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Association of vitamin D receptor (VDR) gene polymorphism with blood lead levels in occupationally lead-exposed male battery workers in Delhi – National capital region, India

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Lead is a well-known environmental pollutant due to its widespread industrial uses and persistent nature. Studies have underlined the toxicities caused due to occupational exposure to lead and have also reported the association of blood lead levels (BLL) with Vitamin D Receptor (VDR) gene polymorphism; however, such data is scarce from India. This maiden attempt aims to investigate the association of different VDR polymorphic variants on BLL in the north-Indian lead-exposed population. We recruited 100 occupationally lead–exposed battery workers (LEBW) and 100 non-lead exposed controls (NLEC). BLL, serum Vitamin D, calcium and phosphorous levels were measured. Further, VDR polymorphisms namely, *FokI*, *TaqI*, *ApaI*, and *BsmI* polymorphic variants were studied. Results demonstrated that BLL was significantly increased in LEBW as compared to NLEC. Chi–square test results show that frequencies of Ff *FokI* VDR genotype and bb *BsmI* VDR genotype were significantly more in LEBW as compared to NLEC (P =0.02 and 0.03, respectively). Furthermore, FF, aa and bb genotype showed the highest BLL in LEBW indicating higher lead levels in some VDR polymorphisms.

Keywords: *ApaI*, Blood lead levels (BLL), *BsmI*, *FokI*, Occupational exposure, *TaqI*, Vitamin D receptor (VDR) polymorphism, Vitamin D

Lead (Pb) is a ubiquitous, persistent and major environmental pollutant. Its omnipresent nature and widespread use cause millions of people to be exposed to lead[1]. Multiple ways of exposure including dermal contact, inhalation, and ingestion are involved in lead– induced health effects[2,3]. Occupational exposure is a major contributor to exposure in workers engaged in industries including lead smelting, paints, ceramics, lead battery manufacturers, automobile technicians *etc*[4-6]. The population of lead-exposed battery workers (LEBW) has recently increased considerably due to increased usage of lead batteries in industries like automobiles and electronics. In humans, lead causes a wide range of biological effects depending upon the levels and duration of exposure *viz*. oxidative stress[7], cognitive deficits[8], intelligence, and memory deficits, attention disorders[9], anemia,immune toxicity[10], effects on fetus and child growth[11] and cardiovascular effects[12]. As per the report given by CDC 2011, lead poisoning refers to a condition when lead levels are >10 µg/dL in blood[13]. This has recently been further reduced to 5 µg/dL by National Institute for Occupational Safety and Health (NIOSH) in 2015.

Upon entering the body, lead distributes itself throughout the body and the major proportion is stored in bone[14,15]. Therefore, bone comprises the chief reservoir of lead and is mobilized to the circulation whenever it is needed. Pb mobilizes from bone to bloodstream *via* bone resorption which is similar to minerals like Ca^{2+}[,16]. Pb and Ca^{2+} follow similar metabolic pathways for their toxicity. Multiple *in vitro* and *in vivo* studies have proved that these two minerals compete for similar locations inside the body[17,18]. Vitamin D and its active form (1, 25-dihydroxy vitamin D or calcitriol) play a significant role in absorption and regulation of calcium in the body. Studies are reporting that vitamin D by binding to its receptor (vitamin D receptor), a

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Abbreviations: BLL, Blood lead level; CDC, Centers for Disease Control; LEBW, Lead-exposed battery workers; NLEC, Non lead– exposed controls; VDR, Vitamin D Receptor

nuclear transcription factor, activates a large number of target genes including calcium– binding protein calbindin-D which plays a crucial role in the intestinal absorption of calcium[19,20]. Earlier, studies have reported that, increased synthesis of calbindin-D significantly enhanced the absorption of calcium in the gut and ultimately its deposition in bone. Furthermore, VDRs are distributed in most cells in the body including osteoblasts, intestinal epithelial cells, and kidney tubular cells[21].

