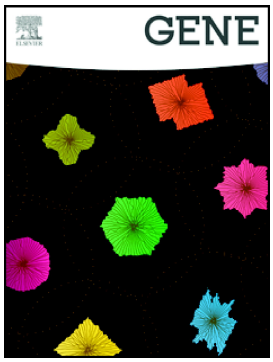

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PII: S0378-1119(17)30933-2
DOI: doi:10.1016/j.gene.2017.10.087
Reference: GENE 42308

To appear in: *Gene*

Received date: 20 June 2017
Revised date: 27 October 2017
Accepted date: 31 October 2017

Please cite this article as: N.R. Javorski, C.A.D. Lima, L.V.C. Silva, S. Crovella, J. de Azêvedo Silva , Vitamin D receptor (VDR) polymorphisms are associated to spontaneous preterm birth and maternal aspects. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Gene(2017), doi:10.1016/j.gene.2017.10.087

Vitamin D receptor (VDR) polymorphisms are associated to spontaneous preterm birth and maternal aspects

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Running Tittle: ***VDR SNPs and SPTB***

ABSTRACT

Preterm birth (PTB) is featured by less than 37 weeks of gestational age or fewer than 259 days since the first day from the last menstrual period. Complications of PTB are the major cause of neonatal deaths, several factors are linked to PTB increased risk including immunological and genetics. Vitamin D plays an important role in immune response modulation and its action occurs through the vitamin D receptor (VDR), which recently has been described as overexpressed in human placenta during the pregnancy. Herein we assessed two single nucleotide polymorphisms (SNPs) FokI (rs2228570 A>G) and Cdx-2 (rs11568820 T>C), within *VDR*, using TaqMan fluorogenic probes, and differential susceptibility to SPTB. We assessed 104 pregnant women with SPTB and 85 women with normal birth in a Northeastern Brazilian population. Statistically significant differences for both SNPs where found when comparing allele and genotype frequencies in both groups: the T allele for rs2228570 and A allele for rs11568820 were significantly more frequent in SPTB group than in normal birth group (p=0.000013 and p=0.00466, respectively). The rs11568820 A/A genotype was associated to clinical/demographic variables such as: premature birth (p=0.007), neonate weight (p=0.039), presence of infection during pregnancy (p=0.011) and premature birth among multiparous (p=0.015). The rs2228570 T/T genotype associated with gestational diabetes mellitus (p=0.044) and chorioamnionitis during pregnancy (p=0.043). In conclusion our findings indicate an association between polymorphisms FokI and Cdx-2 within *VDR* gene and SPTB, suggesting their involvement in the triggering of these syndrome.

KEYWORDS: SNPs, VDR, Spontaneous preterm birth, FokI and Cdx-2.

1. INTRODUCTION

Preterm birth (PTB) is the major cause of death among children and is a condition characterized by less than 37 weeks of gestational age or fewer than 259 days since the first day of women's last menstrual period. Complications in PTB are the major cause of death among children, being responsible for 35% from 3.1 million deaths per year that occurs worldwide . The precise aetiology of PTB is not completely known and is classified as a multifactorial syndrome divided into spontaneous preterm birth (SPTB) and provider-initiated PTB, with the last one deriving for maternal or fetal indications . Among them, about 30-35% of preterm birth are in fact indicated, 40-45% follow spontaneous preterm labour, and 25-30% follow premature rupture of membranes (PROM) .

PTB may occurs naturally, or spontaneously and represent the outcome, or final common pathway of a syndrome that constitutes a variety of pathologic processes .. Several factors contributes to an increased risk of a woman experiencing PTB, for instance, demographics, such as ethnicity , immunological, like infection and inflammation processes , and genetic factors, represented by maternal history of PTB . Therefore accurate prediction and prevention of this syndrome is rather difficult .

