Vitamin D Receptor Fok1 Polymorphism and Blood Lead Concentration in Children

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Variation in blood lead concentration is caused by a complex interaction of environmental, social, nutritional, and genetic factors. We evaluated the association between blood lead concentration and a vitamin D receptor (VDR) gene polymorphism. Environmental samples and blood were analyzed for lead, nutritional and behavioral factors were assessed, and VDR-Fok1 genotype was determined in 245 children. We found a significant interaction between floor dust lead and genotype on blood lead concentration. For every 1 µg/ft² increase in floor dust, children with VDR-FF genotype had a 1.1% increase in blood lead [95% confidence interval (CI), 0.69–1.5], VDR-Ff 0.53% increase (95% CI, 0.1–0.92), and VDR-ff 3.8% increase (95% CI, 1.2–6.3); however, at floor dust levels < 10 µg/ft², children with VDR-ff had the lowest blood lead concentrations. These data suggest that VDR-Fok1 is an effect modifier of the relationship of floor dust lead exposure and blood lead concentration.

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Despite a dramatic decline in blood lead concentration in U.S. children over the last three decades, lead toxicity remains a significant risk for some infants and children (Pirkle et al. 1998). Moreover, there is increasing evidence that blood lead concentrations < 10 µg/dL, the current level of concern [Centers for Disease Control and Prevention (CDC) 1997], are associated with deficits in cognition, hearing, and academic skills (Canfield et al. 2003; Lanphear et al. 2000a; Schwartz 1994; Schwartz and Otto 1991). Scientists have long recognized that children’s blood lead concentrations vary widely by race, socioeconomic status, environmental exposure, mouthing behaviors, and unidentified biologic or genetic factors (CDC 1997; Lanphear et al. 2002; Lin-Fu 1973). But biologic factors that account for differences in blood lead concentrations are poorly understood.

Gastrointestinal lead absorption varies across individuals. Variation in lead absorption results from nutritional deficiencies (Fullmer 1992; Six and Goyer 1970), fasting conditions (Flanagan et al. 1982; Rabinowitz et al. 1980), and genetic factors that influence mineral metabolism (Bouton and Pevsner 2000; Flanagan et al. 1982; Schwartz et al. 2000). Observational and experimental studies have shown that dietary intake of calcium and iron are inversely associated with lead absorption (Fullmer 1992; Mahaffey et al. 1986; Six and Goyer 1970; Watson et al. 1980; Wright et al. 1999; Ziegler et al. 1978), indicating that variation in blood lead concentration may be caused partly by nutritional intake of calcium and iron or genetic factors that influence their absorption (Bouton and Pevsner 2000; Flanagan et al. 1982; Schwartz et al. 2000).

Lead follows the biologic pathway of other bivalent cations, such as calcium, competing for absorptive and protein-binding sites (Godwin 2001). Although the relationship between calcium and lead is complex, in vivo and in vitro studies have demonstrated that cellular lead uptake increases when calcium stores are reduced or depleted (Fullmer 1992; Kerper and Hinkle 1997). The high-affinity binding of lead to calcium-binding protein (Richardt et al. 1986) suggests that lead absorption would be increased in times of low calcium intake (Fullmer 1997). Indeed, children with elevated blood lead concentration have been reported to have significantly lower dietary calcium intake (Fullmer 1992; Johnson and Tenuta 1979; Mahaffey et al. 1986; Rosen et al. 1980). Calcium metabolism is governed, in part, by the vitamin D endocrine system and the vitamin D receptor (VDR) (DeLuca 1979; Henry 1982). Ames et al. (1999) reported that the VDR-Fok1 genotype FF was associated with increased bone mineral density (p = 0.02) and a 30–40% increase in calcium absorption (p = 0.04) in healthy children 7–12 years old.

The purpose of this study was to test whether the VDR-Fok1 polymorphism was associated with increased blood lead concentration in a cohort of 275 children. We hypothesized that the VDR-Fok1 polymorphism would modify blood lead concentrations. In particular, we predicted that children homozygous for the F allele—a marker for increased calcium absorption—would have higher blood lead concentrations than heterozygotes and children homozygous for the f allele, after adjusting for environmental lead exposure. We examined the interaction of floor dust lead loading, the major source of lead intake for children, and VDR-Fok1 to determine if VDR-Fok1 was an effect modifier of the relationship of floor dust lead exposure and blood lead concentration.