The gene for VDR is very large and localized on chromosome 12q13.1 and consists of one noncoding exon (1a - 1f) along with eight protein– coding exons (2-9). Four among the many known single nucleotide polymorphisms (SNP) include *ApaI* (RS7975232), *BsmI* (RS1544410), *TaqI* (RS731236), and *FokI* (RS2228570). Multiple studies reported that these SNPs are associated with the regulation of calcium absorption in many diseases[22-25]. Further, a few studies have also emphasized the role of these SNPs in modulating the Pb levels in several populations[26,27]. Several reports also indicate an underlying potential role of these SNPs in regulating the circulatory levels of lead in exposed populations. However, globally only a few reports have tried to establish an association between VDR SNPs and BLL and this is a maiden effort in Indian scenario which attempts to show the effects of different genotypes of VDR on blood lead levels in occupationally exposed battery workers[28-31].

Hence in the present study, the association of VDR polymorphisms with the blood lead levels inoccupationally lead exposed battery workers was investigated.

Materials and Methods

Study Subjects

In this cross-sectional study, 200 adult males who had no obvious health problems were recruited after clearance from the Institutional Ethical Committee (IEC) and written informed consent was obtained from all the subjects. The study had two arms: 100 occupationally lead– exposed male workers working in the battery industry (LEBW) and 100 age–matched males, not occupationally exposed to lead as controls (NLEC), recruited from the Delhi-NCR region of India. On recruitment, anthropometric measurements were recorded, and all participants were asked to complete a questionnaire having questions related to demographic characteristics, duration of employment, smoking status, alcohol consumption, food preferences (vegetarian and non-vegetarian) and current illness. Subjects who reported any medical disorders or were found to have any obvious medical conditions from clinical history and examinations and those on any medications continuing for more than a week were excluded from the study.

Clinical laboratory analysis

5 mL of the venous blood sample was collected from all participants. From the total of 5 mL blood taken for study, 2.0 mL of whole blood was stored in EDTA vial at −80°C for blood lead levels estimation until analysis. Again 2.0 mL of whole blood taken in the plain vial was used for the serum separation by centrifugation at 3000 rpm at 4°C for 10min and then stored at −20°C for estimation of various clinical parameters including serum vitamin D, calcium, phosphorous. Rest 1.0 mL of whole blood taken in EDTA vial was used for DNA isolation. Routine biochemistry parameters including serum calcium and phosphorous were measured using a Modular P biochemistry auto-analyzer (Roche diagnostic, Indianapolis, IN).

Blood lead levels (BLL) estimation

Whole blood lead levels were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 8000, Perkin Elmer, Waltham, MA). Briefly, 2.0 mL whole blood was digested in the presence of 2.0 mL nitric acid and 0.2 mL hydrogen peroxide at specified power, temperature and duration of time in Microwave Digestive System 3000 (Anton Paar, Graz, Austria) (Table 1). Digested samples were made up to 5 mL with triple distilled water and analyzed in ICP-OES machine along with known standards having defined concentration.

Enzyme linked immune sorbent assay (ELISA)

Estimation of serum total Vit D was done using Vitamin D ELISA kit (Cal Biotech, El Cajon, CA). The principle of detection was a competitive binding detection method and was performed as per manufacturer's instruction.

Table 1 — Operating conditions for the microwave digestion system

S. No.	Power (W)	Ramp (min)	Hold (min)	Fan
1	400	5	10	1
2	800	5	10	1
3	0	0	10	3

Genotyping

Genomic DNA was extracted from whole blood using commercially available kits (Qiagen, Hilden, Germany). DNA quality and quantity were determined using NanoDrop 1000 UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., USA). VDR gene polymorphisms (*FokI*, *TaqI*, *ApaI*, and *BsmI*) were studied using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Primers were commercially synthesized and procured from Eurofins MWG Operon (Germany). The sequence of forward and reverse primers for all studied VDR polymorphic variants is given in (Table 2). For restriction digestion, all *FokI*, *TaqI*, *ApaI*, and *BsmI* primer-specific amplicons were digested by incubating with their respective restriction enzymes and separation was done on 2% agarose gel electrophoresis. Staining was done using EtBr and the image was captured on a gel documentation system (Protein Simple, San Jose, California).

