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Vitamin D receptor haplotypes affect lead levels during pregnancy

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ABSTRACT

Pregnant women are particularly susceptible to toxic effects associated with lead (Pb) exposure. Pb accumulates in bone tissue and is rapidly mobilized from bones during pregnancy, thus resulting in fetal contamination. While vitamin D receptor (VDR) polymorphisms modify bone mineralization and affect Pb biomarkers including blood (Pb-B) and serum (Pb-S) Pb concentrations, and %Pb-S/Pb-B ratio, the effects of these polymorphisms on Pb levels in pregnant women are unknown. This study aimed at examining the effects of three (*FokI*, *BsmI* and *ApaI*) VDR polymorphisms (and VDR haplotypes) on Pb levels in pregnant women. Pb-B and Pb-S were determined by inductively coupled plasma mass spectrometry in samples from 256 healthy pregnant women and their respective umbilical cords. Genotypes for the VDR polymorphisms were determined by PCR and restriction fragment length digestion. While the three VDR polymorphisms had no significant effects on Pb-B, Pb-S or %Pb-S/Pb-B ratio, the haplotype combining the *f*, *a*, and *b* alleles for the *FokI*, *ApaI* and *BsmI* polymorphisms, respectively, was associated with significantly lower Pb-S and %Pb-S/Pb-B ($P<0.05$). However, maternal VDR haplotypes had no effects on Pb levels in the umbilical cords. To our knowledge, this is the first study showing that a combination of genetic polymorphisms (haplotype) commonly found in the VDR gene affects Pb-S and %Pb-S/Pb-B ratios in pregnant women. These findings may have major implications for Pb toxicity because they may help to predict the existence of a group of subjects that is genetically less prone to Pb toxicity during pregnancy.

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1. Introduction

Pregnant women are particularly susceptible to the toxic effects resulting from lead (Pb) exposure. This is because increased demands for calcium during pregnancy increase bone turnover, thus increasing the circulating Pb levels by removing Pb from bones (Gulson et al., 1997; Tellez-Rojo et al., 2004). Pb from the mother can easily cross the placenta and expose the fetus to Pb (Goyer, 1990). Indeed, Pb accumulates in bones and has a half-life of years or decades, so that Pb transference to the fetus may take place even years after maternal exposure (Rothenberg et al., 2000; Tellez-Rojo et al., 2004). This exposure is known to affect the embryonic development of multiple organ systems and cause retardation of cognitive development (Banks et al., 1997). Recent results clearly indicate reduced intellectual development in children with history of prenatal lead exposure (Schnaas et al., 2006). Therefore assessing Pb levels during pregnancy may help to predict these harmful effects associated with Pb, especially in pregnant women with some predisposing factors (Montenegro et al., 2008).

While Pb levels are usually measured in blood (Pb-B), plasma (Pb-P), or serum Pb (Pb-S) concentrations, or %Pb-S/Pb-B ratios are considered very relevant to assess Pb toxicity. This is because the most toxic Pb fraction is reflected by these biomarkers, which correspond to the diffusible Pb fraction in the body (Barbosa et al., 2005; Smith et al., 2002). Importantly, pregnancy is associated with significant increases in these biomarkers (Amaral et al., 2010; Montenegro et al., 2008), which are clearly affected by genetic polymorphisms (Montenegro et al., 2006; Onalaja and Claudio, 2000; Rezende et al., 2008).

Vitamin D and its active metabolites (especially 1,25-dihidroxyvitamin D, or calcitriol) are primarily involved in maintaining calcium homeostasis (Uitterlinden et al., 2004), and vitamin D receptor (VDR) polymorphisms affect bone mineralization and resorption (Morrison et al., 1994; Valdivielso and Fernandez, 2006). However, although VDR polymorphisms apparently modify Pb toxicity (Onalaja and Claudio, 2000; Rezende et al., 2008; Schwartz et al., 2000a,b), no previous study has examined whether VDR polymorphisms affect the circulating levels of Pb in pregnant women.