Materials and Methods

Study population. The study population is based on a cohort of 275 children enrolled in an ongoing longitudinal study (Lanphear et al. 1999). Families who lived in the city of Rochester, New York, were identified and recruited using sequential lists of live births from five urban hospitals in Rochester. Four home visits were made to families when the child was 6 (baseline), 12, 18, and 24 months old. A certified phlebotomist drew a blood sample (5–10 cc) from each infant at every visit, and a trained interviewer conducted a face-to-face survey with the primary caretaker to assess risk factors for lead exposure, including mouthing behaviors (e.g., soil ingestion, paint chip ingestion), time spent outdoors, and attainment of developmental milestones. The primary caretaker was questioned about the content of the child’s diet using a food frequency checklist (Willet 1990) that was modified to reflect the dietary content of children in the study population. Nutritional contributions to infants’ diets from breast milk were calculated based on published data (Lawrence 1994). The estimated breast milk intake of 6-month-old infants who were not exclusively breast-fed, as defined by Labbok and Krasovec (1990), was based on individual surveys and was 769 ml/day (Heinig et al. 1993). Address correspondence to E.N. Haynes, University of Cincinnati, Department of Environmental Health, Division of Epidemiology and Biostatistics, PO Box 670056, Cincinnati, OH 45267-0056. Telephone: (513) 558-1986. Fax: (513) 558-6272. E-mail: erin.haynes@cchmc.org

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Contributions from formula were calculated by ounces of formula (brand and type) consumed daily at 6 and 12 months (Johnson 1993).

At each of the four visits, an environmental technician collected three or four composite interior dust wipe samples from surfaces that were accessible to a child (i.e., carpeted floors, noncarpeted floors, and window sills) or known to be heavily contaminated with lead (window troughs) in the child’s bedroom, the kitchen, and the living room. A composite dust sample consisted of a maximum of three wipe samples collected from the same surface (i.e., carpeted floor, noncarpeted floor, and interior window sill or window wells). Because floor dust lead values were highly skewed and the most extreme floor dust values exerted a high degree of influence on regression coefficients, we truncated the extreme floor dust lead values (> 98.5th percentile) rather than log-transforming the data. Floor samples (carpeted and noncarpeted floor dust lead loading values) were combined to form a single floor dust lead variable. Dust samples were analyzed first by flame atomic absorption, followed by graphite furnace if levels were < 5 µg/sample. The detection limit of graphite furnace for the dust wipe was 0.5 µg/sample.

Soil and water samples were measured at baseline and when a child moved to a new residence. Three soil samples were taken from each side of the home along the perimeter of the foundation where bare soil was present. The samples were combined to form a single composite soil sample. Soil was analyzed separately with flame atomic absorption spectrometry. The detection limit for lead in soil was 25 µg/g. Caregivers collected a water sample (250 cc) in the morning from the kitchen tap after the water flowed for 1 min. Water samples were analyzed for lead by graphite furnace if levels were < 5 µg/sample. The detection limit for lead in water was 0.23–0.26 µg/dL, and the between-run precision, based on duplicate measurements, ranged from 0.1 to 0.5 µg/dL for blood lead concentrations < 20 µg/dL. The detection limit for lead in blood was 1 µg/dL. Blood samples were stored in a −70°C freezer.

**VDR-Fok1 genotyping.** Whole blood samples were analyzed for VDR-Fok1 polymorphism by the Cincinnati Children’s Hospital Medical Center, Division of Human Genetics. Genomic DNA was isolated from 3 ml of whole blood collected in EDTA-coated tubes by a standard phenol–chloroform extraction procedure. The 265-bp fragment of genomic DNA containing the polymorphic portion of exon 2 on the VDR gene was amplified by polymerase chain reaction (PCR), as described by Ames et al. (1999). Primers VDRa: 5’-AGCTGGGCCCTCCACACTGACTCTGCTCT-3’ and VDRb: 5’-ATGGAAACACCTTGCTTTCTCTCCCTC-3’ were used to amplify the Fok1 polymorphic restriction site (Gibco BRL Custom Primers, Carlsbad, CA). In summary, 2 µl genomic DNA was added to 47.6 µl PCR mixture nucleoside containing 1 µl dNTP, 2 µl MgCl₂, and 5 µl 10% dimethyl sulfoxide. After 3 min of thermocycling at 95°C in the PCR machine, 0.4 µl Tag DNA polymerase was added to the reaction. Thermocycling conditions were 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec. A final elongation period of 5 min at 72°C was added after 30 cycles. PCR products were digested with 4 µl Fok1 and 5 µl New England Biolabs buffer (Beverly, MA) for 1.5 hr at 37°C and then electrophoresed through a 3% agarose gel for 2 hr at 80 V.