Table 2 — Forward and reverse primers sequence

Name of the gene	Primers	Primer sequences	Amplicon size; Annealing temperature (°C)
FokI	FP	5′-AGCTGGCCCT GGCACTGACTC TGCTCT-3′	269 bp; 58°C
	RP	5′- ATGGAAACAC CTTGCTTCTTCT CCCTC-3′	
TaqI & ApaI	FP	5′-CAACCAAGAC TACAAGTACCG CGTCAGTGA-3′	2000 bp; 63°C
	RP	5′-CACTTCGAGC ACAAGGGGCG TTAGC-3′	
BsmI	FP	5′-CAACCAAGAC TACAAGTACCG CGTCAGTGA-3′	825bp; 58°C
	RP	5′-AACCAGCGGG AAGAGGTCAA GGG-3′	

Statistical analysis

Results were analyzed statistically using GraphPad Prism 6 and SPSS version 21.0. Data were expressed as mean, median (range) for study subjects. Independent *t*-test (for parametric data) and Mann-Whitney U test (for nonparametric data) were used to compare the available data. Chi-square analysis was used to check for the distribution of genotypes (Hardy–Weinberg equilibrium) and alleles. Regression analysis was used to determine the association between BLL and VDR SNPs along with other clinical parameters. Statistical significance was defined at a $P < 0.05$.

Results

The demographic data of the recruited subjects have been published in our previous study[32]. The mean duration of exposure for LEBW was 14.8 ± 9.5 years. Results detailing the genotypic and allelic frequency of *FokI*, *TaqI*, *BsmI*, and *ApaI* variants of the VDR gene are given in (Table 3). Results

Table3 — Prevalence of VDR polymorphisms (*FokI*, *TaqI*, *ApaI* and *BsmI*) in occupationally lead exposed battery workers (LEBW) and occupationally non-exposed controls (NLEC)

Genotype	Genotype frequency		HWE *P* -value	Allele frequency			
	NLEC (100)	LEBW (100)		NLEC		LEBW	
Fok1				F	f	F	f
FF	69	49					
Ff	28	47	0.02*	0.83	0.17	0.73	0.28
ff	3	4					
Taq1				T	t	T	t
TT	48	48					
Tt	43	43	0.99	0.70	0.31	0.70	0.31
tt	9	9					
Apa1				A	a	A	a
AA	37	32					
Aa	37	35	0.50	0.56	0.45	0.50	0.51
aa	26	33					
Bsm1				B	b	B	b
BB	35	21					
Bb	43	43	0.03*	0.57	0.44	0.43	0.58
bb	22	36					

*P <0.05. Chi square test was used to determine the genotypic difference.

Table 4 — Evaluation of Blood lead levels for all genotypes of VDR gene polymorphic variants (*FokI*, *TaqI*, *ApaI* and *BsmI*) in occupationally exposed lead battery workers (LEBW). Data is expressed as Median (Range). Levels of significance is obtained if $P < 0.05$

Parameters	Median (Range)			*P* -value		
FokI	FF (n = 49)	Ff (n = 47)	ff (n = 4)	FF & Ff	FF & ff	Ff & ff
BLL (µg/dL)	35.1 (5.5-139.7)	21 (7.7-107.8)	28.8 (16.5-58.7)	< 0.0001	0.45	0.68
TaqI	TT (n = 48)	Tt (n = 43)	tt (n = 9)	TT & Tt	TT & tt	Tt & tt
BLL (µg/dL)	31.1 (14.3-139.7)	25.4 (5.5-99)	28.9 (10.8-107.8)	0.08	0.95	0.78
ApaI	AA (n = 32)	Aa (n = 35)	aa (n = 33)	AA & Aa	AA & aa	Aa & aa
BLL (µg/dL)	25.6 (9.9-107.8)	26.8 (5.5-105.6)	37.7 (14.3-139.7)	0.99	0.03	0.04
BsmI	BB (n = 21)	Bb (n = 43)	bb (n = 36)	BB & Bb	BB & bb	Bb & bb
BLL (µg/dL)	27.9 (10.8-104.4)	25.4 (5.5-107.8)	37.5 (14.3-139.7)	0.99	0.05	0.01