Even though several aspects are involved in SPTB, immunological and genetic factors seem to play a pivotal role in SPTB triggering. A widely proposed hypothesis links infections and inflammation with SPTB, including associations with subclinical intrauterine infection, intra-amniotic, and extrauterine maternal infections, such as kidney infection. The association of microorganisms with PTB have been postulated to originate the reproductive or genitourinary tract, ascending upward through the cervix . Substantial data provide evidence for a causal role of lower genital tract infection in the triggering of PTB, they suggest that the initial infection usually ascends from vagina

and cervix to the choriodecidual space and may affect the uterine tissues, such as myometrium, fetal membrane and amniotic fluids, resulting in intra-amniotic infection and immune stimulation . The maternal history of preterm birth is also associated to PTB increased risk , with several studies showing that genetic factors are likely to be important not only solely but also in combination with other genetic or environmental factors contributing to SPTB triggering .

Vitamin D_3 (cholecalciferol) is a hormone produced in the skin after sun exposure . D_3 classic function is to regulate calcium homeostasis and thus bone formation, and recently has been shown to also play an important role in other metabolic pathways, such as those involved in the immune response and cancer . Vitamin D function is through vitamin D receptor (VDR) binding and leads to transcriptional regulation of targeted genes. VDR is present in multiple tissues, such as skin epithelial cells, osteoblasts and chondrocytes, muscle, cells from the immune system and more recent in human placenta . Many studies correlated vitamin D levels with pregnancy outcomes, such as placentary function and fetal growth, suggesting an important role for the VDR and its signalling pathways in the placenta .

VDR is located in chromosome 12 (12q13) and displays an extensive promoter region capable of generating multiple tissue-specific transcripts. Polymorphisms found within *VDR* are distributed in eight coding exons and in two promoters regions. Thus far, several polymorphisms with unknown function were described including *BsmI*, *ApaI* and *TaqI*, the most studied ones. The polymorphisms named *Cdx-2* and *FokI*, are functional polymorphisms since they are able to alter either mRNA levels or protein structure, respectively . The *FokI* polymorphism creates an alternative transcription start site, leading to a VDR protein with 427 aminoacids, 3 aminoacids shorter then the one without the polymorphism. The Cdx-2 A>G polymorphism is located at promoter region

and increases *VDR* expression due to its possible action upon transcription factors binding .

Therefore, believing that vitamin D pathway might be closely linked with SPTB, the present study evaluated whether the *VDR* polymorphisms, rs2228570 (FokI) and rs11568820 (Cdx-2), and maternal clinical and demographic features are associated with differential risk of SPTB.

2. MATERIAL AND METHODS

2.1 Study design

Herein we performed a case-control study including 189 pregnant women derived from two hospitals from Recife, Brazil (Hospital das Clínicas and Hospital Agamenon Magalhães). The subjects enrolled were derived into two groups: the first one enclosed 104 women who have SPTB, and the second one with 85 women who have normal birth. Women with multi-fetal pregnancy, fetal anomalies and chronic infections were excluded from this study (Ethical committee number: 05719112.1.0000.5208).

2.2 DNA Extraction, Polymorphism selection and genotyping

Genomic DNA was extracted from 5 mL of peripheral whole blood, collected from February 2013 to March 2014, using the Mini-Salting out method adapted from Miller et al., 1988. The SNP rs2228570 (FokI) and rs11568820 (Cdx-2) located at promotor region and exon 2 of *VDR* gene, respectively, were selected for this study by their consequence in the final product and due their Minimum Allelic Frequency (MAF) >10%. Genotyping was performed by commercially available TaqMan probes (Applied Biosystem). All reactions were set up based on the manufacture's protocol and the samples were run at 7500 Real-Time PCR instrument (Applied Biosystem).