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In the present study, we examined the effects of three single nucleotide polymorphisms (*BsmI*, *ApaI* and *FokI*) in the *VDR* gene on Pb-B, Pb-S, and %Pb-S/Pb-B ratio in pregnant women. We then examined whether there is a relationship between *VDR* gene haplotypes and Pb-B, Pb-S, and %Pb-S/Pb-B ratios in pregnant women, and between maternal *VDR* gene haplotypes and these biomarkers of Pb exposure measured in the respective umbilical cords.

2. Materials and methods

2.1. Subjects

Approval for the use of human subjects was obtained from the Institutional Review Board of the Faculty of Medicine of Ribeirao Preto and each subject provided written informed consent. We studied 256 peripartum healthy pregnant with at least 38 weeks of gestation, living in the city of Ribeirao Preto, State of Sao Paulo, Brazil, and their respective umbilical cord samples. Although previous studies showed evidence indicating that some exposure to lead would be detected in Ribeirao Preto (Costa de Almeida et al., 2010; Costa de Almeida et al., 2009; Gomes et al., 2004), the peripartum women included in the present study came from different regions.

Maternal blood samples were drawn from each volunteer before delivery. Umbilical cord blood samples were drawn from the umbilical vein immediately after delivery. Maternal and umbilical cord samples were collected into evacuated trace metal free tubes containing EDTA (Vacutainer, Becton-Dickinson, Brazil) to obtain whole blood, and into trace metal free tubes containing no anticoagulants and centrifuged ($800 \times g$, 6 min) to obtain serum. Each blood sample or serum fraction was then pipetted into ultra-cleaned Eppendorfs tubes (2 mL) and immediately frozen at −80 °C until used for analysis (Rezende et al., 2010).

Genomic DNA was extracted from the cellular component of 1 mL of whole blood by a salting-out method and stored at −20 °C until analyzed.

2.2. Instrumentation

Analysis were carried out with an inductively coupled plasma mass spectrometer equipped with a reaction cell (DRC-ICP-MS ELAN DRCII, PerkinElmer, SCIEX, Norwalk, CT, USA) operating with high-purity argon (99.999%, Praxaair, Brazil). Sample introduction system included a quartz cyclonic spray chamber and a Meinhard® nebulizer connected by Tygon® tubes to the ICP-MS's peristaltic pump (set at 20 rpm). The ICP-MS was operated with Pt sampler and skimmer cones purchased either from Perkin Elmer. A radiofrequency (rf) of 1100 watts power was selected in pulse mode with autolens one. Sample data were acquired by using 20 sweeps/reading, 1 reading/replicate and a 50 ms dwell time. Argon nebulizer gas flow rate was optimized daily from 0.5 to 0.9 L min^{-1}. Data were acquired in counts per second (cps). The ^{208}Pb isotope was selected. The detection limit for lead was 0.0001 µg/dL.

2.3. Materials and reagents

High-purity de-ionized water (resistivity 18.2 MΩ cm^{-1}) used for the preparation of samples and solutions was obtained using a Milli-Q water purification system (Millipore RiOs-DITM, Bedford, MA, USA). All used reagents were of analytical-reagent grade, except HNO_3, which was previously purified in a quartz sub-boiling stills (Kürner Analysentechnik) before use. A clean laboratory and laminar-flow hood capable of producing class 100 was used to prepare solutions. Rhodium (1000 mg L^{-1}) and a multi-element (10 mg L^{-1}) solution were obtained from PerkinElmer (Shelton, CT, USA). Triton® X-100 was purchased from Sigma-Aldrich (St. Louis, USA), and gaseous 5% hydrogen/95% argon (99.999%, Praxair, Brazil). Plastic bottles and cryogenic vials were cleaned by soaking in 10% (v/v) HNO_3 for 24 h, rinsing five times with Milli-Q water, and dried in a class 100 laminar-flow hood before use. Sample preparation and analysis were performed in a clean class 1000 room.

Serum and blood samples were stored in 2 mL tubes at −80 °C. All tubes, plastic bottles, autosampler cups, and glassware materials were cleaned by soaking in 10% v/v HNO_3 for 24 h, rinsing five times with Milli-Q water, and dried in a class 100 laminar-flow hood located within the class 10,000 clean room.