demonstrate that allelic frequencies of wild type *FokI* F allele and mutant f allele were 0.73% and 0.28% in LEBW while in NLEC, it was observed to be 0.83% and 0.17% implicating that allelic frequency of f was significantly higher among the LEBW. Genotypic frequencies of *FokI* FF and Ff genotype were 0.49% and 0.47%, respectively, in LEBW and 0.69% and 0.28%, respectively, in NLEC, whereas, the frequency of ff genotype was 0.04% in LEBW and 0.03% in NLEC. Chi−square test results concluded that Ff genotype was significantly associated with LEBW compared to NLEC.

Further, allelic frequencies of wild type *TaqI* T allele and mutant t allele were 0.70% and 0.31%, respectively, in LEBW and in NLEC it was 0.70% and 0.31%, respectively. Genotypic frequencies of *TaqI* TT and Tt genotypes were 0.48% and 0.43%, respectively, in LEBW and 0.48% and 0.43%, respectively, in NLEC, whereas, the frequency of tt genotype was 0.09% in battery workers and 0.09% in controls. Chi square test showed no significant association of *TaqI* polymorphisms in LEBW compared to NLEC.

Similarly, allelic frequencies of wild type *ApaI* A allele and mutant a alleles were 0.50% and 0.51%, respectively, in LEBW and 0.56% and 0.45%, respectively, in NLEC. The genotypic frequencies of *ApaI* AA and Aa genotypes were 0.32% and 0.35%, respectively, in LEBW and 0.37% and 0.37%, respectively, in NLEC groups. The frequencies of aa genotypes were 0.33% in battery workers and 0.26% in controls. Chi−Square test revealed no significant association of *ApaI* polymorphism with LEBW.

Lastly, allelic frequencies of wild-types *BsmI* B allele and mutant b allele were 0.43% and 0.58%, respectively, in LEBW and NLEC they were 0.57% and 0.44%, respectively. Genotypic frequencies of *BsmI* BB and Bb genotypes were found to be 0.21% and 0.43%, respectively, in battery workers and 0.35% and 0.43% in controls, whereas, frequencies of bb genotype were 0.36% in battery workers and 0.22% in controls. Chi− square test demonstrated a statistically significant increase in the bb genotype frequency in LEBW compared to NLEC.

Comparison of blood lead levels in VDR polymorphic variants in LEBW and NLEC

Results of BLL for the same set of subjects along with their serum vitamin D, calcium and phosphorous levels have been analyzed and published by us recently as mentioned above. In the present study, the association of BLL in LEBW and NLEC groups with the VDR gene polymorphic variants and results are shown in (Table 4 & Fig. 1). Results show that in the occupationally exposed group BLL was significantly higher ($P \leq 0.05$) in some of the polymorphic variants (FF compared to Ff, aa compared to AA and Aa, and bbcompared to Bb). Further FF, aa and bbgenotypes showed the highest BLL in LEBW study subjects.

Analyses of Serum vitamin D, calcium and phosphorous levels in all genotypes of VDR polymorphism variants in LEBW and NLEC

Serum calcium also showed significantly lower levels in LEBW for only Ff, TT, Tt, Aa, and Bb genotypes whereas phosphorous levels were significantly lowered in FF, Ff, TT, Aa, aa, and Bb genotypes of LEBW in comparison to NLEC (Table 5). Significant differences were not observed between different VDR polymorphic variants for vitD, Ca and Phosphorus.

