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**ORIGINAL ARTICLE**

# Influence of vitamin D receptor haplotypes on blood lead concentrations in environmentally exposed children of Uygur and Han populations

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**Abstract**

*Objective*: To explore the association between polymorphism of vitamin D receptor (VDR) and lead poisoning in Uygur and Han children in China.

*Methods:* The *BsmI*, *ApaI* and *TaqI* restriction sites of VDR genotyping were determined by polymerase chain reaction–restriction fragment length polymorphism in 443 Uygur and 469 Han children from Xinjiang province. The correlation between the polymorphism of *VDR* haplotypes and blood lead levels was explored.

*Results:* The genotype frequencies of *VDR* had significant differences in Han and Uygur children ($p$ <0.01). According to *VDR–BsmI*, *ApaI* and *TaqI* haplotype analysis in Han children, haplotypes Atb and AtB were considerably decreased in the lead poisoning group ($p$ <0.05) while haplotype aTb and ATb were significantly increased in the lead poisoning group ($p$ <0.01). However, such results were not found in Uygur children ($p$ < 0.05).

*Conclusion:* A significant difference was seen in the frequency distribution of the *VDR* genotype among the different races. Haplotypes Atb and AtB might be protective factors while haplotypes ATb and aTb might be risk factors in Han children.

**Keywords:** *Vitamin D receptor; haplotype; lead poisoning; Uygur; Han*

## Introduction

Lead is a common environmental pollutant, which has been known for thousands of years. It is well documented that lead causes a wide variety of detrimental effects on the developing central nervous system, including cognitive deficits (Finkelstein et al. 1998, Bressler et al. 1999, Lidsky & Schneider 2003, Marchetti 2003). Lead poisoning is diagnosed if the blood lead level is higher than 10 μg $dl^{-1}$ (US Centers for Disease Control and Prevention (CDC) 1997). It is more frequent in children than in adults, because of hand-to-mouth activities, a faster respiratory rate and better intestinal absorption of lead, and these factors increase the uptake of lead (Lin-Fu 1973, Ziegler et al. 1978). It is noteworthy that in children, the blood lead level is determined by multifactors, including the exposure and some unidentified genetic susceptibilities (CDC 1997, Lanphear et al. 2002, Guidotti & Ragain 2007).

The secosteroid hormone vitamin D, its receptor (VDR) and the metabolizing enzymes involved in the formation of the biologically active form of the hormone together are major players in the vitamin D endocrine system. This system plays an important role in the skeletal metabolism, including intestinal calcium absorption (Haussler et al. 1998). The gene coding for the vitamin D receptor (*VDR*) is located on chromosome 12q13 (Baker et al. 1988; Miyamoto et al. 1997). Previous studies showed that the polymorphism in a *BsmI* restriction site in the intron between exons γ and η (Zmuda et al.

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2000) is associated with the uptake of lead (Weaver et al. 2005, Schwartz et al. 2000). However, little information is available about the association between blood lead levels and the polymorphism in *ApaI* and *TaqI* restriction sites of *VDR* (Rezende et al. 2008).

Linkage disequilibrium (LD) measures describe the association (or co-occurrence) of alleles of polymorphisms adjacent to each other (Wall & Pritchard 2003); haplotypes are blocks of linked alleles of adjacent polymorphisms whereby the length of such a block coincides with the strength of LD across the area. The frequent haplotype alleles can be found around the polymorphic variation within 10–20 kb. In practice, this also means that relatively few polymorphisms have to be genotyped to 'cover' the variance in a certain area. Therefore, a massive effort was made to determine a haplotype map of the human genome (International HapMap Consortium 2003, Wall & Pritchard 2003, Barnes 2006).

In the present cross-sectional study, we investigated the combined effects (haplotype analysis) of three polymorphisms (*BsmI*, *ApaI* and *TaqI*) of *VDR* on blood lead levels in children of two races, Uygur and Han, in order to examine the contribution of the *VDR* haplotype on the variability of blood lead levels.