2.4. Genotyping

2.4.1. Fok I polymorphism (rs 10735810)

Genotypes for the *Fok I* polymorphisms in exon 2 were determined by PCR using the primers: 5′-GATGCCAGCTGGCCCTGGCACTG-3′ and 5′-ATGGAAACACCTTG CTTCTTCTCCCTC-3′ (Rezende et al., 2007). The PCR was performed in a 25 µl reaction volume containing 0.20 µM of each primer, 200 µM of each dNTP, 1 X PCR buffer supplied by Invitrogen Corp. (10 mM Tris–HCl, pH 8.8, 50 mM KCl), 2.0 mM MgCl2, and 2.5 U of DNA Taq polymerase (Biosystems). The running conditions were: predenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 69 °C for 30 s, and extension at 72 °C for 30 s. Finally, extension was conducted at 72 °C for 3 min. The amplified products were digested with *FokI* (Fermentas Life Sciences) for 3 h at 55 °C, producing fragments of 272 bp for wild-type allele (allele "F"), or 198 and 74 bp in the case of a polymorphic variant (allele "f"). The fragments were separated by electrophoresis in 8% polyacrylamide gels and visualized by silver staining (Fig. 1).

2.4.2. BsmI polymorphism (rs 1544410)

Genotypes for the *BsmI* polymorphism in intron 8 were determined by polymerase chain reaction (PCR) using the primers 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′ and 5′-AACCAGCGGGAAGAGGTCAAGGG-3′ as previous described (Rezende et al., 2007). The PCR reaction was performed in a 25 µl reaction volume that included approximately 500 ng of template genomic DNA, 0.5 µM of each primer, 200 µM of each dNTP, 1 X PCR buffer supplied by Invitrogen Corp. (10 mM Tris–HCl, pH 8.8, 50 mM KCl), 2.0 mM MgCl2, and 2.5 U of DNA Taq polymerase (Biosystems, Curitiba, Brazil). The running conditions were: holding at 94 °C for 5 min, then 35 cycles of denaturation at 94 °C for 30 s, annealing at 67 °C for 30 s and extension at 72 °C for 1 min. The resulting 825-bp fragment was digested with *BsmI* (New England Biolabs) for 2 h at 65 °C, producing fragments of 825 bp for wild-type allele (allele "B"), or 650 and 175 bp in the case of a polymorphic variant (allele "b"). Fragments were separated by electrophoresis in 8% polyacrylamide gels and visualized by silver staining (Fig. 1).

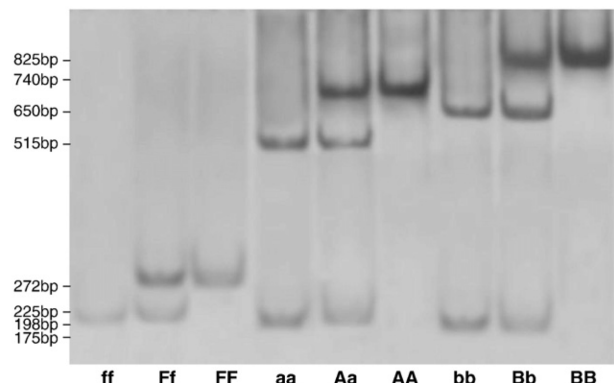

Fig. 1. Genotyping for the *FokI*, *ApaI*, and *BsmI*, VDR gene polymorphisms. The PCR products were digested with restriction enzymes producing different fragments leading to specific genotypes.

2.4.3. ApaI polymorphism (rs 7975232)

For the detection of the *ApaI* polymorphism in intron 8, the primers 5′-CAGAGCATGGACAGGGAGCAAG-3′ and 5′-CAACTCCTCATGGCTGAGGTCTC-3′ were used in a PCR (Rezende et al., 2007). The PCR was performed in a 25 µl reaction volume containing 0.25 µM of each primer, 200 µM of each dNTP, 1X PCR buffer supplied by Invitrogen Corp. (10 mM Tris–HCl, pH 8.8, 50 mM KCl), 2.0 mM MgCl2, and 2.5 U of DNA Taq polymerase (Biosystems). The running conditions were: predenaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 68 °C for 1 min, and extension at 72 °C for 1 min. Finally, extension was conducted at 72 °C for 7 min. The amplified products were digested with *ApaI* (Fermentas Life Sciences) for 2 h at 37 °C, producing fragments of 740 bp for wild-type allele (allele "A"), or 515 and 225 bp in the case of a polymorphic variant (allele "a"). The fragments were separated by electrophoresis in 8% polyacrylamide gels and visualized by silver staining (Fig. 1).