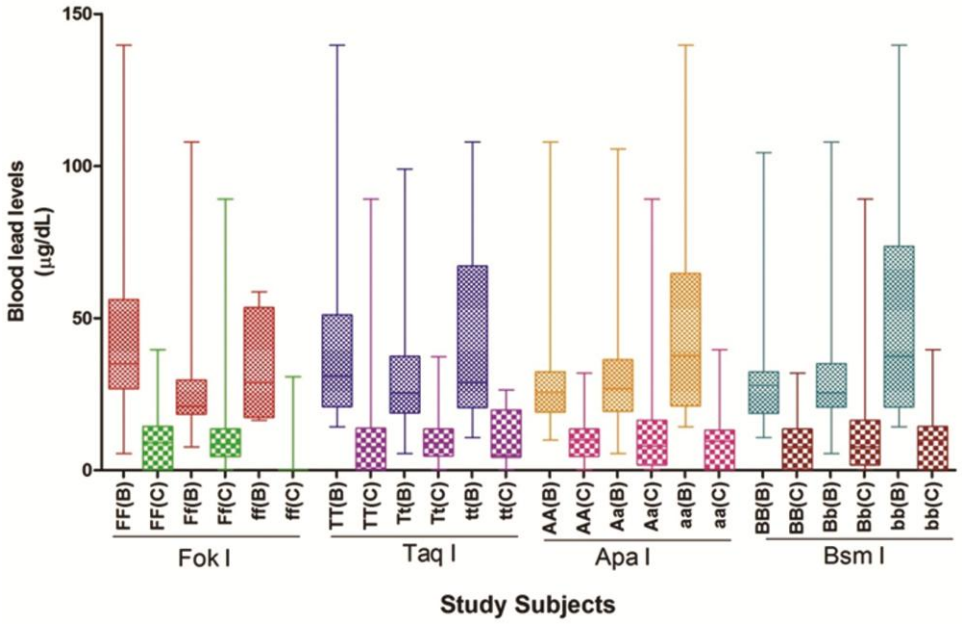

Fig. 1 — Box and whiskers plot demonstrating the comparison of Blood lead levels between VDR genes. FF, TT, AA and BB represent wild homozygous. Ff, Tt, Aa and Bb represent mutant heterozygous and ff, tt, aa and bb represent mutant homozygous. (B) and (C) represent Battery workers and Controls respectively. Within *FokI* polymorphism difference of BLL between FF and Ff was found significant (P <0.0001) amongst LEBW group. *TaqI* polymorphic variants did not show significant differences between themselves. Within *ApaI* polymorphic variants aa showed significantly different BLL compared to both AA (P =0.03) and Aa (0.04). In the *BsmI* polymorphic variants bb showed significant difference in BLL compared to Bb (P =0.01). The P-value between BB and bb was 0.05. *= P < 0.05 significant

Table 5 — Serum Vitamin D, Serum calcium and phosphorus levels amongst VDR gene polymorphic variants among occupationally lead exposed battery workers (LEBW) and occupationally non-exposed controls (NLEC)

