Bone mineral density and polymorphisms in metallothionein 1A and 2A in a Chinese population exposed to cadmium

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ABSTRACT

Cadmium (Cd) effect on bone varies between individuals. We investigated whether genetic variation in metallothionein (MT)1A and MT2A associated with Cd induced bone loss in this study. A total of 465 persons (311 women and 154 men), living in control, moderately and heavily polluted areas, participated. The participants completed a questionnaire and the bone mineral density (BMD) was measured by dual energy x-ray absorptiometry (DXA) at the proximal radius and ulna. Samples of urine and blood were collected for determination of Cd in urine (UCd) and blood (BCd). Genotypes for polymorphisms in MT1A (rs11076161) and MT2A (rs10636) were determined by Taqman allelic discrimination assays. BCd had a weak association with variant alleles for MT1A (rs11076161) and MT2A (rs10636) were determined by Taqman allelic discrimination assays. BCd had a weak association with variant alleles for MT1A (rs11076161) and MT2A (rs10636) in female living in the highly polluted group (p = 0.08 and 0.05, respectively). A weak association was found between bone mineral density and MT2A polymorphisms variation (p = 0.06) in female living in the highly polluted group. Only a weak association was found between bone mineral density and MT1A polymorphisms variation in female. Genetic variation in the MT1A and MT2A genes may not associate with bone loss caused by cadmium exposure.

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1. Introduction

Bone is one of target organs for cadmium (Cd) toxicity (WHO, 1992). It has been showed that Cd exposure could result in bone loss and high risk of osteoporosis (Wang et al., 2003; Staessen et al., 1999; Åkesson et al., 2006; Engstrom et al., 2011; Osada et al., 2011). Once absorbed, Cd could retain in the organism and accumulated throughout life (Wu et al., 2008; Nordberg, 1996). One of the important features of Cd is its long biological half-time (10–30 years) (WHO, 1992; Kazantzis, 2004; Comelekoglu et al., 2007). Metallothionein (MT) is a family of low molecular weight proteins and has the capacity to bind some metals (Margoshes and Vallee, 1957), such as Cd. The synthesis of MTs could be induced by various metals, including Cd which could induce MTs gene expression in liver, kidney and bone (Searle et al., 1984; Oda et al., 2001; Regunathan et al., 2003; Chen et al., 2011). In the cell, most of Cd is bound to MTs and MTs may play important role in Cd transportation (Miyahara et al., 1988; Lu et al., 2005; Oda et al., 2001).

Experimental data suggested that MTs may provide protection against metal toxicity and oxidative stress (Margoshes and Vallee, 1957; Lu et al., 2005; Kaasen et al., 1999). It has been indicated that MT genes knockout mice are more sensitive to Cd toxicity than wild-type mice (Liu et al., 2000). Bone loss induced by Cd in MT genes knockout mice was much more than that in wild-type mice (Habeebu et al., 2000). Those finding supported that MT is one of main determinants of sensitivity and toxicity of cell to Cd. Lu et al. (2005) found that, at similar urinary Cd level, N-acetyl-beta-D-glucosaminidase level would be higher in workers with low level of MT mRNA than those with high level of MT mRNA. Those finding indicated that variation in the expression of MT may be related with Cd toxicity to renal dysfunction.

The main MTs related with Cd toxicity are MT1A and MT2A (Nordberg, 1989, 1998). Several single nucleotide polymorphisms (SNPs) (rs8052394 and rs11076161 in the MT1A gene, and rs10636 in MT2A gene) have been reported to be involved in aging, diabetes and atherosclerosis (Mazzatti et al., 2008; Yang et al., 2008; Giacconi et al., 2007; Kita et al., 2006). Some studies have indicated that certain genetic polymorphisms may modify metal toxicokinetics (Onalaja and Claudio, 2000; Gundacker et al., 2009). Individual characteristic and genetic variation are important factors for accumulation of toxic elements (Whitfield et al., 2010). Bjorkman et al. (2000) reported that genes are one of important factors for concentration of Cd in blood. However, it is not yet clear if these SNPs may modify Cd-induced bone loss. In this study, we investigated whether
polymorphisms in MT1A and MT2A were associated with variation of BMD in a Chinese population exposed to Cd.