The F genotype was indicated by the presence of the restriction site that generates two fragments of 196 bp and 69 bp. The F genotype was indicated by a single uncleaved 265-bp fragment. Determination of VDR genotype FF, Ff, or ff was indicated based on the Fok1 cleavage pattern (Figure 1). The following safeguards were instituted to maintain consistency of the results: a) Genomic DNA, which was previously genotyped at the VDR locus, was repeated twice per month alongside new samples, to confirm previous results; b) ”no DNA” controls were routinely run with samples to ensure that DNA contamination was prevented. The reproducibility of these methods is 100%.

**Statistical analysis.** We used descriptive statistics to examine the differences in characteristics of 24-month-old children by their VDR-Fok1 genotype. We used analysis of variance to make comparisons among the three genotypes for continuous variables, and chi square tests for categorical variables. For all statistical analyses, blood lead concentration was log transformed.

We developed multiple regression models to predict blood lead concentration as a function of environmental, nutritional, behavioral, and demographic variables. Data were analyzed using repeated measures analysis, a mixed model regression method that accounts for the correlation among outcomes measured on the same child over time. The subjects in this study were considered random effects, and the VDR-Fok1 genotype, environmental lead exposure variables, nutritional intake, and mouthing behaviors were considered fixed effects. The PROC MIXED procedure in SAS (version 8; SAS Institute, Cary, NC) was used to conduct the repeated measures analysis.

To examine effect modification of the VDR-Fok1 genotype, we tested numerous interactions, including calcium intake (lagged by one visit), vitamin D intake, race, environmental exposure to lead-contaminated floor dust, and the VDR-Fok1 genotype. Covariates were retained in the final model if they caused a meaningful change in the VDR coefficient or were significant predictors of blood lead, as determined by a two-tailed p-value of < 0.05. Final model covariates included age, African-American race, calcium intake, iron intake, renting a home, floor dust lead loading, window-well dust lead loading, soil ingestion, paint chip ingestion, and water lead concentration. Interactions included the VDR-Fok1 genotype by floor dust age by total iron intake, age by African-American race, age by renting a home, age by paint ingestion, and age by window-well dust lead loading. After adjustment, we compared the geometric mean blood lead concentration for each age group by the VDR-Fok1 genotype.

**Results**

**Study population.** Of the 275 children who were enrolled at 6 months of age, 245 (89%) were available for the 24-month follow-up blood test (Lanphear et al. 1999). The attrition rate was similar for African-American (4%) and non–African-American children (6%). Six children were removed from the analysis because their residences had floor dust loading values > 100 µg/ft², which were considered to be outliers in this data set.

![Figure 1. Influence of floor dust lead loading and VDR-Fok1 genotype on blood lead concentration.](image-url)
There were adequate blood samples for 239 (97%) of the children (145 African American, 47 white, and 47 other, which included Asian, Latino, American Indian, and unknown). The overall prevalence of the VDR-Fok1 genotypes in the study population was 51% FF, 42% Ff, and 8% ff. Allele distributions in the study population were in Hardy-Weinberg equilibrium.

