Modifying Effect of COMT Gene Polymorphism and a Predictive Role for Proteomics Analysis in Children’s Intelligence in Endemic Fluorosis Area in Tianjin, China


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The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

Cumulative fluoride exposure has adverse influences on children’s intelligence quotient (IQ). In addition, catechol-O-methyltransferase (COMT) gene Val158Met polymorphism (rs4680) is associated with cognitive performance. This study aimed to evaluate the associations of COMT polymorphism and alterations of protein profiles with children’s intelligence in endemic fluorosis area. We recruited 180 schoolchildren (10–12 years old) from high fluoride exposure (1.40 mg/l) and control areas (0.63 mg/l) in Tianjin City, China. The children’s IQ, fluoride contents in drinking water (W-F), serum (S-F), and urine (U-F); serum thyroid hormone levels, COMT Val158Met polymorphism, and plasma proteomic profiling were determined. Significant high levels of W-F, S-F, and U-F were observed in the high fluoride exposure group compared with those in control (all P < 0.05). S-F and U-F were inversely related with IQ (rS = −0.47, P < 0.01; rU = −0.45, P = 0.002). Importantly, higher fluoride exposure was associated with steeper cognitive decline among children with the reference allele Val compared with those homozygous or heterozygous for the variant allele Met (95% CI, −16.50 to 2.55; P interaction < 0.01). Additionally, 5 up-regulated protein spots related to cell immunity and metabolism were detected in children with high fluoride exposure compared with the control. In conclusion, fluoride exposure was adversely associated with children’s intelligence, whereas the COMT polymorphism may increase the susceptibility to the deficits in IQ due to...
Fluoride is widely dispersed in nature and is known for both beneficial and detrimental effects on health. The primary adverse effects of chronic, excess intake of fluoride in drinking water are dental and skeletal fluorosis. Impairments in intellectual function are also one of the hallmark effects, even at low levels (Ozsvath, 2009), which is further demonstrated recently by a systematic review and meta-analysis with regard to developmental fluoride neurotoxicity based on numerous epidemiologic studies (Choi et al., 2012). Moreover, the evidence of fluoride neurotoxicity is also supported by animal experiments, which showed that fluoride can penetrate the blood-brain barrier and significantly influence the learning and memory in laboratory animals and their offsprings (Gui et al., 2010; Liu et al., 2010).

The reasons for cognitive deficits caused by excessive intake of fluoride from drinking water are still poorly understood. However, there is evidence that it may involve the alterations of neurotransmitters metabolism in the central nervous system (CNS). It is reported that fluoride is capable of changing the levels of dopamine, norepinephrine, epinephrine, serotonin, homovanillic acid, and 5-hydroxyindoleacetic acid in the hippocampus and neocortex regions of the rat brain (Chirumari and Reddy, 2007; Flora et al., 2009; Kaur et al., 2009; Reddy et al., 2014). Of particular concern is dopamine, since it plays a prominent role in modulating processes within the prefrontal cortex (PFC), which is the central system of human cognitive activity and is directly associated with human intelligence (Seamans and Yang, 2004; Sternberg, 2000).

Availability of dopamine is regulated by 2 crucial proteins: dopamine transporter and catechol-O-methyltransferase (COMT), controlling reuptake and degradation, respectively. In the PFC, COMT enzymatic activity is thought to be particularly important for determining dopamine availability, due to the relative absence of dopamine reuptake transporter (Yavich et al., 2007). The human COMT gene on chromosome 22q11.2 contains a functional Val158Met polymorphism (rs4680), causing a valine (Val) to methionine (Met) substitution at codon 158 that affects the activity of the enzyme (Lachman et al., 1996). As a result, the variant allele Met has one-fourth the enzymatic activity of the reference allele Val, resulting in slower degradation and greater availability of dopamine within the brain, especially in the PFC (Chen et al., 2004; Lachman et al., 1996). As expected, numerous studies have shown that individuals with Val allele performed worse on neurocognitive tests than Met carriers due to lesser dopamine available (Barnett et al., 2007). Fluoride has been found to decrease the level of dopamine in brain (Flora et al., 2009; Kaur et al., 2009; Reddy et al., 2014). Nonetheless, whether children with Val allele are more susceptible to the cognitive deficits following fluoride exposure is unclear. We sought to test whether the Val158Met polymorphism is a modifier of fluoride exposure on intellectual impairments.