2.3 Statistical analysis

Allelic and genotypic frequencies as well as Hardy-Weinberg Equilibrium were calculated using Genotyping Transposer software. The genotype and allele distributions were compared using χ^2 test between the two groups. Odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression analysis in genetic models for adjustment of confounding factors such as ethnicity, previous premature birth, premature mother, family history of prematurity, urinary tract infection during pregnancy, leucorrhoea, premature birth among primiparous and multiparous, categorized parity, neonate weight, presence of infections during pregnancy, chorioamnionitis, PROM and gestational diabetes. Microsoft Excel and SPSS 24.0 (SPSS, Chicago, IL) were used to perform statistical analysis. In all analysis, P-value < 0.05 were considered statistically significant.

3. RESULTS

In this work we analysed two functional polymorphisms within *VDR*, the allele and genotype frequencies from rs2228570 (FokI) and rs11568820 (Cdx-2) were in Hardy-Weinberg equilibrium in both group (Table 1). FokI and Cdx-2 VDR allele frequencies were significantly different in the SPTB group when compared with the control maternal group (women with normal birth) (OR=7.49; p-value 0.00013; OR=3.11; p-value: 0.01724, respectively). The SNP rs2228570 T>C, the T allele was more frequent in the group with SPTB (44%) than in the group with normal birth in general (25%; OR=2.36, p-value= 0.00014) and the SNP rs11568820 G>A, also had the G allele was more frequent in SPTB (47%) group than in the normal birth group (36%; OR=1.55, p-value= 0.00466), being both polymorphisms associated with a major susceptibility to development the SPTB.

We also identified statistical differences in the T/T genotype, from FokI, and A/A genotype, from Cdx-2, when we analysing with the demographic data obtained from the mothers. We observed that the rs2228570 T/T genotype were more frequent between the SPTB group women who had gestational diabetes mellitus (GDM) (OR= 4.71, CI95%= 1.1-22.30; p-value= 0.044) and also among those women who developed chorioamnionitis during pregnancy (OR= 4,9, CI95%=1.2-22.30; p-value= 0.043). The rs11568820 A/A genotype was associated with: premature birth (OR=3.62; CI95%= 1.41-.9.27; p-value= 0.007), neonate weight, being more frequent among premature with low birth weight (OR=1.50; CI95%= 1.17-2.24; p-value=0.039), presence of infection during pregnancy (OR= 3.17, CI95%= 1.30-7.69, p-value=0.011) and premature birth among multiparous (OR=4.01; CI95%=1.30-12.33; p-value= 0.015), as demonstrated in table 2.

4. DISCUSSION

SPTB it is a multifactorial condition that includes genetic, environmental and hormonal factors as influencing upon its triggering. Has been suggested that the gene-environment interactions may better explain the risk of preterm birth, and if so, polymorphisms within critical genes might be used to ascribe risk .

Both tested SNPs, namely rs2228570 (FokI) and rs11568820 (Cdx-2) within *VDR*, were associated with increased susceptibility to SPTB in this study. Up until now, just one genetic association study performed in Israeli women was performed assessing FokI and SPTB. The authors performed, investigated the influence of four SNPs (FokI, ApaI, TaqI and BsmI), within *VDR* in 33 woman with SPTB and 98 woman with normal birth, but only FokI was associated (p= 0.01) . They also assessed umbilical cord samples from the primiparous full-term births and identified and increased risk for FokI

carried OR=3.31, indicating that this variant may represent a maternal risk trait for SPTB among these women . We identified similar results in our study population whereas FokI variant carried present an OR=7.49 and p-value=0.00013, indicating that this variant also may be influencing upon SPTB in Northeastern Brazilian population.

Regarding Cdx-2 SNP we also found an association with SPTB (OR=3.11, p-value=0.017), in the presence of Cdx2 variant A/A. This particular allele displays an impact in *VDR* expression, since the A-allele promotes an increasing binding to the Cdx-2 protein and thus increased transcription activity of the *VDR* promoter . In the immune system the vitamin D promotes monocyte differentiation and inhibits lymphocyte proliferation and secretion of cytokines, such as interleukin 2 (IL2), interferon-γ and IL12, all characterized as pro-inflammatory cytokines . Up until now no association study assessed this SNP in SPTB, even though it has been associated with immune unbalanced disorders such as carcinogenic processes as well as in infectious diseases, whereas in both pathologies the immune system plays an important role in establishment and/or combating of these disorders . Considering the fact that the maternal-fetal interface is a prime area of immune regulation, the role performed by vitamin D in immune system and that pregnant women possess vitamin D deficiency , the Cdx-2 polymorphism can be responsible for promoting, or even being a part of, a deregulation of immune system in those pregnant women and thus influencing the SPTB.