VDR genotypes	Serum Vitamin D (ng/mL)			Serum calcium (mg/dL)			Serum Phosphorus (mg/dL)		
	NLEC Mean ± SD	LEBW Mean ± SD	*P* -value	NLEC Mean ± SD	LEBW Mean ± SD	*P* -value	NLEC Mean ± SD	LEBW Mean ± SD	*P* -value
FokI									
FF	53.0 ± 11.6	19.1 ± 8.5	< 0.0001	9.2 ± 0.8	8.8 ± 0.5	0.16	4.1 ± 1.3	3.8 ± 0.9	0.03*
Ff	55.0 ± 12.5	18.6 ± 9.4	< 0.0001	8.9 ± 0.9	8.8 ± 0.4	<0.0001*	4.3 ± 1.2	3.7 ± 0.8	0.001*
ff	51.7 ± 11.9	19.3 ± 8.7	≤ 0.0087	9.5 ± 0.4	8.9 ± 0.4	0.81	4.2 ± 1.3	3.8 ± 1.2	0.64
TaqI									
TT	52.1 ± 12.6	18.1 ± 7.3	< 0.0001	9.3 ± 0.9	8.7 ± 0.5	0.08	4.1 ± 1.2	3.9 ± 0.8	0.001*
Tt	54.1 ± 11.0	19.2 ± 10.5	< 0.0001	8.9 ± 0.6	8.9 ± 0.5	<0.0001*	4.2 ± 1.3	3.7 ± 0.9	0.06
tt	58.2 ± 9.9	21.3 ± 8.7	< 0.0001	9.4 ± 0.8	8.8 ± 0.6	0.11	4.4 ± 1.4	3.4 ± 0.4	0.98
ApaI									
AA	52.4 ± 12.5	18.7 ± 9.4	< 0.0001	9.2 ± 0.6	8.8 ± 0.5	0.001*	4.2 ± 1.3	3.6 ± 0.7	0.19
Aa	55.5 ± 10.2	18.7 ± 10.5	< 0.0001	8.8 ± 0.6	8.9 ± 0.5	<0.0001*	4.2 ± 1.3	4.0 ± 0.9	0.005*
aa	52.3 ± 12.6	19.2 ± 6.6	< 0.0001	9.4 ± 1.1	8.7 ± 0.4	0.22	4.1 ± 1.2	3.7 ± 0.9	0.04*
BsmI									
BB	55.4 ± 10.3	19.7 ± 8.2	< 0.0001	9.4 ± 0.9	8.7 ± 0.6	0.27	4.2 ± 1.2	3.4 ± 0.8	0.29
Bb	51.6 ± 12.7	18.8 ± 10.9	< 0.0001	8.9 ± 0.6	8.9 ± 0.5	<0.0001*	4.2 ± 1.3	3.9 ± 0.8	0.008*
bb	54.3 ± 11.8	18.5 ± 6.5	< 0.0001	8.9 ± 0.9	8.7 ± 0.4	0.25	4.1 ± 1.4	3.9 ± 0.9	0.07

Data is expressed as mean ± SD. *P <0.05.

Table 6 — Regression analyses to determine associations between demographic characteristics, VDR SNPs, clinical parameters and blood lead levels (BLL) in occupationally lead exposed battery workers (LEBW). Level of significance is attained if P <0.05.

Dependent variable	BLL (μg/dL)			
Independent variable	Univariate coefficient (95% CI)	P -value	Multivariate coefficient (95% CI)	P -value
Age (yr)				
20-30	ref		Ref	
30-40	−5.6 (−14.7, 3.56)	0.23	-	
40-60	−0.05 (−9.9, 9.75)	0.99	-	
Exposure Status (yr)				
High Exposure (>20)	32.1 (17.1,47.09)	0.001	26.01 (12.7, 39.2)	0.001
Moderate Exposure (>10-20)	22.7 (8.9, 36.5)	0.001	22.5 (10.34, 34.6)	0.001
Low Exposure (<10)	ref		Ref	
Smoking Status				
Non smoker	ref		Ref	
smoker	17.5 (10.13,24.7)	0.001	-	
Alcohol consumption				
Non drinker	ref		Ref	
Drinker	9.9 (2.3, 17.5)	0.01	-	
VDR genotype				
Fok1				
FF	ref		Ref	
Ff	−5.2 (−13.3, 2.8)	0.20	−12.4 (−18.7, −6.2)	0.001
ff	−3.3 (−24.7, 18.06)	0.76	−25.3 (−53.05, 2.3)	0.07
Taq1				
TT	ref		Ref	
Tt	−6.01 (−14.2, 2.03)	0.15	-	
tt	−0.77 (−14.8, 13.3)	0.92	9.49 (−1.07, 20.06)	0.07
Apa1				
AA	ref		Ref	
Aa	0.74 (−8.4, 9.8)	0.87	-	
aa	12.4 (2.9, 21.8)	0.01	-	
Bsm1				
BB	ref		Ref	
Bb	3.0 (−6.1, 12.07)	0.51	-	
bb	18.7 (8.8, 28.6)	0.001	12.2 (5.49,18.95)	0.001

Level of significance is attained if P <0.05.

