sun, D., Yang, Y., Gao, Y., 2015. Modifying Role of GSTP1 Polymorphism on the Association between Tea Fluoride Exposure and the Brick-Tea Type Fluorosis. PLoS ONE 10, e0128280. https://doi.org/10.1371/journal.pone.0128280.

Xiong, M., Wang, J., Guo, M., Zhou, Q., Lu, W., 2016. TRPM8 genetic variations associated with COPD risk in the Chinese Han population. Int. J. Chron. Obstruct. Pulmon. Dis. 11, 2563–2571. https://doi.org/10.2147/copd.s109026.

Xu, J., Wu, L., Xia, W., 2014. Case control study on combined Raven's test of amblyopic children. Chinese J. Strabismus & Pediatric Ophthalmol. 22, 9–11.

Yang, D., Liu, Y., Chu, Y., Yang, Q., Jiang, W., Chen, F., Li, D., Qin, M., Sun, D., Yang, Y., Gao, Y., 2016. Association between vitamin D receptor gene FokI polymorphism and skeletal fluorosis of the brick-tea type fluorosis: a cross sectional, case control study. BMJ Open. 6, e011980. https://doi.org/10.1136/bmjopen-2016-011980.

Yu, R., Jin, S.B., Lendahl, U., Nister, M., Zhao, J., 2019. Human Fis1 regulates mitochondrial dynamics through inhibition of the fusion machinery. EMBO J. 38, e99748. https://doi.org/10.15252/embj.201899748.

Yu, X., Cao, L., Yu, X., 2013. Elevated cord serum manganese level is associated with a neonatal high ponderal index. Environ. Res. 121, 79–83. https://doi.org/10.1016/j.envres.2012.11.002.

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. https://doi.org/10.1016/j.envint.2018.05.042.

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. https://doi.org/10.1016/j.tox.2007.04.007.

Zhang, M., Wang, A., Xia, T., He, P., 2008. Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-kappaB in primary cultured rat hippocampal neurons. Toxicol. Lett. 179, 1–5. https://doi.org/10.1016/j.toxlet.2008.03.002.

Zhang, S., Zhang, X., Liu, H., Qu, W., Guan, Z., Zeng, Q., Jiang, C., Gao, H., Zhang, C., Lei, R., Xia, T., Wang, Z., Yang, L., Chen, Y., Wu, X., Cui, Y., Yu, L., Wang, A., 2015. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. Toxicol. Sci. 144, 238–245. https://doi.org/10.1093/toxsci/kfu311.

Zhao, W.P., Wang, H.W., Liu, J., Zhang, Z.H., Zhu, S.Q., Zhou, B.H., 2019. Mitochondrial respiratory chain complex abnormal expressions and fusion disorder are involved in fluoride-induced mitochondrial dysfunction in ovarian granulosa cells. Chemosphere 215, 619–625. https://doi.org/10.1016/j.chemosphere.2018.10.043.

Zhu, W., Zhang, J., Zhang, Z., 2011. Effects of fluoride on synaptic membrane fluidity and PSD-95 expression level in rat hippocampus. Biol. Trace Elem. Res. 139, 197–203. https://doi.org/10.1007/s12011-010-8654-9.

Zhu, Y.P., Xi, S.H., Li, M.Y., Ding, T.T., Liu, N., Cao, F.Y., Zeng, Y., Liu, X.J., Tong, J.W., Jiang, S.F., 2017. Fluoride and arsenic exposure affects spatial memory and activates the ERK/CREB signaling pathway in offspring rats. Neurotoxicology 59, 56–64. https://doi.org/10.1016/j.neuro.2017.01.006.