Most of genetic association studies of SPTB have focused on genes involved in immunity and inflammation such as the TNFα, IL1A, IL1B, IL1R, IL2 and IL4 . The reason for choosing those inflammation-related genes relies on physiological role of immunomodulation in the whole SPTB process. However, they are not the only ones responsible for immune modulation in SPTB. Vitamin D acts through both innate and

immune response and polymorphisms in the gene responsible to encoding VDR may compromise vitamin D action and thus unbalance immune response . VDR polymorphisms, particularly the ones assessed in this study, FokI and Cdx-2, provide more affinity with transcriptional factors (FokI) and correctly activate the transcription of target genes responsible for activating the immune response (Cdx-2) . Additionally, vitamin D levels during pregnancy are associated to normal intrauterine growth and skeletal mineralization . VDR and 1-alpha hydroxylase are expressed in the human placenta during all pregnancy, indicating that vitamin D locally influence fetal-placental development, cell growth and differentiation as well as in signalling in maternal-fetal interface .

Among risk factors for SPTB triggering, women who had previous preterm birth are more likely to repeat a preterm delivery, after the first, second and third premature labour the risk increase in 22%, 42% and 62%, respectively, for the recurrence for this outcome . The influences of previous premature birth and number of pregnancies were also found in this study, where there was a direct association between the Cdx-2 polymorphism with previous premature birth (OR: 3.62, p-value=0.007) and premature birth among multiparous (OR: 4.0; p-value=0.015). This result may reinforce the role of VDR variants in triggering the SPTB event.

To date, accumulating data has showed that vitamin D deficiency has been related with numerous health outcomes, including heart disease, cancer, hypertension, autoimmune disease, infectious disease, type 1 diabetes, type 2 diabetes and GDM . A case-control study conducted in the north of China demonstrated an association between some genes related with vitamin D signalling pathway and GDM, such as *CYP2R1*, *CYP24A1* and *VDR* . Recently, a study performed on a population of pregnant Iranian women also showed a significant association between *VDR ApaI* and *TaqI* gene

polymorphisms and the risk of GDM . Our study is the first to show an association between *VDR* FokI polymorphism and the GDM, reinforcing the *VDR* as a biomarker to this disease.

Some investigations highlighted the effect of micronutrients on birth weight, vitamin D affects fetal growth by its interaction with Ca^{2+} homeostasis and parathyroid hormone . In a recent study, performed in Iranian women, were detected that vitamin D deficiency was widespread among them, especially in rural women (61.1%) in compare to the urban resident ones (46.2%). Vitamin D deficiency and low birth weight were recently reported in Iranian population, the vitamin D serum level among women who delivered low birth weight neonates was significantly lower than in those with normal birth weight newborns (p=0.001) . In our study we observed an association between neonate weight and *VDR* Cdx-2 polymorphism, where newborns with lower weight were frequently found among women who had premature birth and also presented the A/A genotype for this polymorphism.

Acute inflammation and bacterial infection have been implicated in the mechanisms involved in preterm and term parturition, as well as fetal injury . Of all suspected causes of preterm labour and delivery, infection and/or inflammation is the only pathologic process for which there is causal link with preterm birth established and a molecular pathophysiology defined . Our findings regarding the association from Cdx-2 and infection during the pregnancy (OR:3.17; p-value=0.011) and the FokI T/T genotype with presence of chorioamnionitis (OR:4.7; p-value=0.043) agrees with the fact that infections are able to induce the SPTB. At last, our study may reinforce *VDR* variant FokI as potential marker in this condition and brings up the first genetic association data associating the Cdx-2 polymorphism with SPTB outcome, contributing in understanding the correlation of maternal aspects and these variants.