2.5. Haplotype inference

Haplotypes were inferred using the Bayesian statistical based program PHASE version 2.1 (http://www.stat.washington.edu/stephens/software.html) (Stephens et al., 2001) to estimate the haplotype frequencies in the population and the two haplotypes for each subject (Metzger et al., 2007; Palei et al., 2010). These results were used to evaluate a possible relationship between haplotypes and Pb-B or Pb-S, and %PbS/PbB ratio. The possible haplotypes including genetic variants of three VDR polymorphisms studied (*FokI, ApaI*, *BsmI*) were: H1(FAB), H2(FAb), H3(FaB), H4(Fab), H5(fAB), H6(fAb), H7(faB), and H8 (fab).

2.6. Statistical analysis

The distribution of genotype for each polymorphism was assessed for deviation from the Hardy–Weinberg equilibrium by using chi-squared tests (StatView, Cary, NC, USA). To assess the potential relationship between each polymorphism or haplotype and Pb-B, Pb-S and %Pb-S/Pb-B ratio, we used the Kruskal–Wallis test followed by Dunn's multiple comparison tests. To reduce the degrees of freedom and increase the power of our haplotype-based analysis, we excluded a priori uncommon haplotype (haplotype frequency <10%) from the analysis. Data were reported as the mean ± SEM. A *P*-value less than 0.05 was considered to be statistically significant.

Table 1
Demographic characteristics of the participants.

Variables	Total (*n* = 256)
Pregnant mothers	Mean ± S.E.M. (or %)
Age (years)	24.0 ± 4.1
Hemoglobin (g/dL)	12.7 ± 1.4
Hematocrit (%)	37.1 ± 1.1
Use of ferrous sulfate	231 (98.0)
Never smoked (%)	202 (79.0)
Smoke or smoked during the pregnancy (%)	54 (21.0)
Blood lead concentration (µg/dL)	1.889 ± 0.079
Serum lead concentration (µg/dL)	0.041 ± 0.009
Serum/blood lead ratio (%)	2.490 ± 0.091
Umbilical cord	
Blood lead concentration (µg/dL)	1.129 ± 0.069
Serum lead concentration (µg/dL)	0.025 ± 0.009
Serum/blood lead ratio (%)	2.390 ± 0.990
Newborns	
Birthweigth (g)	3212 ± 0.037
Apgar scores for newborns (at first minute)	8.4 ± 1.0
Apgar scores for newborns (at five minutes)	9.3 ± 0.6

3. Results

We studied two hundred and fifty-six peripartum healthy pregnant with at least 38 weeks of gestation. The distribution of genotypes for the three polymorphisms studied here showed no deviation from the Hardy–Weinberg equilibrium. The allele frequencies for *F, A* and *B* alleles for the *FokI, ApaI,* and *BsmI* polymorphisms were 0.66, 0.53 and 0.37 respectively. Correspondingly, the allele frequencies for the *f, a,* and *b*

Table 2
Whole blood lead concentrations (Pb-B), serum lead concentrations (Pb-S) and %Pb-S/Pb-B ratios in pregnant mothers and in the respective umbilical cords grouped by pregnant mothers genotype.