None of the lead exposure variables differed significantly by genotype except soil ingestion: 47% of children with VDR-ff reportedly ingested soil compared with approximately 20% in children with VDR-FF and VDR-Ff (p = 0.05; Table 1). The VDR-Fok1 polymorphisms also differed significantly by race (Table 2). The frequency of FF homozygotes in African-American children was significantly greater than that in non–African-American children (chi square = 6.451, 1 df, p = 0.01). African-American children also had a higher percentage of the F allele than did non–African-American children (chi square = 9.688, 1 df, p = 0.003). Genotype frequencies for the FF, Ff, and ff genotypes were 57%, 39%, and 4%, respectively in African-American children, and 40%, 47%, and 13%, respectively in non–African-American children (Table 2). There was no significant difference among the frequencies of genotypes between white and other (Asian, Latino, American Indian, and unknown; chi square = 0.845, 2 df, p = 0.66).

Blood lead concentration increased during the first 24 months of life (p < 0.001), with the steepest increase occurring between 6 and 12 months. In an unadjusted repeated measures analysis, there was a significant difference in blood lead concentrations among the VDR-Fok1 genotypes (p = 0.04; Table 3). At 24 months, children with VDR-FF genotype had blood lead concentrations that were 1.2 µg/dL and 2.3 µg/dL higher than children with VDR-Ff and VDR-ff, respectively (Table 3).

**Multivariable analysis.** There were no changes in the determinants of blood lead concentrations from our previous publication (Lanphear et al. 2002), except for calcium intake. In this VDR model, calcium intake was inversely associated with children's blood lead (p = 0.03).

After adjusting for environmental lead exposure, there was no significant difference in blood lead concentrations among the VDR-Fok1 genotypes (p = 0.58; Table 3). At 24 months, children with VDR-FF genotype had blood lead concentrations that were 0.5 µg/dL and 0.1 µg/dL higher than in children with VDR-Ff and VDR-ff, respectively (Table 3).

To determine whether the VDR-Fok1 polymorphism was an effect modifier of the relationship between lead exposure and children's blood lead concentrations, we included interaction terms between environmental lead exposures and VDR-Fok1 genotypes. We examined all interactions of lead exposure and VDR-Fok1 genotypes. Only floor dust lead loading and paint chip ingestion were significant. Only 6% of children were reported to ingest paint chips, and the largest increase in blood lead compared with those with reportedly no paint chip ingestion was for those children with the VDR-ff genotype (a = 2, or 12%; Table 1). Therefore, we did not examine interactions of VDR with paint chip ingestion.

There was a significant interaction between floor dust lead loading and VDR-Fok1 genotypes on blood lead concentration (p = 0.009). With every 1 µg/ft² increase in floor dust, children with the FF genotype had a 1.1% increase in blood lead [95% confidence interval (CI), 0.69–1.5], whereas children with the ff genotype had a 0.53% increase in blood lead (95% CI, 0.1–0.92; Figure 1). Children with the ff genotype had a 3.8% increase in blood lead concentration (95% CI, 1.2–6.3) for each 1 µg/ft² increase in floor dust lead loading (Figure 1). Mean blood lead concentrations among children with VDR-ff were lower than the other VDR-Fok1 genotypes at floor dust lead levels < 10 µg/ft²; however, when floor dust lead levels were > 10 µg/ft², children with VDR-ff had the highest mean blood lead concentration. To address the issue of small sample size of VDR-ff group, we conducted secondary analyses without the VDR-ff group. The difference between the exposure–response functions for VDR-FF and VDR-Ff decreased in significance from p = 0.0009 to p = 0.03. The slope relating log of blood lead to floor dust loading for VDR-FF was 0.012; that for VDR-Ff was 0.005.

To examine the effect of race on the interaction between VDR genotype and floor dust lead loading, we ran the model for African Americans and non-African Americans separately. Because of the small sample size of the VDR-ff group, we removed it from the analysis. The floor dust lead loading interaction with VDR-FF and VDR-Ff was not significant for African-American children (p = 0.272), but was significant for non–African-American