## Materials and methods

### *Materials*

High purity deionized water (18.2 mΩcm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All reagents used were of high purity analytical grade. All tubes, plastic bottles, cups and glassware materials were cleaned by soaking in 10% v/v $HNO_3$ for 24 h and rinsing five times with Milli-Q water.

### *Study population*

A total of 443 Uygur children and 469 Han children from the different schools in the same community, aged 6–10 years, were enrolled in the study after informed consent was obtained from April to June 2005 at Urumqi, China. Each subject completed an interviewer-administered questionnaire including information about age, gender, living conditions, lifestyle, and health status, etc.

### *Blood collection*

Venous blood samples were collected from each subject in two separated fractions of 4 ml: one evacuated tube containing lyophilized heparin (Vacutainer BD, Franklin Lakes, NJ, USA; trace metals free) for metal analysis, and one containing EDTA (Vacutainer BD,) for genotyping. Before collection, the skin of the children was cleaned with alcohol and MilliQ water. And blood samples were stored at −20°C, until used for blood lead measurement and *VDR* genotyping.

### *Determination of lead in blood*

Blood lead level was measured by graphite furnace atomic absorption spectrophotometry (Zhang et al. 2000).

### *DNA extraction*

Genomic DNA was extracted from blood samples by a routine phenol–chloroform method (Miller et al. 1988).

### *VDR genotyping*

An assay based on polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to determine the genotype of *BsmI*, *ApaI* and *TaqI* restriction sites in the *VDR* gene. The primer sequence was designed by Oligo 6.0 software. Briefly, the genotypes were determined by PCR-RFLP, and the primers, annealing temperature, length of amplified fragments, restriction pattern and restriction enzymes used are listed in Table 1. Genomic DNA (50–100 ng) was used as a

**Table 1** Primer sequences, condition for amplification, restriction pattern and restriction enzymes used.

| Polymorphisms | Primer sequence | Annealing temperature (°C) | Length (bp) | Restriction enzyme | Restriction or PCR pattern (bp) |
|---|---|---|---|---|---|
| *VDR* | F: 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′ | 58 | 800 | *BsmI* | BB: 800 |
| *BsmI* site | R: 5′-AACCAGCGGGAAGAGGTCAAGGG-3′ | | | | Bb: 800, 650, 150 |
| | | | | | bb: 650, 150 |
| *VDR* | F: 5′-GTGCGCCCATGGAAGGACCTAGGTCTG-3′ | 70 | 461 | *ApaI* | AA: 461 |
| *ApaI* site | R: 5′-AAACACTTCGAGCACAAGGGGCGTTA-3′ | | | | Aa: 461, 174, 287 |
| | | | | | aa: 174, 287 |
| *VDR* | F: 5′-GTGCGCCCATGGAAGGACCTAGGTCTG-3′ | 70 | 461 | *TaqI* | TT: 451, 10 |
| *TaqI* site | R: 5′-AAACACTTCGAGCACAAGGGGCGTTA-3′ | | | | Tt: 451, 250, 201, 10 |
| | | | | | tt: 250, 201, 10 |

PCR, polymerase chain reaction.

template. PCR was performed in a 25-μl reaction volume containing 50–100 ng genomic DNA, 0.2 μmol $l^{-1}$ of each primer, 1 × PCR buffer, 0.2 μmol $l^{-1}$ of each deoxynucleotide triphosphate, 2.0 mmol $l^{-1}$ $MgCl_2$ and 0.75 units DNA Taq polymorphism. The PCR programme was a 5-min denaturation step at 95°C followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 45 s and a final extension step at 72°C for 5 min.

The variant alleles in VDR create *BsmI*, *ApaI* and *TaqI* sites, respectively. After digestion at 37°C for hours the products were resolved on 2–3% agarose gels and the digested PCR products were then observed under an ultraviolet image system. All genotypes were evaluated and agreed on by at least two people independently. Ten per cent of the DNA samples were selected randomly for repeats and the concordance was 100%.