FokI (*VDR*)	Pregnant *FF* (*N* = 106)		Pregnant *Ff* (*N* = 127)		Pregnant *ff* (*N* = 23)		
	Pregnant	Umbilical cord	Pregnant	Umbilical cord	Pregnant	Umbilical cord	*P*
Pb-B (µg/dL)	1.91 ± 0.11	1.11 ± 0.06	1.92 ± 0.09	1.16 ± 0.05	1.78 ± 0.19	1.19 ± 0.10	NS
Pb-S (µg/dL)	0.041 ± 0.002	0.026 ± 0.002	0.041 ± 0.002	0.025 ± 0.001	0.033 ± 0. 005	0.022 ± 0.003	NS
Pb-S/ Pb-B (%)	2.68 ± 0.19	2.72 ± 0.20	2.52 ± 0.14	2.48 ± 0.15	2.05 ± 0.33	2.10 ± 0.35	NS
ApaI (*VDR*)	Pregnant *AA* (*N* = 70)		Pregnant *Aa* (*N* = 130)		Pregnant *aa* (*N* = 56)		
	Pregnant	Umbilical cord	Pregnant	Umbilical cord	Pregnant	Umbilical cord	P
Pb-B (µg/dL)	1.85 ± 0.10	1.14 ± 0.06	1.87 ± 0.09	1.18 ± 0.05	2.04 ± 0.16	1.07 ± 0.06	NS
Pb-S (µg/dL)	0.045 ± 0.003	0.027 ± 0.002	0.040 ± 0.002	0.026 ± 0.001	0.036 ± 0.003	0.022 ± 0.002	NS
Pb-S/ Pb-B(%)	2.78 ± 0.20	2.60 ± 0.21	2.68 ± 0.17	2.54 ± 0.16	2.14 ± 0.22	2.48 ± 0.26	NS
BsmI (*VDR*)	Pregnant *BB* (*N* = 30)		Pregnant *Bb* (*N* = 129)		Pregnant *bb* (*N* = 97)		
	Pregnant	Umbilical cord	Pregnants	Umbilical cord	Pregnant	Umbilical cord	P
Pb-B (µg/dL)	1.81 ± 0.18	1.20 ± 0.11	1.81 ± 0.09	1.16 ± 0.08	2.03 ± 0.13	1.19 ± 0.06	NS
Pb-S (µg/dL)	0.047 ± 0.003	0.030 ± 0.003	0.040 ± 0.002	0.025 ± 0.001	0.040 ± 0.002	0.027 ± 0.002	NS
Pb-S/ Pb-B(%)	3.38 ± 0.41	3.06 ± 0.38	2.67 ± 0.16	2.55 ± 0.18	2.49 ± 0.21	2.66 ± 0.20	NS

P: Kruskal–Wallis test followed by the Dunn's multiple comparison tests to compare the three genotype groups.

Table 3
Estimated haplotype frequency in pregnant women.

	Haplotype			Frequency (%)
	FokI	*ApaI*	*BsmI*	
H1 -	*F*	*A*	*B*	12.1
H2 -	*F*	*A*	*b*	21.9
H3 -	*F*	*a*	*B*	9.3
H4 -	*F*	*a*	*b*	17.6
H5 -	*f*	*A*	*B*	12.0
H6 -	*f*	*A*	*b*	8.1
H7 -	*f*	*a*	*B*	5.2
H8 -	*f*	*a*	*b*	13.8

alleles were 0.34, 0.47, and 0.63, respectively. Table 1 shows the characteristics of the pregnant women and the respective umbilical cord samples. No significant difference was found in age, hematological parameters, and smoking status when the genotype groups were compared (data not shown; all $P>0.05$).

Table 2 shows the values of Pb-B, Pb-S and %Pb-S/Pb-B ratio in pregnants and the respective umbilical cord according to the genotype for the three VDR gene polymorphisms in pregnant mothers. We found that VDR polymorphisms had no effects on Pb-B, Pb-S, or %Pb-S/Pb-B ratios (all $P>0.05$) in pregnant women or in the respective umbilical cord samples (Table 2; all $P>0.05$).

The estimated haplotype frequencies for this group of pregnant women are shown in Table 3. Three haplotypes (H3, H6 and H7) were relatively uncommon (frequency <10%) and therefore were excluded from the analysis.

Fig. 2 shows Pb-B, Pb-S and %Pb-S/Pb-B (panels *A*, *B* and *C*, respectively) levels for each haplotype group. Vitamin D receptor gene haplotypes had no effects on Pb-B levels (all $P>0.05$; Fig. 2A). However, the H8 haplotype (which includes the *f*, *a* and *b* alleles for the *FokI*, *ApaI* and *BsmI* polymorphisms, respectively) was associated with lower Pb-S levels than H1 and H2 haplotypes (which include the *F, A,* and *B,* and the *F*, *A* and *b* alles, respectively, for the *FokI*, *ApaI* and *BsmI* polymorphisms; Fig. 2B; both $P<0.05$). In addition, the H8 and the H4 (which includes the *F*, *a* and *b* alleles for the *FokI*, *ApaI* and *BsmI* polymorphisms, respectively) haplotypes were associated with lower %Pb-S/Pb-B ratios than the H1 haplotype (Fig. 2C; both $P<0.05$).