### Table 1. Characteristics of study children at 24 months of age by VDR genotype.

| Characteristic                | VDR-FF (n = 114) | VDR-FF (n = 98) | VDR-FF (n = 17) | p-Value |
|------------------------------|------------------|-----------------|-----------------|---------|
| Floor dust lead loading (µg/ft²)² | 8.7 (6.8–10.7)   | 10.800 (7.200–14.100) | 3.3 (1.4–5.2) | 0.12    |
| Window-well lead loading (µg/ft²)² | 806 (735–877)    | 867 (792–944)    | 8,400 (3,100–13,600) | 0.29    |
| Calcium intake (mg/day)³     | 9.3 (9.0–10.8)   | 10.2 (9.4–11.3)  | 887 (611–1,163) | 0.45    |
| Iron intake (mg/day)³        | 97 (88)          | 79 (62)          | 9.4 (6.6–12.2)  | 0.79    |
| Rent home³                   | 77 (68)          | 72 (75)          | 15 (18)         | 0.54    |
| Income² ≥ $15,000            | 36 (32)          | 24 (26)          | 2 (3)           | 0.20    |
| ≥ $15,000                   | 15 (14)          | 15 (15)          | 3 (3)           | 0.28    |
| African-American race        | 77 (68)          | 55 (57)          | 5 (5)           | 0.01    |

*Mean (95% CI). **Number (%).*!

### Table 2. Race by VDR-Fok1 genotype [no. (%)].

| Race             | VDR-Fok1 genotype | VDR-Fok1 genotype | VDR-Fok1 genotype | Total |
|------------------|-------------------|-------------------|-------------------|-------|
|                  | FF                | Ff                | ff                |       |
| African American | 83 (67)           | 56 (49)           | 6 (4)             | 145 (60) |
| Non-African American | 38 (40)       | 44 (47)           | 12 (13)           | 94 (40)  |
| White            | 17 (36)           | 23 (49)           | 7 (15)            | 47 (20)  |
| Other⁴          | 21 (45)           | 21 (45)           | 5 (11)            | 47 (20)  |
| Total            | 121 (51)          | 100 (42)          | 18 (8)            | 239    |

*Non–African-American race category combines the white and other categories. The test of homogeneity of VDR-Fok1 genotypes between African Americans and non-African Americans is chi square = 9.74, df = 2, p = 0.008. **Other race category includes Asian, Latino, American Indian, and responses marked “unknown.” The test for homogeneity of VDR-Fok1 across all three race groups (African American, white, and other) is chi square = 10.74, df = 3, p = 0.003.

### Table 3. Unadjusted and adjusted geometric mean (95% CI) blood lead concentration (µg/dL) VDR-Fok1 genotype at 24 months of age.

| Genotype | Unadjusted | Adjusted² |
|----------|------------|-----------|
| FF       | 8.1 (7.1–9.0) | 7.0 (6.2–7.0) |
| Ff       | 6.9 (6.1–7.8) | 6.5 (5.6–7.6) |
| ff       | 5.8 (4.3–7.8) | 5.9 (5.2–9.2) |
| p-Value  | 0.04        | 0.58      |

*Adjusted for environmental lead exposure (floor dust lead loading, window-well lead loading, soil ingestion, paint chip ingestion, and water lead concentration), nutritional intake (calcium and iron), race, and renting a home.*
Based on our model, calcium intake was a significant predictor of blood lead concentration (p = 0.03). We did not, however, find a significant effect modification of calcium intake on blood lead concentration by VDR-Fok1 genotype, perhaps because of the complexity of the lead–calcium relationship. Also, the mean calcium intake for study children was well above the dietary reference intakes (DRIs) for children. Similarly, in a study by Dawson-Hughes et al. (1995) the impact of the VDR-Bsm1 BB genotype was reduced at calcium intake of > 300 mg/day. The mean calcium intake for children in the present study was 679 mg/day (95% CI, 649–712) at 6 months, 987 mg/day at 12 months (95% CI, 940–1,034), and 968 mg/day (95% CI, 913–1,023) at 18 months (Lanphear et al. 2002). The DRIs are 210 mg/day for children 0–6 months old, 270 mg/day for children 6–12 months old, and 500 mg/day for children 1–3 years old (Institute of Medicine 1997). Although the calcium intake for children in our study was greater than the DRI, the values are comparable with calcium intake levels in the third National Health and Nutrition Examination Survey (NHANES III). In NHANES III, children 5–7 months old had a mean calcium intake of 691 mg/day; 11–13 months, 933 mg/day; 17–19 months, 874 mg/day; and 23–25 months, 784 mg/day (Lanphear BP. Unpublished data).