2. Materials and methods

2.1. Area and study population

The following areas were included in the present study: Dezheng (a heavy polluted area), Jinzhu (a moderately polluted area), and a control area with lower exposure (Yantou). A smelter that began operating in 1961 was located in the heavily polluted area and industrial wastewater was regularly discharged into the river. The residents living in the polluted areas used the polluted river to irrigate their rice fields from 1961 to 1995. Rice was the main staple food of the local residents and considered to be the major source of Cd intake. 10 Rice samples in each area were collected from 3 to 10 families. The Cd concentrations in these three areas were 3.7(1.9–5.4) mg/kg, 0.51(0.31–0.7) mg/kg and 0.072(0.061–0.083) mg/kg (n = 10) in 1996, respectively. From 1996 onward, however, the residents of the Cd-contaminated areas were asked to stop producing rice in their own fields and to eat commercial rice (0.03 mg Cd/kg) from non-polluted areas. A non-Cd-polluted village (Yantou) was selected as a control area having many conditions in common with the polluted areas (social, economic conditions and living habits) but with a lower Cd concentration in its rice. More detailed information has been provided previously (Wang et al., 2003; Jin et al., 2004).

532 Original participants were recruited with the help of local staff. A total of 465 natives (311 women and 154 men), aged 40 years and older were included in this study. Subjects with impairment of kidney and liver, hyperparathyroidism and those who had conditions known to affect bone mineral density (BMD), including a history of chronic disease, use of medications known to alter bone metabolism were excluded. The participants gave their informed consent and completed a questionnaire including information on medical and drug history, cigarette smoking and menopause status in women.

2.2. Sample collection and analysis

Following a strict sampling protocol (Cornelis et al., 1996; Jin et al., 2002), spot urine samples were collected from each subject in acid-washed containers and stored at −20 °C until analysis. A total of 2 ml of venous whole blood was collected in a vacutainer containing anticoagulant (heparin): one part sample was taken for blood cadmium (B-Cd) analyses and one part was extracted for genomic DNA. Rice was ashed in muffle oven at 500 °C for 6 h and then the ash was dissolved in 1 ml HNO3 (70% HNO3 for trace metal analysis). Five hundred microliters of whole blood and one milliliter of urine were wet digested with HNO3. Cd content in rice, urine and blood were measured by graphite-furnace atomic absorption spectrometry (GF-AAS, ShimadzuAA-670, Kyoto, Japan) with peak area evaluation. A reference urine sample (Seronorm trace elements urine, Nycomed, Oslo, Norway) was inserted in each run of 10 samples. UCd concentrations were adjusted for creatinine (measured by Jaffe assay) and expressed as μg/g creatinine (μg/g crea). All analyses were conducted with consistent methods and by the same trained technicians in the same laboratories.

2.3. Bone measurements

Areal BMD (g/cm²) was measured by dual energy x-ray absorptiometry (DXA, Norland) at the proximal radius and ulna; measurement precision, expressed as the coefficient of variation (CV) was below 1%. Repetition of the measurements in the same person showed that the reproducibility of the results was 99.5%. The apparatus was calibrated every day, including quality assurance and phantom scanning, and all measurements were performed by same experienced technician.