To date, a critical gap in the field of fluoride exposure-associated cognitive decline research is the identification of molecules that will provide valuable insights into mechanisms of fluoride neurotoxicity. Although such indicators can contribute to diagnostic, prognostic, and therapeutic determinations, the complexity of the nervous system and its distinctive peculiarities have hindered their development (Gil and Pla, 2001). In this regard, emerging technologies such as proteomics have acquired great interest in terms of its applications and may help to identify proteins involved in the formation of cognitive impairment. Proteomic analysis has previously been applied to kidney and brain of rats exposed to high fluoride. More importantly, it has identified several proteins mainly related with cellular signaling, cell proliferation, metabolism, and oxidative stress, which provide a valuable clue to explore the mechanism of fluorosis (Ge et al., 2011; Xu et al., 2005). However, it has not been reported for proteomic analysis in human plasma.

In the present study, therefore, we focused on associations of COMT gene polymorphism and alterations of protein profiles with children’s intelligence in endemic fluorosis area, with an attempt to discover the modification of cognitive performance by COMT gene polymorphism and to find the early biomarkers of fluorosis in children.

MATERIALS AND METHODS

Study population. Endemic fluorosis is very serious in Jinnan District, Tianjin City, China, fluoride concentration in drinking water in this region is higher than the upper limit of 1 mg/l prescribed in Chinese Standards for Drinking Water Quality (GB 5749-2006) for a long period. In accordance with the principles of matching social and natural factors such as educational standard, economic situation, geological environments as much as possible, we selected 2 areas with different fluoride concentrations in the groundwater by a stratified cluster randomly sampling of this region. Taking the results of a pilot study into consideration, we calculated the sample size for the present study to achieve a 5% level of significance and 80% power. A cross-sectional design was applied in the current study to obtain a total of 180 children aged from 10 to 12 years among the fifth grade in 2 primary schools: Gegu Second Primary School (from an endemic fluorosis area, drinking water fluoride >1 mg/l) and Shuanggang Experimental Primary School (from a non-endemic fluorosis area, drinking water fluoride <1 mg/l), located 18 km apart in Jinnan District, based on the geological survey report of the Government of China. Each extended household has one or more tube wells and members of extended families rely on well water as their main source of drinking water. None of these 2 sites was exposed to potential neurotoxins that are recognized as contaminates affecting intelligence quotient (IQ) value, like arsenic in drinking water, nor delimitated into endemic areas of iodine deficiency disorders. All subjects were unrelated ethnic Han Chinese and residents in Tianjin, which had similar physical and mental health status. We excluded known neurological conditions including pervasive developmental disorders and epilepsy. Information about age and other personal data (educational level of parents and knowledge of fluorosis) were collected from self-completed, structured questionnaire at the time of the check-up. The data were collected in 2011.

The study protocols were approved by 2 collaborative institutions, namely the ethics review boards of Tongji Medical College Ethics Committee and Tianjin Center for Disease...
Control and Prevention, and complied with Chinese regulations and laws regarding research in human subjects. Written informed consent was obtained from all parents and their children.

Sample collection. The drinking water samples (10 ml) collected from the tube wells of each child’s household were kept in clean plastic bottles and analyzed within 1 week. Three fasting venous blood samples were collected: 1 (1 ml) was drawn by venipuncture into an anticoagulant vacuum tube and stored at −80 °C for later genotyping; another 2 (2–2.5 ml) were drawn into anticoagulant and anticoagulant-free vacuum tubes, respectively. After standing at room temperature for natural solidification for 30 min, the latter (2–2.5 ml) were centrifuged at 3000 rpm for 10 min at 4 °C to isolate the plasma (for proteomic analysis) and serum (for fluoride content and serum thyroid hormones determination), respectively. Urine samples were collected in the early morning before breakfast and kept in −20 °C until used for analysis.