The VDR gene has been cloned (Miyamoto et al. 1997) and several genetic variants have been described in humans (Morrison et al. 1992). Variation in the VDR gene has been reported, including sites cleaved by Bsm1 (Morrison et al. 1992), Apal (Furaco et al. 1989), Taq1 (Morrison et al. 1992), and Fok1 (Saijo et al. 1991), and a poly(A) site at the 5’ end of the gene (Ingles et al. 1997). Located within VDR translation initiation site on exon II, the Fok1 polymorphism, a C→T transition, creates an upstream initiation codon, resulting in a VDR molecule elongated by three amino acids (B compared with those initiating translation from the downstream site (F) (Arai et al. 1997; Gross et al. 1996). Unlike the Bsm1, Apal, and Taq1 sites, the Fok1 polymorphism affects the amino acid sequence altering the VDR protein structure (Gennari et al. 1999). VDR-Fok1 is not genetically linked to the Bsm1/Apal/Taq/poly(A) cluster (Whitelaw et al. 2001). Very few studies have examined the association with the Apal/Bsm1/Taq cluster and the Fok1 site.

Lead-contaminated house dust is a major source of childhood lead exposure (Adams 1991; Lanphear 1998; Lanphear et al. 1998a, 1998b; Manton et al. 2000; Sayre and Katzel 1979; Succop et al. 1998). Lead-contaminated house dust can be ingested during normal hand-to-mouth activity or on foods (Melnik et al. 2000). Lead-contaminated floor dust was the primary source of exposure in our cohort. We ran the repeated measures model with each of the four lead exposure variables, floor dust, window-well dust, water lead, and soil lead, individually. Based on the likelihood ratio statistic, the floor dust model was the best fit for the data.

The prevalence of the VDR-Fok1 alleles has previously been determined for African-American women (Harris et al. 1997) and children (Ames et al. 1999). We found 55% of the African-American children in our study to be homozygous for the F allele, 40% to be heterozygous, and 4% to be homozygous for the f allele. The distributions reported by Harris et al. (1997) and Ames et al. (1999) were 65% Ff, 31% ff, 4% ff for African-American women and 67% FF, 33% Ff, and 0% ff for African-American children. Consistent with these other reports, we found that African-American children were significantly more likely to have VDR-FF than were non–African-American children (p = 0.02). Thus, although it is beyond the scope of this study, the ability of African-American individuals to have increased calcium absorption may partially explain the higher blood lead concentration seen in African-American children (Lanphear et al. 1996, 2000b, 2002; Pirkle et al. 1998).

This study has some limitations. The semi-quantitative food frequency survey required caregivers to recall a child’s food intake over a 6-month period, which is subject to error. The food frequency surveys did not correlate with a 3-day diary for a random sample of 26 children 18 months old (Lanphear et al. 2002). However, the nutritional intake in our study population was similar for those surveyed in NHANES III (Lanphear BP. Unpublished data). Another limitation is the small number of African-American children (n = 83; African-American FF, n = 33; African-American Ff, n = 38; African-American ff, n = 14) and African-American women (n = 56; African-American FF, n = 38; African-American Ff, n = 14).
of children in our study homogenous for the f allele (n = 17). These low numbers may, in part, explain the higher blood lead concentrations among children who were exposed to floor dust lead loading > 10 µg/ft². Only 2 of the 17 children with VDR-Fk were exposed to floor dust lead loading > 10 µg/ft². In contrast 44 and 43% of children with VDR-FF and VDR-Ff were exposed to floor dust lead loading > 10 µg/ft². The low floor dust lead loading (mean = 3.3 µg/ft²) in the VDR-f children may also contribute to the differences we observed in blood lead concentration. In addition, we did not examine the correlation between the other VDR polymorphisms (BsmI, Apal, TaqI, poly(A)) and FokI (Kerr Whitlefield et al. 2001).

In conclusion, this study suggests that VDR-Fok1 polymorphism is an effect modifier of the relationship between floor dust lead exposure and blood lead concentration in non–American–American children, possibly through its effect on gastrointestinal absorption. Further research is necessary to evaluate the gene–environment interaction among other markers of calcium metabolism, the gene–gene interaction between the Fok1 and other VDR polymorphic sites, and blood lead concentration in children, and to examine the contribution of calcium metabolism and the VDR on racial differences in blood lead concentration.

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