### *Statistical analysis*

All data were analysed by using SPSS 10.0 software (SPSS Inc., Chicago, IL, USA). The linkage disequilibrium was done by using 2 LD and the haplotype analysis was done by using Phase 2.0. Values are shown as mean ± SD; $p < 0.05$ was considered statistically significant.

## Results

### *The blood lead levels in Uygur and Han children*

In the 912 children, the blood lead levels ranged from 0.5 to 48.2 μg $dl^{-1}$, with an average of 5.45 ± 0.22 μg $dl^{-1}$, while 23.36% (213) of the children had a blood lead level >10 μg $dl^{-1}$ (lead poisoning). The blood lead levels were the same in Uygur children (5.57 ± 0.223 μg $dl^{-1}$, $n = 443$) and Han children (5.30 ± 0.224 μg $dl^{-1}$, $n = 469$) ($t = 0.90$, $p = 0.37$). The incidence of lead poisoning was also the same in Uygur (23.26%) and Han (23.45%) children ($\chi^2 = 0.0053$, $p = 0.99$). The blood lead levels at different ages (<7, 8–9, >10 years) were the same in Uygur ($F = 2.36$, $p > 0.05$, ANOVA, df = 2)) and Han children ($F = 9.64$, $p > 0.05$, ANOVA, df = 2). Also the blood levels in different genders were the same in Uygur ($t = 1.96$, $p = 0.16$) and Han children ($t = 0.00$, $p = 0.96$).

### *VDR genotype and blood lead concentration*

All of the allele frequencies of VDR in the three restriction sites complied with Mendel's law (Table 2). At *BsmI*, *ApaI* and *TaqI* restriction sites, the allele frequencies of B, A, and T were significantly different between Uygur (B: 0.18; A: 0.46; T: 0.54) and Han children (B: 0.06; A: 0.28; T: 0.94) children. As the polymorphisms of *BsmI*, *ApaI* and *TaqI* are associated with *VDR* function, including expression and efficacy (Howard et al. 1995, Matsuyama et al. 1995, Tokita et al. 1996, Uitterlinden et al. 2004), we tested whether these polymorphisms contribute to the variable blood lead levels. As illustrated in Table 3, in Uygur and Han children, those with the BB/Bb genotype have higher blood lead levels than those who do not ($p = 0.00$), and the blood lead levels are the same between the Uygur and Han children with *ApaI* site AA/Aa or *TaqI* site TT genotype.

### *VDR BsmI–ApaI–TaqI haplotype and lead poisoning in Uygur and Han children*

The haplotype analysis was done because LD occurred on the *BsmI–ApaI–TaqI* loci of the *VDR* gene (Uitterlinden et al. 2004). 2 LD analysis also showed that there were significant linkages among the genotypes of *VDR* in three loci in both Han and Uygur children. The combination of alleles in the three loci generated eight haplotypes – ATb, ATB, Atb, AtB, aTb, aTB, Atb, atB – and the frequency of these haplotypes were, respectively, 0.09, 0.01, 0.31,

**Table 2.** The genotype frequencies of VDR *BsmI*, *ApaI* and *TaqI* sites in Uygur and Han children.

| | | Uygur children | | Han children | | | |
|---|---|---|---|---|---|---|---|
| Gene | Genotype | *n* | % | *n* | % | $\chi^2$ | *p*-Value |
| *VDR* (*BsmI*) | BB | 17 | 3.94 | 0 | 0 | 332.06 | 0.00 |
| | Bb | 124 | 28.77 | 54 | 11.51 | | |
| | bb | 290 | 67.29 | 415 | 88.49 | | |
| *VDR* (*ApaI*) | AA | 92 | 21.90 | 40 | 8.53 | 199.45 | 0.00 |
| | Aa | 200 | 47.62 | 182 | 38.81 | | |
| | aa | 128 | 30.48 | 247 | 52.66 | | |
| *VDR* (*TaqI*) | TT | 125 | 29.76 | 411 | 87.63 | 506.76 | 0.00 |
| | Tt | 208 | 49.52 | 58 | 12.37 | | |
| | tt | 87 | 20.72 | 0 | 0 | | |