Fluoride concentrations in drinking water, serum, and urine. Fluoride contents in drinking water (W-F), serum (S-F), and urine (U-F) were measured using an ion analyzer EA940 with a fluoride ion selective electrode (Shanghai constant magnetic electronic technology Co, Ltd, China) according to the China standard GB 7489-87. All reference solutions for the fluoride determinations were double-deionized water. Parallel samples were set for determination and averages were taken. The quantitation limits of this method for W-F, S-F, and U-F were 0.2, 0.012, and 0.5 mg/l, respectively. Recovery rate of this method was in the range of 94.3%–106.4%. The intra- and inter-assay coefficients of variation for fluoride were 2.7% and 6.7%, respectively.

Genotyping of COMT. The genomic DNA was isolated via commercial DNA Extraction Kit (Tiangen Biotech, Beijing, China). Genotypes were examined by using polymerase chain reaction-restriction fragment length polymorphism methods as previously described (Lee and Kim, 2011). To ensure laboratory quality control, 2 independent readers interpreted the gel photographs and 10% of the samples were selected randomly and genotyped again, with identical results.

Plasma proteomic. Plasma samples from 10 children of each area (high fluoride and control) were collected for complete proteomic analysis. Two-dimensional electrophoresis (2-DE) was performed with the Amersham Biosciences IPGphor IEF system and Ettan DALT Six electrophoresis unit (GE Healthcare Amersham Biosciences, Little Chalfont, UK). Spot detection, quantification, and image matching were analyzed by using the Image Master 2D-Platinum software version 5.0 (GE Healthcare Amersham Biosciences). Protein spots of interest were analyzed on an ABI 4800 matrix-assisted laser desorption ionization-time of flight/time of flight Proteomics Analyzer mass spectrometer (Applied Biosystems). A database search against NCBI was performed with Mascot software.

Serum thyroid hormone levels. Serum levels of total triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) were determined by standard radioimmunooassay kits (North Institute of Biological Technology, Beijing, China). The extract samples were run on radioimmunooassay in triplicates, and results were interpolated from the standard curve with recoveries in the range of 95.0%–110.0%.

The intra-assay coefficients of variation for T3, T4, and TSH were 5.8%, 3.6%, and 2.7%, and the inter-assay coefficient variations were 6.2%, 5.3%, and 4.2%, respectively.

Children’s IQ scores. A Combined Raven’s Test for Rural China (CRT-RC) was taken to evaluate the IQ of each child (Wang, 2007). All tests were administered at school by a trained examiner who was masked to participants’ drinking water fluoride levels. The 7 categories of this test scores are as follows: ≤69 retarded (low); 70–79 borderline (below average); 80–89 dull normal (low average); 90–109 normal (average); 110–119 high normal (high average); 120–129 superior (good); and ≥130 very superior (excellent).

Statistical analysis. Data from both high fluoride and control regions were analyzed separately and then combined. Normality test (Kolmogorov-Smirnov test) was executed for all continuous variables. Genotype frequencies were calculated and compared against expected counts with the χ² statistic to test adherence to the principles of Hardy-Weinberg equilibrium. Descriptive analyses were given for characteristics of the study children, and differences according to the status were evaluated. After descriptive analyses, associations among exposure markers, IQ, and risk factors were evaluated using partial correlation coefficients with an adjustment of age and gender.

We estimate the associations between S-F or U-F levels and IQ scores with 2 progressive methods. First, we performed a general linear model aimed at analyzing the difference in mean IQ scores across different levels of fluoride. Full ranges of S-F or U-F concentrations were included as independent variable, age as covariate variable, and IQ scores were seen as the dependent variable.