Regression analysis was performed to study the effects of VDR genotypes on BLL and to identify the polymorphisms which were independent predictors of blood lead levels. Results of univariate and multivariate analyses of various characteristics is shown in (Table 6). Univariate analysis showed that *ApaI* (aa) and *BsmI* (bb) genotypes had significant association with the BLL in the LEBW (P = 0.01 and 0.001, respectively), whereas in multivariate analysis with adjustment for age, duration of exposure, smoking and alcohol consumption we found that genotypes like Ff and bb were independently associated with higher blood lead level (P <0.05).

Further, in Table 7**,** we summarize the different genotypic combinations obtained in our LEBW subjects along with their mean BLL. Although the sample numbers in the different combinations do not merit statistical analysis some combinations reveal remarkably high or low BLL. 14 samples with aa/bb/FF/TT combination showed very high blood lead level (82.26 ± 45.32 μg/dL). However, the highest BLL (107.8 μg/dL) was observed in one sample with AA/Bb/Ff/tt combination. 9 samples with

Table 7 — Blood lead levels in occupationally exposed subjects with different genotypic combinations of VDR gene

Genotypic combination	n	Blood lead level (μg/dL)
AA/BB/FF/Tt	7	32.54 ± 12.17
AA/BB/FF/tt	4	24.08 ± 8.89
AA/BB/Ff/TT	1	20.90
AA/BB/Ff/Tt	4	29.38 ± 21.22
AA/BB/Ff/tt	3	48.83 ± 48.76
AA/Bb/FF/Tt	4	30.20 ± 21.46
AA/Bb/Ff/TT	3	21.43 ± 1.89
AA/Bb/Ff/Tt	4	21.90 ± 8.24
AA/Bb/Ff/tt	1	107.8
AA/bb/FF/TT	1	33.00
Aa/BB/FF/Tt	1	18.00
Aa/BB/Ff/tt	1	29.70
Aa/Bb/FF/TT	3	55.73 ± 43.40
Aa/Bb/FF/Tt	8	47.46 ± 27.26
Aa/Bb/Ff/TT	5	23.72 ± 5.61
Aa/Bb/Ff/tt	9	20.28 ± 8.46
Aa/Bb/ff/Tt	1	19.8
Aa/bb/FF/TT	1	36.0
Aa/bb/FF/Tt	1	78.60
Aa/bb/Ff/TT	4	24.53 ± 7.91
aa/Bb/FF/TT	2	24.60 ± 4.67
aa/Bb/FF/Tt	2	26.10 ± 0.0
aa/Bb/ff/TT	1	37.80
aa/bb/FF/TT	14	82.26 ± 45.32
aa/bb/FF/Tt	1	37.50
aa/bb/Ff/TT	11	32.35 ± 21.26
aa/bb/Ff/Tt	1	19.8
aa/bb/ff/TT	2	37.60 ± 29.84

Combinations in bold have very high or very low BLL

Aa/Bb/Ff/tt combination sowed surprisingly low BLL (20.28 ± 8.46 μg/dL) than most other combinations.

Discussion

Apart from multiple modes of exposure ranging from inhalation, ingestion, and dermal contact and a potential to enter every organ system of our body, multiple gene mutations and polymorphisms including those in Vitamin D receptors, Amino levulenic acid dehydratase (δ-ALAD), glutathione S-transferase (GST), and metallothioneins have been shown to affect the bioavailability of lead[33-36]. Studies have reported that genetic differences among the population might sensitize them towards Pb exposure[37].

This is the first study to report the association of vitamin D receptor (VDR) gene polymorphism with blood lead levels in occupationally exposed battery workers in the Delhi-NCR area. In our previous study, we measured BLL, serum vitamin D, calcium and phosphorous levels in LEBW and compared them with NLEC. In the present study, VDR gene polymorphism has been studied along with BLL analysis, Vit D levels, and biochemical parameters estimation on the basis of different genotypes of each polymorphic variant of the VDR gene. Regression analysis was performed to study the association between the above parameters and blood lead levels in LEBW.