**Table 3.** VDR *BsmI*, *ApaI* and *TaqI* sites and blood lead levels of Uygur and Han children.

| | | Han population | | | Uygur population | | |
|---|---|---|---|---|---|---|---|
| | | | Blood lead level (μg $dl^{-1}$), | | | Blood lead level (μg $dl^{-1}$), | |
| Genotype | | *n* | mean ± SD | *p*-Value | *n* | mean ± SD | *p*-Value |
| *VDR* (*BsmI*) | BB/Bb | 54 | 9.55 ± 0.14 | 0.00 | 141 | 8.81 ± 0.16 | 0.0000 |
| | bb | 415 | 4.90 ± 0.22 | | 290 | 4.47 ± 0.23 | |
| *VDR* (*ApaI*) | AA/Aa | 222 | 5.03 ± 0.22 | 0.39 | 292 | 5.49 ± 0.22 | 0.87 |
| | aa | 247 | 5.62 ± 0.22 | | 128 | 5.75 ± 0.24 | |
| *VDR* (*TaqI*) | TT | 411 | 5.25 ± 0.22 | 0.39 | 125 | 5.75 ± 0.22 | 0.84 |
| | Tt/tt | 58 | 5.75 ± 0.21 | | 295 | 5.49 ± 0.23 | |

0.25, 0.28, $0.14 \times 10^{-2}$, 0.03 and 0.02 in Uygur children, and 0.22, 0.04, 0.15, 0.04, 0.24, 0.05, 0.21 and 0.05 in Han children. Han and Uygur children have different frequencies in some haplotypes, e.g. ATb ($\chi^2 = 6.45$, $p = 0.01$), Atb ($\chi^2 = 7.23$, $p = 0.0046$) and AtB ($\chi^2 = 17.79$, $p < 0.001$) (Table 4).

To test the relationship between the haplotypes and the susceptibility of lead poisoning, we compared the frequencies of the haplotypes in children with lead poisoning and normal Han and Uygur children. In Han children, the frequencies of both haplotypes Atb and AtB were considerably lower in children with lead poisoning than in normal children, while the frequencies of haplotypes aTb and ATb were significantly higher in children with lead poisoning ($p < 0.05$, $p < 0.01$) (Tables 5 and 6). However, the frequencies of all haplotypes were not significantly different in children with lead poisoning and normal Uygur children (Table 7).

## Discussion

The present study is the first to report the polymorphism of *VDR* in three restriction loci and the haplotypes in a Uygur population. Similarly to Han children, Uygur children with the B allele at the *BsmI* restriction site have higher blood lead levels. In Han children, the haplotype analysis shows that haplotypes Atb and AtB might be protective factors while haplotypes ATb and aTb might be risk factors. These may provide a biomarker for screening and protecting the susceptible population.

**Table 4.** Haplotype frequencies in Uygur and Han children.

| Haplotype | Frequency in Uygur children | Frequency in Han children | $\chi^2$ | *p*-Value |
|---|---|---|---|---|
| ATb | 0.22 | 0.09 | 6.45 | 0.01 |
| ATB | 0.04 | 0.01 | 0.82 | 0.73 |
| Atb | 0.15 | 0.31 | 7.23 | 0.0046 |
| AtB | 0.04 | 0.25 | 17.79 | 0.0000 |
| aTb | 0.24 | 0.28 | 0.42 | 0.53 |
| aTB | 0.05 | $0.14 \times 10^{-2}$ | 6.11 | 0.01 |
| Atb | 0.21 | 0.03 | 15.34 | 0.0000 |
| atB | 0.05 | 0.02 | 0.59 | 0.42 |