In summary, *VDR* polymorphisms are associated to SPTB and some maternal demographic variables in the Northeastern Brazilian population. Our results provide evidence for considering VDR as a potential marker in SPTB.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ACKNOWLEDGMENT

This work was supported by the Brazilian funding agencies: Capes, CNPq and FACEPE.

Tables Captions

Table 1. Allele and genotype frequencies from *VDR* rs2228570 and rs11568820 SNPs in SPTB women and normal birth women groups.

Table 2. Binary logistic regression analysis of the association between clinical characteristics and *VDR* genotypes.

REFERENCES

Annells MF, Hart PH, Mullighan CG, et al (2004) Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-beta, FAS, and mannose-binding protein C gene polymorphisms in Australian women: Risk of preterm birth. Am J Obstet Gynecol 191:2056–67. doi: 10.1016/j.ajog.2004.04.021

Arai H, Miyamoto KI, Yoshida M, et al (2001) The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J Bone Miner Res 16:1256–64. doi: 10.1359/jbmr.2001.16.7.1256

Bediako PT, BeLue R, Hillemeier MM (2015) A Comparison of Birth Outcomes Among Black, Hispanic, and Black Hispanic Women. J Racial Ethn Heal Disparities 2:573–582. doi: 10.1007/s40615-015-0110-2

Bezold KY, Karjalainen MK, Hallman M, et al (2013) The genomics of preterm birth: from animal models to human studies. Genome Med 5:34. doi: 10.1186/gm438

Crider KS, Whitehead N, Buus RM (2005) Genetic variation associated with preterm birth: a HuGE review. Genet Med 7:593–604. doi: 10.1097/01.gim.0000187223.69947.db

Dai Z-M, Fei Y-L, Zhang W-G, et al (2015) Association of Vitamin D Receptor Cdx-2 Polymorphism With Cancer Risk: A Meta-Analysis. Medicine (Baltimore)

94:e1370. doi: 10.1097/MD.0000000000001370
Evans KN, Nguyen L, Chan J, et al (2006) Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biol Reprod 75:816–822. doi: 10.1095/biolreprod.106.054056
Fang YUE, Meurs JBJVAN, Bergink AP, et al (2003) Cdx-2 Polymorphism in the Promoter Region of the Human Vitamin D Receptor Gene Determines Susceptibility to Fracture in the Elderly. J Bone Miner Res 18:1632–1641.
Goldenberg RL, Culhane JF, Iams JD, Romero R (2008) Epidemiology and causes of preterm birth. Lancet 371:75–84. doi: 10.1016/S0140-6736(08)60074-4
Green NS, Damus K, Simpson JL, et al (2005) Research agenda for preterm birth: recommendations from the March of Dimes. Am J Obstet Gynecol 193:626–35. doi: 10.1016/j.ajog.2005.02.106
Gross C, Krishnan a V, Malloy PJ, et al (1998) The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. J Bone Miner Res 13:1691–1699. doi: 10.1359/jbmr.1998.13.11.1691
Guo B, Jiang X, Hu X, et al (2015) Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. Int J Clin Exp Med 8:8020–4.
Harrison MS, Goldenberg RL (2015) Global burden of prematurity. Semin Fetal Neonatal Med 6–11. doi: 10.1016/j.siny.2015.12.007
Hewison M (2011) Vitamin D and innate and adaptive immunity. Vitam Horm 86:23–62. doi: 10.1016/B978-0-12-386960-9.00002-2
Hey H, Schmedes A, Horn P, Brandslund I (2009) Vitamin D deficiency. Ugeskr Laeger 171:2179–2184. doi: 10.1056/NEJMra070553
Jefferson KK (2012) The Bacterial Etiology of Preterm Birth. pp 1–22
Kalish RB, Vardhana S, Gupta M, et al (2004) Interleukin-4 and -10 gene polymorphisms and spontaneous preterm birth in multifetal gestations. Am J Obstet Gynecol 190:702–6. doi: 10.1016/j.ajog.2003.09.066
Khalessi N, Kalani M, Araghi M, Farahani Z (2015) The Relationship between Maternal Vitamin D Deficiency and Low Birth Weight Neonates. J Fam Reprod Heal 9:113–117.
Knight AK, Smith AK (2015) Insights into genetic susceptibility in the etiology of spontaneous preterm birth. 283–290.
Lewis S, Lucas RM, Halliday J, Ponsonby AL (2010) Vitamin D deficiency and pregnancy: From preconception to birth. Mol Nutr Food Res 54:1092–1102. doi: 10.1002/mnfr.201000044
Liu L, Johnson HL, Cousens S, et al (2012) Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 379:2151–61. doi: 10.1016/S0140-6736(12)60560-1
Lucock M, Jones P, Martin C, et al (2015) Vitamin D : Beyond Metabolism. doi: 10.1177/2156587215580491
Macones GA, Parry S, Elkousy M, et al (2004) A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. Am J Obstet Gynecol 190:1504–8; discussion 3A. doi: 10.1016/j.ajog.2004.01.001
Manzon L, Altarescu G, Tevet A, et al (2014) Vitamin D receptor polymorphism FokI is associated with spontaneous idiopathic preterm birth in an Israeli population. Eur J Obstet Gynecol Reprod Biol 177:84–8. doi: 10.1016/j.ejogrb.2014.03.008
McManemy J, Cooke E, Amon E, Leet T (2007) Recurrence risk for preterm delivery. Am J Obstet Gynecol 196:576.e1-6; discussion 576.e6-7. doi:

10.1016/j.ajog.2007.01.039
Mojibian M, Soheilykhah S, Fallah Zadeh MA, Jannati Moghadam M (2015) The effects of vitamin D supplementation on maternal and neonatal outcome: A randomized clinical trial. Iran J Reprod Med 13:687–96.
Muglia LJ, Katz M (2010) The Enigma of Spontaneous Preterm Birth. N Engl J Med 529–535. doi: 10.1056/NEJMra0904308
Muscogiuri G, Mitri J, Mathieu C, et al (2014) Mechanisms in endocrinology: vitamin D as a potential contributor in endocrine health and disease. Eur J Endocrinol 171:R101-10. doi: 10.1530/EJE-14-0158
Mysorekar IU, Cao B (2014) Microbiome in parturition and preterm birth. 32:50–55. doi: 10.1055/s-0033-1361830
Pasco JA, Wark JD, Carlin JB, et al (2008) Maternal vitamin D in pregnancy may influence not only offspring bone mass but other aspects of musculoskeletal health and adiposity. Med Hypotheses 71:266–269. doi: 10.1016/j.mehy.2008.01.033
Pennell CE, Jacobsson B, Williams SM, et al (2007) Genetic epidemiologic studies of preterm birth: guidelines for research. Am J Obstet Gynecol 196:107–118. doi: 10.1016/j.ajog.2006.03.109
Pilz S, Verheyen N, Grübler MR, et al (2016) Vitamin D and cardiovascular disease prevention. Nat Rev Cardiol 13:404–417. doi: 10.1038/nrcardio.2016.73
Prince AL, Antony KM, Chu DM, Aagaard KM (2014) The microbiome, parturition, and timing of birth: more questions than answers. J Reprod Immunol 104–105:12–19. doi: 10.1016/j.jri.2014.03.006
Ramagopalan S V, Heger A, Berlanga AJ, et al (2010) A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 20:1352–60. doi: 10.1101/gr.107920.110
Romero R, Espinoza J, Gonçalves L, et al (2007) The role of inflammation and infection in preterm birth. Semin Reprod Med 25:21–39. doi: 10.1055/s-2006-956773.
Romero R, Espinoza J, Kusanovic JP, et al (2006) The preterm parturition syndrome. BJOG 113 Suppl:17–42. doi: 10.1111/j.1471-0528.2006.01120.x
Romero R, Friel LA, Velez Edwards DR, et al (2010) A genetic association study of maternal and fetal candidate genes that predispose to preterm prelabor rupture of membranes (PROM). Am J Obstet Gynecol 203:361.e1-361.e30. doi: 10.1016/j.ajog.2010.05.026
Rubens CE, Sadovsky Y, Muglia L, et al (2014) Prevention of preterm birth: harnessing science to address the global epidemic. Sci Transl Med 6:262sr5. doi: 10.1126/scitranslmed.3009871
Tamblyn J a, Hewison M, Wagner CL, et al (2015) Immunological role of vitamin D at the maternal-fetal interface. J Endocrinol 224:R107-21. doi: 10.1530/JOE-14-0642
Uitterlinden AG, Fang Y, van Meurs JBJ, et al (2004) Genetics and biology of vitamin D receptor polymorphisms. Gene 338:143–156. doi: 10.1016/j.gene.2004.05.014
Wadhwa PD, Culhane JF, Rauh V, et al (2001) Stress, infection and preterm birth: a biobehavioural perspective. Paediatr Perinat Epidemiol 15 Suppl 2:17–29. doi: 10.1046/j.1365-3016.2001.00005.x
Wang Y, Zhu J, DeLuca HF (2012) Where is the vitamin D receptor? Arch Biochem Biophys 523:123–133. doi: 10.1016/j.abb.2012.04.001
Wei S-Q, Qi H-P, Luo Z-C, Fraser WD (2013) Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. J Matern Neonatal Med 26:889–899. doi: 10.3109/14767058.2013.765849
Zehnder D, Evans KN, Kilby MD, et al (2002) The Ontogeny of 25-Hydroxyvitamin D3