In addition, multivariate models with ordinary least squares regression directed to the associations of U-F levels with IQ scores according to COMT polymorphism were carried out. Our first step was to create a multivariate main effects model to estimate the effects of U-F levels and COMT genotypes (categorized as val/val [reference], val/met, or met/met) on IQ (model 1). Next we estimated effects of U-F on IQ according to genotype (3 categories) using stratified models (model 2). We then combined the COMT allele categories into 2 categories (val/met vs val/met and met/met combined). In Model 3, we estimated the effects of U-F on IQ stratified across these categories, and in Model 4 we evaluated the interaction between U-F and COMT polymorphism. The interaction term (U-F × COMT category) was modeled first in separate model to assess joint interactions. Covariates in the regression model were chosen as follows: First, the risk factors associated with U-F levels or IQ scores were identified from the literature. Second, the variable was considered to be a potential confounder if the variables changed the R² and/or main effect estimate by >10%. Covariates included the indicator variables for age, gender, educational levels of parents (primary and below, junior high school, senior high school, and above), and continuous variables for drinking water fluoride (mg/l) and levels of thyroid hormones (T3, T4, and TSH). All models were adjusted for the covariates above.

For 2-DE, among the resulting protein spots, only those differentially expressed with fold changes ≥1.5 were chosen, and further analyzed using Student’s t test. The x-level for all statistical tests of significance was set at 5%. Statistical analyses were performed using SPSS for windows software version 12.0 (SPSS Inc, Chicago, Illinois).
RESULTS

Sociodemographic and Clinical Characteristics

Descriptive data of the children and fluoride contents are listed in Table 1. The average age of children was 11.35 years, and approximately one-third of the subjects were boys. Children in the 2 groups were well matched for age and gender. According to their age and exposure duration, children on average have been exposed since birth to the wells used by the household. S-F and U-F were significantly higher in children exposed to a higher drinking W-F concentration. Interestingly, there is a pretty clear trend that U-F > W-F > S-F, both in the 2 groups. In regard to the thyroid hormones, we found that TSH in the high fluoride group was notably increased (P < 0.03), whereas T4 was somewhat decreased but did not reach statistical significance (P = 0.06). However, the individual values of thyroid hormones were all within the normal range. For children exposed to high fluoride drinking water, the mean IQ value was notably decreased, whereas the percentage of poor IQ (IQ scores fell below the normal range of 90) was markedly increased, when compared with those of control cohorts (both P < 0.01).

TABLE 1. Demographic, Baseline Lifestyle, and Phenotypic Information by Fluoride Exposure Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>High Fluoride</th>
<th>Control</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>84</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Age (years) (means, SD)</td>
<td>11.37 ± 0.19</td>
<td>11.34 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>11.50 ± 0.51</td>
<td>11.50 ± 0.50</td>
<td>0.63</td>
</tr>
<tr>
<td>Girls</td>
<td>11.32 ± 0.32</td>
<td>11.23 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Gendera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>34 (40.48)</td>
<td>40 (41.67)</td>
<td>0.87</td>
</tr>
<tr>
<td>Girls</td>
<td>50 (59.52)</td>
<td>56 (58.33)</td>
<td></td>
</tr>
<tr>
<td>Years of residencec</td>
<td>11.32 ± 1.85</td>
<td>11.25 ± 1.26</td>
<td>0.98</td>
</tr>
<tr>
<td>Water fluoride (mg/l)e</td>
<td>1.40 (1.23-1.57)</td>
<td>0.63 (0.58-0.68)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Serum fluoride (mg/l)f</td>
<td>0.18 ± 0.11</td>
<td>0.06 ± 0.03</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Urinary fluoride (mg/l)g</td>
<td>2.40 ± 0.10</td>
<td>1.10 ± 0.67</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Education levels of parents (%)h</td>
<td>Primary and below</td>
<td>12 (14.28)</td>
<td>12 (12.50)</td>
</tr>
<tr>
<td></td>
<td>Junior high school</td>
<td>36 (42.86)</td>
<td>40 (41.67)</td>
</tr>
<tr>
<td></td>
<td>Senior high school and above</td>
<td>36 (42.86)</td>
<td>44 (45.83)</td>
</tr>
<tr>
<td>Having knowledge of fluorosis (%)h</td>
<td>Yes</td>
<td>75 (89.29)</td>
<td>86 (90.58)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9 (10.71)</td>
<td>10 (9.42)</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T_3 (\mu g/ml)^j )</td>
<td>2.22 ± 0.36</td>
<td>2.15 ± 0.42</td>
<td>0.29</td>
</tr>
<tr>
<td>( T_4 (\mu g/ml)^j )</td>
<td>85.65 ± 16.80</td>
<td>90.79 ± 18.85</td>
<td>0.06</td>
</tr>
<tr>
<td>( TSH (\mu U/ml)^j )</td>
<td>3.11 (2.60-4.09)</td>
<td>2.59 (2.24-3.16)</td>
<td>0.03*</td>
</tr>
<tr>
<td>COMT(^d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>val/val</td>
<td>18 (19.05)</td>
<td>12 (12.50)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>met/met</td>
<td>48 (57.14)</td>
<td>40 (41.67)</td>
<td></td>
</tr>
<tr>
<td>met/met</td>
<td>20 (23.81)</td>
<td>44 (45.83)</td>
<td></td>
</tr>
<tr>
<td>IQ scores(^e)</td>
<td>93.33 ± 13.46</td>
<td>90.42 ± 13.30</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>&lt;90 (%)</td>
<td>24 (28.57)</td>
<td>8 (8.33)</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