**Table 5.** Haplotype frequencies of Han children in lead poisoning and non-lead poisoning groups.

| Haplotype | Frequency | Lead poisoning | Non-lead poisoning | $\chi^2$ | *p*-Value |
|---|---|---|---|---|---|
| ATb | 0.09 | 0.16 | 0.09 | 4.89 | 0.03 |
| ATB | 0.01 | 0.01 | 0.01 | 0.00 | 0.99 |
| Atb | 0.31 | 0.07 | 0.34 | 31.56 | 0.00 |
| AtB | 0.25 | 0.09 | 0.26 | 15.04 | 0.00 |
| aTb | 0.28 | 0.60 | 0.25 | 49.71 | 0.00 |
| aTB | $0.14 \times 10^{-2}$ | $0.01 \times 10^{-2}$ | $0.16 \times 10^{-2}$ | 0.15 | 0.70 |
| Atb | 0.03 | 0.03 | 0.03 | 0.08 | 0.78 |
| atB | 0.02 | 0.03 | 0.02 | 0.43 | 0.51 |

The cellular actions of hormonal vitamin D depend on its interaction with the gene of VDR that regulates the production for calcium-binding proteins. The *VDR* gene exists in several polymorphic forms in humans – *BsmI*, *ApaI* and *TaqI* restriction sites. The current study found the allele frequencies of *VDR* restriction sites *BsmI*, *ApaI* and *TaqI* among Uygur children are, respectively, 0.18 for B, 0.46 for A and 0.54 for T, which is significantly different from Han children (0.06 for B, 0.28 for A and 0.94 for T), in keeping with the notion that *VDR* polymorphism has been demonstrated to vary with race. The B allele of *BsmI* RFLP has a lower frequency in Asians (4–8%) compared with Caucasians and Africans (17–22% ) (Morrison et al. 1994, Garnero et al. 1995, Zhang et al. 2006), The A allele of *ApaI* RFLP has a frequency in Asians of 27% compared with 31–44% in Caucasians and Africans (Wang et al. 2006, Uitterlinden et al. 2004), and also the T allele of *TaqI* RFLP has a higher frequency in Asians (85–96%) compared with Caucasians and Africans (29–41% ) (Nishijima et al. 2002, Uitterlinden et al. 2004). Taken together, the frequency of *VDR* restriction sites *BsmI*, *ApaI* and *TaqI* among Uygur children are similar to the Caucasian population, while those in Han children are similar to the Asian population.

In our study, the blood lead level of children varied from 0.5 to 48.2 μg $dl^{-1}$, the mean blood lead concentration

**Table 6** Haplotype analysis of VDR genotyping and lead poisoning in Han children.

| | Atb | | ATb | | AtB | | aTb | |
|---|---|---|---|---|---|---|---|---|
| Group | + | − | + | − | + | − | + | − |
| Lead poisoning | 8 | 106 | 10 | 104 | 10 | 104 | 69 | 45 |
| Non-lead poisoning | 116 | 229 | 54 | 291 | 91 | 254 | 86 | 259 |
| Total | 124 | 335 | 64 | 395 | 101 | 358 | 155 | 304 |
| $\chi^2$ | 30.76 | | 3.38 | | 15.47 | | 48.56 | |
| p-Value | 0.00 | | 0.03 | | 0.00 | | 0.00 | |
| Odds ratio | 0.15 | | 0.52 | | 0.27 | | 4.62 | |
| 95% Confidence interval | 0.07-0.32 | | 0.26–1.06 | | 0.13–0.54 | | 2.95–7.23 | |