2.4. Genotype analyses

SNPs located within the MT1A and MT2A gene were selected from the NCBI LocusLink (http://NCBI.nlm.nih.gov/LocusLink) and HapMap (http://hapmap.org). Genomic DNA was extracted from the whole blood using QIAamp blood DNA mini kits (QIAGEN, Hilden, Germany) according to the methods recommended by the manufacturer. Taqman allelic discrimination assay (ABI 7900; Applied Biosystems, Foster City, CA, USA) was used to separately analyze all the SNPs: MT1A (rs11076161) and MT2A (rs10636). Each polymerase chain reaction (PCR) was performed with a reaction volume of 5 μl supplemented with 1× Universal Taqman mix (Applied Biosystems), 1 ng genomic DNA, 0.4 μM of each primer (rs11076161: 5′-TTCCGGAATAGACCAAAATGAC-3′ and 5′-GAAATGGATCATTTGGCGCTCTC-3′; rs10636: 5′-TTCCGAAACGAGCTGCTG-3′ and 5′-CCCTCCAGGTCAATCCCT-3′), and 0.1 μM of each probe. Amplification reactions were performed using the following program: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Controls were included for each genotype in each run, and genotyping was repeated on 5% of the samples.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of the study population.a</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Male</strong></td>
</tr>
<tr>
<td><strong>Control</strong> (n = 40)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>67.8 ± 9.7</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
</tr>
<tr>
<td>164.7 ± 5.9</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td>64.0 ± 11.9</td>
</tr>
<tr>
<td><strong>BMI (kg/cm²)</strong></td>
</tr>
<tr>
<td>23.5 ± 4.0</td>
</tr>
<tr>
<td>**B-Cd (μg/l)**b,c</td>
</tr>
<tr>
<td>1.30 (0.12–5.35)</td>
</tr>
<tr>
<td>**UCd (μg/g crea)**b,c</td>
</tr>
<tr>
<td>2.42 (0.061–10.17)</td>
</tr>
<tr>
<td><strong>BMD</strong></td>
</tr>
<tr>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
</tr>
<tr>
<td>26</td>
</tr>
</tbody>
</table>

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a The values presented are arithmetic average.

b The abbreviations used were B-Cd = blood cadmium; U-Cd = urinary cadmium. The values presented are geometric mean.

* The values presented are arithmetic average.

* Compared with control, p < 0.05.
2.5. Ethical consideration

During this study, Declaration of Helsinki was followed. This study was carried out with the permission of the local authority and the Institutional Review Board of Fudan University.

2.6. Statistical analysis

Deviations from Hardy–Weinberg equilibrium were evaluated by the χ² test. B-Cd and U-Cd were log-transformed to fulfill the criteria for parametric analysis. Each polymorphism was modeled as a categorical variable (zero, one, or two variant alleles). Analysis of covariance (ANCOVA) was employed to analyze, within the different exposure groups, the effects of genotype of each polymorphism on log(B-Cd), log(U-Cd) or BMD, as the dependent variables. Adjustments were made for other potentially influential variables (age, body mass index and smoking). Multiple linear regression analysis was employed to explore the association between BMD and different variables. We then analyzed multiplicative effect modification in a multivariate model with an interaction term between ln (B-Cd) and genotype on BMD as dependent variables. Regression analysis was used for trend analysis. All statistical analyses were performed using SPSS software (version 11.5; SPSS, Chicago, IL, USA). Statistical significance refers to p < 0.05.

3. Results

3.1. Study population characteristics

The characteristics of the study population are shown in Table 1. There were 33.1% men and 66.9% women in the total study population. The age of subjects living in control area was significantly different with those living in moderately and heavily polluted areas. There was increasing median levels of B-Cd and U-Cd from low to high exposure groups, and the contrary trends were seen for BMD.

The genotype and allele frequencies of MT1A rs11076161 and MT2A rs10636 were tabulated in Table 2. All the SNPs demonstrated allele frequencies >5%. The Chi square test showed that the genotypic distributions of all three SNPs did not deviate from the Hardy–Weinberg equilibrium (p > 0.05).