Data collected in 2011.
\( ^a \)Data were presented as mean ± SD for parametrically distributed data.
\( ^b \)Number (percentage) for categorical data.
\( ^c \)Median (interquartile range) for nonparametrically distributed data.
\( ^d \)Genotype frequencies for COMT conform to the Hardy-Weinberg equilibrium (all P > 0.05).
\( ^e \)Differences for means were tested with Student’s t test, for medians were evaluated using Mann-Whitney U test, and for percentages were evaluated using \( G^2 \) test, respectively.
\( ^f \)Significant (P < 0.05) difference.
\( ^g \)Significant (P < 0.01) difference.

Associations among Exposure Markers, IQ, and Risk Factors

Data from both high fluoride and control regions were combined for analysis. As shown in Table 2, S-F and U-F were positively correlated, and both increased with fluoride concentration in drinking water. Among all the children, S-F and U-F were positively correlated with years of residence. In addition, IQ had a negative relationship to S-F and U-F (all P < 0.05).

Associations between Fluoride Levels and IQ Scores

To further examine the dose-response relationship of S-F or U-F levels with IQ scores, the children were assigned by their fluoride concentrations in serum and urinary and divided into 9 groups of about 20 in each. The mean value of S-F concentration of the lowest group was 0.003 mg/l and of the highest group was 0.333 mg/l, whereas for U-F was 0.459 mg/l and 3.290 mg/l, respectively. For each group, the IQ difference from mean value was plotted against fluoride concentrations in serum and urinary (Fig. 1). From the first group to the ninth, mean IQ scores declined about 16.40 (S-F) and 14.40 (U-F) points, respectively. Besides, mean IQ scores of the last 5 groups were below the mean value of total IQ scores. Manifestly, there was an inverse dose-response relationship between S-F or U-F levels and IQ scores.

Associations between Fluoride Levels and IQ Scores According to COMT Genotype

The adjusted multivariate main effects of model (Table 3, Model 1) indicated an inverse association between U-F and IQ. Adjusted estimate (95% confidence interval [CI]) of the effect of U-F levels on IQ scores was a decrease of 2.42 (–4.59, –0.24) points for each one increase in U-F (mg/l) (P = 0.030).

In the models stratified by 3-category genotype (Table 3, Model 2), the inverse association between U-F and IQ was stronger among children who carried the reference genotype (val/val) than among children who carried the heterozygous or homozygous variant genotypes (met/val or met/met). Also, the
magnitude and direction of the association between U-F and IQ were similar in children with the met/val and met/met genotypes for the COMT polymorphisms.

This suggested a recessive expression of the genotype, which we modeled by creating categories of variant genotypes (met/val + met/met; n = 152) versus COMT reference genotype (val/val, n = 28).