**Table 7** Haplotype frequencies in Uygur children in lead poisoning and non-lead poisoning groups.

| Haplotype | Frequency | Lead poisoning | Non-lead poisoning | $\chi^2$ | *p*-Value |
|---|---|---|---|---|---|
| ATb | 0.22 | 0.23 | 0.18 | 1.33 | 0.25 |
| ATB | 0.04 | 0.04 | 0.04 | 0.01 | 0.92 |
| Atb | 0.15 | 0.16 | 0.13 | 0.67 | 0.41 |
| AtB | 0.04 | 0.03 | 0.06 | 1.96 | 0.16 |
| aTb | 0.24 | 0.23 | 0.27 | 0.70 | 0.40 |
| aTB | 0.05 | 0.05 | 0.08 | 2.27 | 0.13 |
| Atb | 0.21 | 0.22 | 0.18 | 0.81 | 0.37 |
| atB | 0.05 | 0.04 | 0.06 | 0.42 | 0.52 |

was 5.45 ± 0.22 μg $l^{-1}$ and the blood lead concentration of 23.36% of children was >10 μg $dl^{-1}$. The blood lead level of Uygur children was slightly higher than in Han children, but no significant difference was found. The blood lead concentration of children in Urumqi was higher than in Dalian (4.51 μg $l^{-1}$) and Qingdao (4.74 μg $l^{-1}$), and similar to Beijing (5.26 μg $l^{-1}$) and Guangzhou (5.97 μg $l^{-1}$) in China (Zhang et al. 2005). The high-risk factors of parents' education, parents' occupation, bad habits, housing condition and living environment were associated with blood lead levels. It is important for prevention and control of lead poisoning in children to direct against the selected factors.

Most studies of the *VDR* gene have focused on the *BsmI* polymorphism. The BB genotype has been associated with lower bone mineral density and other diseases, particularly in women and may play a role in the development of osteoporosis (Lurie et al. 2007, Gómez-Vaquero et al. 2007, Morrison et al. 1992). Lead can substitute for calcium, which is a common mechanism underlying its toxic effects Finkelstein et al. 1998, Bressler et al. 1999, Marchetti 2003). It is possible that the genetic polymorphism in *VDR* may change the susceptibility to lead poisoning. In our study, we found that the *VDR* polymorphism of the *BsmI* restriction site affected blood lead levels for Uygur and Han children. It is consistent with previous studies showing that the BB genotype is associated with an uptake of lead and susceptibility to lead toxicity (Rezende et al. 2008, Weaver et al. 2005, Schwartz et al. 2000).

The functional significance of the *BsmI* polymorphism is unclear because it is not located at the exon–intron boundaries, and would not influence the structure of the *VDR*; and recent *in vitro* studies have not demonstrated differences in VDR expression or cellular responsiveness to vitamin D treatment by genotype (Uitterlinden et al. 2004, Zmuda et al. 2000). This suggests that the *BsmI* polymorphism may be in LD with another functional variant at the VDR locus. LD occurred on the *BsmI* site*, ApaI* site and *TaqI* site of the *VDR* gene, which we called the *BsmI–ApaI–TaqI* haplotype. We found eight haplotypes existed in both Uygur and Han children, but haplotypes Atb and AtB were considerably decreased in the lead poisoning group while haplotypes aTb and ATb were significantly increased in the lead poisoning group of Han children; these results were not found in Uygur children.

In the present study, blood lead levels varied from 0.5 to 48.2 μg $dl^{-1}$, and the mean blood lead level was 5.45 ± 0.22 μg $dl^{-1}$, which is barely below the normal reference level currently set at 10 μg $dl^{-1}$. However, recent studies have reported the possibility of adverse effects, including intellectual impairment in young children, at blood lead levels <10 μg $dl^{-1}$, suggesting that there is no safe level of exposure (Fulton et al. 1987, Lanphear et al. 2000, Koller et al.2004). In addition, measurements of the whole blood are still the most reliable indicator of recent lead exposure, although some alternatives include lead determinations in plasma/serum, saliva, bone, teeth, feces and urine (Barbosa et al. 2005, Costa de Almeida et al. 2007).

In conclusion, different *VDR BsmI* RFLP genotypes were shown to be associated with blood lead levels in environmentally exposed Uygur and Han children, especially the *BsmI–ApaI –TaqI* haplotype for Han children. A significant *VDR* allele difference existed in Uygur and Han populations; it is important for researchers to provide the basic information of *VDR* genotype for Chinese populations, especially Chinese Uygur populations.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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