We found no significant associations between maternal VDR genotypes or haplotypes and Pb-B, Pb-S, or %Pb-S/Pb-B ratios in their respective umbilical cords (Fig. 3; all $P>0.05$).

4. Discussion

To our knowledge, this is the first study showing that genetic polymorphisms commonly found in the VDR gene affect Pb-S and % Pb-S/Pb-B ratios in pregnant women. These findings may have major implications for lead toxicity.

While B-Pb has been the primary fluid to diagnose lead exposure, largely because blood lead sampling is recognized as a relatively easy procedure, other biomarkers of internal dose have been proposed (Barbosa et al., 2005). Pb-P may be a more relevant index of exposure, distribution, and health risks associated with Pb because the toxic effects of Pb are primarily associated to the most rapidly exchangeable fraction of Pb in the bloodstream (Barbosa et al., 2005; Schutz et al., 1996). Indeed, previous studies have shown the importance of plasma (or serum) Pb concentrations, especially during pregnancy, because Pb in plasma promptly allows endogenous contamination of the fetus (Gulson et al., 2003; Hu et al., 2006; Lamadrid-Figueroa et al., 2006; Tellez-Rojo et al., 2004). In the present study, we found similar %Pb-S/Pb-B ratios in the maternal blood and in the umbilical cords. These findings support previous results from our group (Amaral et al., 2010). Interestingly, significant correlations exist between biomarkers of Pb (Pb-S, Pb-B, and %Pb-S/Pb-B ratios) exposure measured in the umbilical cords and in the respective mothers (Amaral et al., 2010). Therefore, it is highly probable that the VDR haplotype associated with lower Pb levels protects the fetus against the toxic effects associated with Pb exposure.

It is now known that VDR polymorphisms affect bone mineralization and resorption (Morrison et al., 1994; Valdivielso and Fernandez, 2006), and that Pb-S and %Pb-S/Pb-B ratio depend on the release of lead from the bone (Barbosa et al., 2005; Cake et al.,