In the subpopulation carrying the COMT reference genotype (Model 3), 1 unit increase in U-F (1 mg/l) was associated with a decrease of 9.67 points of IQ and was significant after controlling for covariates (P = 0.003). Among children carrying variant genotypes, 1 unit increase in U-F resulted in a decrease of 1.85 IQ points, but this was not statistically significant in this stratum. We also observed a significant interaction between U-F and COMT genotype (Model 4). From the interaction model including the cross product term (U-F × COMT homozygous reference), 1 unit increase in U-F was associated with 2.63 IQ points decrease (P = 0.002), after controlling for other variables.

Identification of Differentially Expressed Proteins

Seven differentially expressed protein spots (B1–B7) matched on the 2-DE were detected in high fluoride group contrasted with those in the control (Fig. 2). Of these high expression proteins, B1 was not identified; whereas B3 and B4 were the same protein and COMT genotype (Model 4). From the interaction model including the cross product term (U-F × COMT homozygous reference), 1 unit increase in U-F was associated with 2.63 IQ points decrease (P = 0.002), after controlling for other variables.

DISCUSSION

A strong body of evidence suggests that exposure to high levels of fluoride in drinking water associates with deficits in children’s intelligence (Choi et al., 2012; Tang et al., 2008). Consistently, the present work demonstrated that the IQ scores of children exposed to high fluoride drinking water were significantly lower than those who lived in control area, and this decreased intelligence was accompanied by elevated levels of fluoride in serum and urine. It is well accepted that fluoride contents in serum and urine, as internal exposure indicators, integrate all sources and changes in exposure, and reflect the actual exposure levels (Ding et al., 2011; Mehta, 2013). There is also evidence that internal concentrations are crucial to understanding toxicity (Escher and Hermens, 2004; Mehta, 2013). In support of this, our findings further showed that, across the full range of serum and urinary fluoride, children’s IQ decreased gradually with the increase of fluoride contents in serum and urine, in a dose-dependent manner, even when the external exposure levels were not relatively high. These are in agreement with a recent study (Ding et al., 2011) showing a very close mean value of fluoride in drinking water (1.31 ± 1.05 mg/l) to our study.

Accumulating evidence has showed that COMT Val158Met polymorphism is a striking candidate influencing intelligence-related neural function (Winterer and Goldman, 2003). Our study showed that the COMT genotype was correlated to total IQ scores before and after adjusting for age and gender. In particular, we found that children with Val/Val homozygote showed lower IQ scores than those with Met/Met homozygote or Met/Val heterozygote, which coincides with the findings from previous results. A meta-analysis of 16 studies (n > 9,000) demonstrated that COMT polymorphism was markedly and robustly associated with IQ scores (Barnett et al., 2008). Further evidence for a contribution of COMT polymorphism to intelligence has been validated by brain-imaging studies in humans, pharmacological, transgenic, and gene-knockout studies in animals (Goldman et al., 2009).

The steep decrease in IQ associated with increasing urinary fluoride among the children with the reference allele (Val) of COMT suggests that these children may be more susceptible to the influences of fluoride on cognition and, more importantly, this points toward COMT polymorphism as a modifier of the effect of cumulative fluoride exposure on cognition. In animal studies, fluoride has been shown to alter dopamine system. Fluoride treatment is reported to reduce dopamine level in rat brain with increased fluoride deposition (Reddy et al., 2014). Additionally, in a combined study of fluoride and aluminum, chronic post-weaning fluoride exposure was associated with decreased dopamine in cerebral, cerebellum, and medulla oblongata of rat brain, and this effect was more pronounced in animals given fluoride and aluminum together (Kaur et al., 2009). Since the reference Val allele carriers with high enzymatic activity induce faster degradation and less availability of dopamine within the brain, it is conceivable that the children with Val allele had a higher risk for cognitive impairments after fluoride exposure.