The SNPs in the VDR gene are known to affect the activity of a protein and modify bone mineralization, which is regulated by vitamin D and may further interact with lead[38]. The genetics and biology of VDR polymorphism have been studied previously and the effects of different SNPs in the VDR gene have been reported. *Fok*I RFLP is reported not to show any linkage disequilibrium with other VDR gene polymorphism because it is an independent marker of the VDR gene. The authors also suggested that the *Fok*I allele had some effect on transcription activity. On the other hand, *BsmI*, *ApaI*, and *TaqI* RFLPs, which are located near the 3′UTR have strong linkage disequilibrium. These explain associations observed with these three VDR RFLPs *Bsm1*, *Apa1* and *Taq1* SNPs regulate the mRNA stability to affect VDR gene expression[39].

In this study, we found the association of high blood lead levels in VDR variants in a setting of occupational exposure however, no significant association of *Taq1* variants with blood lead levels (BLL) was observed. In has been reported earlier that homozygous mutant (denoted as tt in our study) VDR *Taq1* genotype had higher lead levels in maternal and cord blood in women, however, no statistically significant difference was observed[40].

In our study, we have also found that aa genotype of *Apa1* variants had significantly increased blood lead levels than those of the other genotypes. The finding corroborates with a previous study report that *Apa1* aa genotype had higher blood lead levels in workers and the exposure status, smoking, and alcohol drinking are the major factors associated with blood lead levels[29].

We also found that the FF genotype of the *Fok1* variant had significantly higher BLL than other genotypes, which corroborates with the previous study reports that VDR *Fok1* is an effect modifier of the relationship of lead exposure *via* floor dust and blood lead levels[41].

Further, VDR *BsmI* and *TaqI* polymorphisms has been described in a Polish children cohort to modify the relationship between IQ and BLL[27]. The effects of VDR SNPs *Fok1*, *Apa1* and *Bsm1*have been demonstrated on blood lead levels among healthy pregnant women and are reported having lower lead levels with the association of the combination of f, a, and b alleles for the VDR *Fok1*, *Apa1*, and *Bsm1*SNPs than other VDR haplotypes[26]. Thus, all these studies demonstrate the variation of lead accumulation due to different VDR polymorphic variants.

At a molecular level, evidence show that VDR is a DNA binding transcription factor of the nuclear receptor superfamily[42]. Heterodimerization occurs through vitamin D activation with retinoid X receptor that is required for DNA binding, nuclear translocation, and transcriptional activation or suppression[43]. The *FokI* restriction site is found in exon 2 of the VDR gene that causes a change in the start codon (a T/C transition polymorphism [ATG to ACG]) and results in a VDR protein that binds to three amino acids[44]. This can cause altered activity and modification of mineral homeostasis and the regulation of calcium absorption and regeneration. Although the *FokI* polymorphism is at the NH_2 terminus, where the highly conserved DNA binding domain (DBD) is located, the ligand-binding domain (LBD) is at the variable COOH-terminus of the VDR molecule indicating the independent action of each domain[45]. However, LBD and DBD interact allosterically to regulate VDR gene expression as the *FokI* region is located in the DBD, it may cause changes in molecular mechanisms that ultimately lead to biological effects caused by high BLLs[46]. The variation in lead absorption may be associated with genetic factors that may influence mineral metabolism[47]. Because lead is also a bivalent cation; like calcium, it competes for absorption and protein-binding sites, and cellular lead uptake and its toxicokinetic scan increase when calcium stores are reduced or depleted[48].

Conclusion

This study provides insight into the association of vitamin D receptor polymorphic variants in modulating levels of blood lead (Pb) levels in subjects with occupational exposure. In addition, this study showed that *FokI* and *BsmI* variants are crucial genes associated with high BLL and may be suggested as potential candidates for genetic screening in industries with occupational exposure to lead. The functional implications of these genetic variants need to be elucidated.

Conflict of Interest

All authors declare no conflict of interest.

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