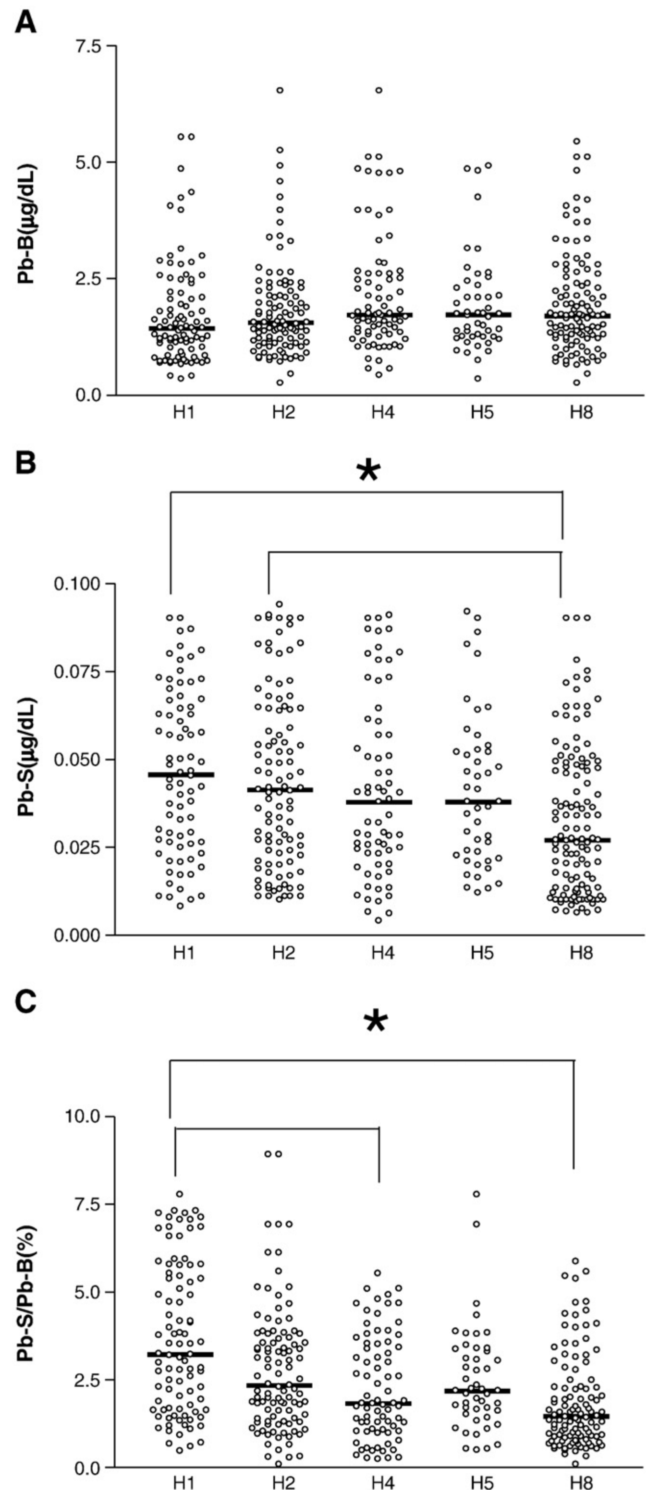

Fig. 2. Whole blood lead (Pb-B; panel A), serum lead (Pb-S; panel B), and %Pb-S/Pb-B ratio (panel C) in pregnants according to VDR haplotype groups H1, H2, H3, H7, and H8. The bar shows the median value. *$P<0.05$ for H8 haplotype versus H1 and H2 haplotype groups (Panel B) and H1 versus H4 and H8 haplotype groups (Panel C).

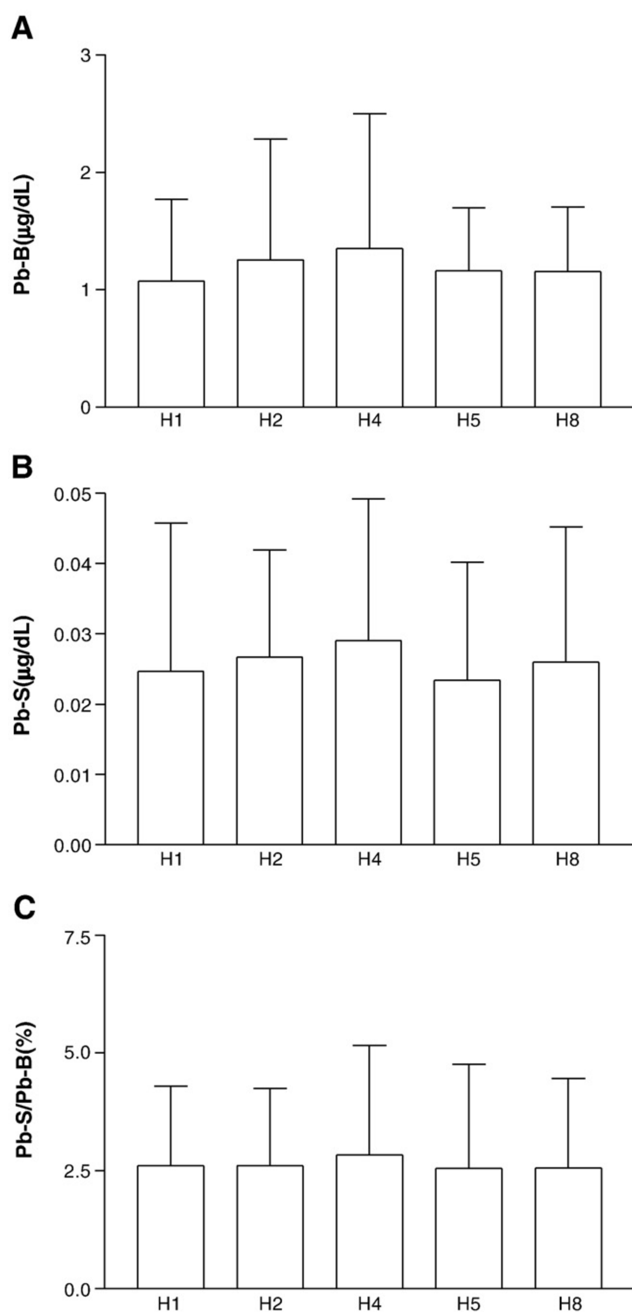

Fig. 3. Whole blood lead (Pb-B; panel A), serum lead (Pb-S; panel B), and %Pb-S/Pb-B ratio (panel C) in the umbilical cords according to maternal VDR haplotype groups H1, H2, H3, H7, and H8. The bars show the means and S.D. No significant differences were found.