In order to better understand the molecular mechanisms of fluoride neurotoxicity and to evaluate potential protein biomarkers, a classical gel-based proteomic approach was applied to determine the children’s plasma proteome profile from the high fluoride and the control groups. The functional significance and potential roles of the altered proteins in fluoride-exposed children are highlighted as follows: (1) immune-associated proteins. Complement C1s subcomponent precursor is a serine protease that combines with C1q and C1r to form C1 complex, which initiates the classical pathway activation of the complement system (Wallis et al., 2010). It is known that complement components play central roles in mediation of inflammation and regulation of the immune response (Ricklin et al., 2010), implying that fluoride exposure may cause immune...
response and inflammatory activation in children with fluorosis. Similar result was obtained in a recent study with regard to Huntington’s disease that plasma complement components elevation reveals neuroinflammatory activation (Dalrymple et al., 2007). Another identified protein Immunoglobulin light chain variable region, representing prototypical domain of the immunoglobulin superfamily, can mediate interactions with antigens and effector molecules (Poljak et al., 1976). This may further support the role of inflammatory reaction in fluoride-exposed children. A1GB belongs to immunoglobulin superfamily but with a hitherto unknown function (Ishioka et al., 1986). However, it was present in the plasma of subjects with multiple sclerosis (Rithidech et al., 2009), a disabling CNS disorder. Thus, it is worthy of further study to determine its association with pathogenesis of fluorosis.

(2) Metabolism-related proteins. HPX is the plasma protein with the highest binding affinity to heme. It is mainly responsible for the transportation of heme thus preventing both heme-catalyzed oxidative damage and heme-bound iron loss (Tolosano and Altruda, 2002). In the current study, the increased level of plasma HPX may be accounted for oxidative...
stress arising from fluoride exposure. This is consistent with previous in vivo and in vitro studies and epidemiologic investigations that fluorid e is an oxidative stress inducer (Barbier et al., 2010). Finally, APOE precursor is a key lipoprotein of lipoprotein complexes that is expressed highest in the liver followed by the brain. Although APOE have substantial effects on peripheral lipid metabolism, it is still involved in diseases such as type III hyperlipoproteinemia and atherosclerosis (Mahley and Rall, 2000).

Furthermore, it is interesting to note that majority of the candidate markers, such as complement components, HPX and APOE, have previously been implicated in Alzheimer’s disease (AD) pathogenesis or related pathological processes or have been found to be altered in AD, though many of which were identified in core cerebrospinal fluid (Finehout et al., 2007; Rohrer et al., 2009). Given that AD is a progressive neurodegenerative disease characterized by cognitive deficits; one can hypothesize that this abnormal expression of plasma proteins can contribute to or be a consequence of impaired cognition, or predict a high risk of neurodegenerative diseases in children following fluoride exposure. Actually, recent works in our laboratory and others have showed that fluoride is capable of inducing neurodegeneration in cognition-related brain regions (El-Jethy et al., 2010; Jiang et al., 2014; Wilson et al., 2012). However, due to the number of samples included, our study should be regarded as a preliminary study that makes further evaluation of the identified candidate markers on a larger cohort of cases necessary.

The present study has several limitations. First, the cross-sectional observational design does not allow us to determine temporal or causal associations between fluoride and cognition. Second, the study has a relatively small sample size, which limits the power to assess effects of gene-environment interactions on children’s IQ. Third, we did not evaluate the cognitive performance of children using other neurocognitive tests except for CRT-RC. Despite the study limitations, this is the first gene-environment study investigating the potential impact of COMT single-nucleotide polymorphism (SNP) on the relationship between children’s cognitive performance and exposure to elemental fluoride.

CONCLUSION

In summary, our data suggest that the intelligence of children is affected by the COMT gene polymorphism and, in particular, this SNP plays a role in modifying the effect of fluoride exposure on cognition. Children with COMT reference allele had a higher risk for cognitive impairments after fluoride exposure. Additionally, proteomics analysis represents early specific markers of developmental fluoride neurotoxicity. Hence, our findings provide a certain basis for clarifying the mechanisms and identifying molecular targets of pharmacological interventions for potential delayed therapy.

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