3.2. Association between MT SNPs and Cd exposure biomarkers

First, the impact of genotype on B-Cd level was evaluated in male and female in each exposure group. For MT1A rs11076161 and MT2A rs10636, an allele-dosage effect could be observed (Fig. 1A) where variant genotypes demonstrated higher B-Cd levels in the moderate and the high exposure groups. The trend for higher B-Cd with increasing number of variant alleles was obvious for MT1A rs11076161 and MT2A rs10636 in the high exposure group (p = 0.07 and p = 0.05, adjusted for age) in female. p-values for trend in the other exposure groups were p > 0.1. Secondly, the same analysis was performed for U-Cd, but no clear allele dosage effect for MT1A rs11076161 or MT2A rs10636 was found (Fig. 1B). The same analysis was performed for Bcd and Ucd in male; no clear allele dosage effect for MT1A rs11076161 or MT2A rs10636 was found (Fig. 2).

3.3. Association between MT SNPs and BMD

Next we evaluated genetic effect modification on BMD in different exposure groups. For rs10636, the genotype was significantly (p = 0.04,
unadjusted for age) associated with BMD in female in the highly polluted group (Fig. 3A), but no significant difference was found if adjusted for age and BMI (p = 0.06). For rs11076161, no obvious association was found between genotype and BMD both in female (Fig. 3A) and male (Fig. 3B). In addition, we also combine all data and analyze them by dividing them by B Cd exposure levels and no obvious interaction was found between MT genotype and BMD (data not shown).

Secondly, we evaluated genetic effect modification on Cd-related bone damage by using Cd in blood as exposure markers. There was no obvious interaction of MT1A rs11076161 (adjusted p = 0.463, 0.710 for female and male, respectively) and MT2A rs10636 (adjusted p = 0.110, 0.527 for female and male, respectively) with B Cd for BMD (Table 3).

3.4. Regression analysis

Results of multiple regression analysis were presented in Table 4. BMD was significantly associated with B Cd and age both in male and female (p < 0.05). However, there was no obvious association between BMD and MT1A and MT2A polymorphism.

Fig. 2. Interval of blood cadmium (A) and urinary cadmium (B) (log transformed) in the control (C, n = 40), moderately (M, n = 40) and heavily (H, n = 74) exposure groups stratified for genotype of MT1A rs11076161 and MT2A rs10636 (0 = AA, 1 = AG and 2 = GG) in male.

Fig. 3. Bone mineral density in the control (C), moderately (M) and heavily (H) exposure groups stratified for genotype of MT1A rs11076161 and MT2A rs10636 (0 = CC, 1 = CG and 2 = GG) in female (A) and male (B).
Table 3  
Influence of genetic variation on the associations between BCd exposure and bone mineral density (BMD).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BMD/SNP</th>
<th>p-value interaction</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD/rs1076161</td>
<td>0.623</td>
<td>0.395</td>
<td>0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>BMD/rs10636</td>
<td>0.527</td>
<td>0.110</td>
<td>0.003</td>
<td>0.009</td>
</tr>
</tbody>
</table>

4. Discussion

MTs play important role against Cd toxicity (Nordberg, 1989). However, little is known about the role of MT genotype for sensitivity toward Cd. In this study, we investigated the association of BMD with MTs SNP. We found no genetic variant in MT that accounted for BMD decrease caused by Cd exposure.

Cadmium-induced bone effects may be mediated via renal tubular dysfunction (Jin et al., 2004; Berglund et al., 2000). Cd may reduce the normal activation of vitamin D and lead to decreasing Ca absorption from the intestines and low bone mineralization (Kjellstrom, 1986). In our study, kidney damage also was found after Cd exposure (data not shown), especially for tubule dysfunction. In addition, renal dysfunction caused by cadmium was related with MTs expression in kidney (Nordberg et al., 1992; Liu et al., 2000; Lu et al., 2005). MT is an important determinant of individual susceptibility to Cd toxicity (Miura, 2009). Miura (2009) and Kita et al. (2006) reported that MT2A genotype was correlated with cadmium induced various biological dysfunctions. Thus, genetic variant in MT may account for variation in renal dysfunction induced by similar Cd exposure.