1996; Hernandez-Avila et al., 1998), which accounts for >94% of adult body burden of Pb (Barbosa et al., 2005). Therefore, it is reasonable to expect that VDR polymorphisms may affect Pb mobilization (Chuang et al., 2004; Onalaja and Claudio, 2000; Theppeang et al., 2004; Weaver et al., 2006), particularly during pregnancy. We have previously reported that the three VDR polymorphisms studied here (*BsmI*, *ApaI* and *FokI* polymorphisms) affect Pb-B and Pb-P in subjects exposed to Pb. Interestingly, we found lower Pb-P, Pb-B, and %Pb-P/Pb-B levels in the carriers of the H8 haplotype (which combines the *a*, *b*, and *f* alleles for the *ApaI*, *BsmI*, and *FokI* polymorphisms, respectively) (Rezende et al., 2008). Our present findings in pregnant women agree with these previous findings, thus supporting the idea that VDR haplotypes may modify the levels of relevant markers of Pb exposure, even though individual VDR polymorphisms had no significant effects. Although we have not assessed the clinical consequences of lead exposure in the present study, it is possible that pregnants with high Pb-S and %Pb-S/Pb-B expose their children to increased health risks associated with Pb exposure. Conversely, mothers carrying the H8 haplotype may protect their children against this risk.

In the present study, we have not addressed the molecular mechanisms explaining the possible functionality of VDR polymorphisms. However, while there is evidence that the *FokI* polymorphism leads to less active VDR in the presence of the *f* allele (Gross et al., 1998), the mechanism implicated in the effects of the other VDR polymorphisms on Pb levels are unknown. In addition, while we found no effects associated with individual VDR polymorphisms, we found significant effects associated with VDR haplotypes. This is probably explained by the fact that that analysis of haplotypes has been valued as a more powerful approach in genetic studies, especially because this analysis takes into consideration the combined effects associated with genetic variants (Crawford and Nickerson, 2005; Sandrim et al., 2008a,b; Vasconcellos et al., 2010).

Our findings showing that VDR gene polymorphisms affect Pb concentrations in pregnant women may have pathophysiological implications. Since Pb is commonly found all over the world, we could speculate that this metal may increase the susceptibility to the development of hypertensive disorders of pregnancy. For example, we have previously shown that Pb exposure may decrease nitric oxide (NO) bioavailability (Barbosa et al., 2006b,c) and increase the concentrations of matrix metalloproteinases (MMPs) (Barbosa et al., 2006a; Rizzi et al., 2009). Interestingly, either decreased NO bioavailability (Sandrim et al., 2010a,c; 2008b) or increased MMP activities (Palei et al., 2008) have been implicated in the pathophysiology of hypertensive disorders of pregnancy. However, this suggestion remains to be proved.

The lack of significant effects of maternal VDR genotypes or haplotypes and Pb-B, Pb-S, or %Pb-S/Pb-B ratios in their respective umbilical cords suggests that maternal VDR polymorphisms are not predictive of Pb levels in the umbilical cords. It is possible that fetal genotypes (or haplotypes) may interact with maternal genotypes (or haplotypes) and modulate Pb levels, thus making this a much more complex issue to be studied.

5. Conclusion

In conclusion, we found that the H8 haplotype (which combines the *f*, *a*, and *b* allels for the *ApaI*, *BsmI*, and *FokI* VDR polymorphisms, respectively) is associated with lower Pb-S and %Pb-S/Pb-B ratio than other VDR haplotypes in pregnant women. This toxicogenetics finding may help to predict the existence of a group of subjects that is genetically less prone to lead toxicity during pregnancy.

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