Cadmium may have direct effects on bone (Åkesson et al., 2006). It was been suggested that Cd could induce MT gene expression in bone tissue and osteoblasts in vitro (Oda et al., 2001; Regnathan et al., 2003; Chen et al., 2011). MT may involve in metabolism and transportation of Cd in bone (Oda et al., 2001). MT could protect the body against oxidative damage (Yu et al., 2011) and oxidative stress may involve in the mechanism of damaging Cd action in the skeleton (Brzoska et al., 2010). Thus, MT may be related with Cd effects on bone. Our data showed that the female with AA genotype (rs10636) may be more sensitive to cadmium toxicity than those with the GG genotype. However, only weak association was found between MT2A polymorphisms and BMD in female living in heavily polluted area. One of the reasons may be the small sample size. In addition, it is possible that BMD decrease caused by cadmium may be more influenced by other factors, such as age, menopause and renal dysfunction. Some studies had showed that Cd may account for about 10% of BMD decrease induced by Cd (Åkesson et al., 2006; Chen et al., 2009; Alfvén et al., 2000). However, this explanation remains speculative without further study.

Although it has been showed that MT2A polymorphism may influence accumulation of Cd in human placenta (Tekin et al., 2010) and Cd induced biological dysfunction (Kita et al., 2006), we could not find other studies about the modifying effects of MT1A and MT2A polymorphisms on Cd metabolism or Cd toxicity. The sample size in this study was smaller than our previous investigation and the analysis was based on gender. Only a weak association between MT1A and MT2A polymorphisms and BCd (p = 0.07 and p = 0.05) was found in female. We speculated that genetic variant in MT1A and MT2A may be related with Cd body burden, especially for Bcd. Because the sample size adopted in this study was small, more studies may be needed to confirm this finding. This study could be seen as an exploration.

The major strengths in this study were as follows. First, Cd exposure (Bcd and Ucd) varied widely in this study. Second, the living conditions (housing, temperature and weather), lifestyles (food, drinking and smoking habits), social and economic conditions were similar in the different areas and the study population was ethnically homogenous. Finally, the SNP genotyping was done with Taqman probe assays that are more accurate compared to other methods, such as restriction fragment length polymorphism analysis.

In conclusion, only a weak association was found between MT2A polymorphisms and BMD variation in a Chinese population exposed to Cd. These results suggested that genetic variations in MT may not be related with bone loss caused by Cd exposure.

Acknowledgment

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References

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Table 4  
Multiple linear regression analysis between BMD and different variables.  

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>B</th>
<th>Std. error</th>
<th>Beta</th>
<th>p</th>
</tr>
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<tr>
<td>Male</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.86</td>
<td>0.084</td>
<td>0.00</td>
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<tr>
<td>BMI</td>
<td>0.006</td>
<td>0.002</td>
<td>0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>MT1A</td>
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<td>0.011</td>
<td>0.01</td>
<td>0.819</td>
</tr>
<tr>
<td>MT2A</td>
<td>0.008</td>
<td>0.012</td>
<td>0.05</td>
<td>0.554</td>
</tr>
<tr>
<td>AGE</td>
<td>−0.002</td>
<td>0.001</td>
<td>−0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>BCD*</td>
<td>−0.026</td>
<td>0.008</td>
<td>−0.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
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<tr>
<td>Constant</td>
<td>1.080</td>
<td>0.057</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>BMI</td>
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<td>0.002</td>
<td>0.03</td>
<td>0.446</td>
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<tr>
<td>MT1A</td>
<td>0.001</td>
<td>0.009</td>
<td>0.00</td>
<td>0.906</td>
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<tr>
<td>MT2A</td>
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<td>0.010</td>
<td>−0.02</td>
<td>0.566</td>
</tr>
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<td>0.001</td>
<td>−0.06</td>
<td>0.000</td>
</tr>
<tr>
<td>BCD*</td>
<td>−0.090</td>
<td>0.016</td>
<td>−0.29</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* The abbreviations used were BCD = blood cadmium and BMI = body mass index. For males: n = 145–154; for females n = 290–311.