1α-Hydroxylase Expression in Human Placenta and Decidua. Am J Pathol 161:105–114. doi: 10.1016/S0002-9440(10)64162-4

Table 1. Allele and genotype frequencies of *VDR* rs2228570 and rs11568820 SNPs in SPTB women and normal birth women groups.

rs2228570 (FokI)	Case (n)	Control(n)	OR (CI 95%)	p-value
Alleles	n=208(Freq.)	n=170(Freq.)		
C	117 (0.56)	128 (0.75)	Ref.	
T	91 (0.44)	42 (0.25)	2.36 (1.48-3.79)	**0.00014**[a]
Genotypes	n=104(Freq.)	n=85(Freq.)		
CC	37 (0.36)	47 (0.55)	Ref.	
CT	43 (0.41)	34 (0.40)	1.60 (0.82-3.14)	0.1568
TT	24 (0.23)	4 (0.05)	7.49 (2.29-32.32)	**0.00013**[a]
rs11568820 (Cdx-2)	Case (n)	Control(n)	OR (CI 95%)	p-value
Alleles	n=208(Freq.)	n=170(Freq.)		
G	110 (0.53)	108 (0.64)	Ref.	
A	98 (0.47)	62 (0.36)	1.55 (1.01-2.40)	**0.0466**[a]
Genotypes	n=104(Freq.)	n=85(Freq.)		
GG	32 (0.31)	31 (0.36)	Ref.	
GA	46 (0.44)	46 (0.54)	0.96 (0.48-1.93)	1
AA	26 (0.25)	8 (0.10)	3.11 (1.14-9.22)	**0.01724**[a]

OR = Odds Ratio, CI = 95% Confidence Interval, HWE = Hardy-Weinberg Equilibrium.

[a] *Statistically significant p-value (highlighted in bold)*

Table 2: Binary logistic regression analysis of the association between clinical characteristics and *VDR* genotypes.

VDR *versus* Previous premature birth

Gene	SNP_ID	Model	Genotype	Case (%)	Control (%)	B	OR (95%IC)	P*
VDR	rs2228570 (FokI)	Dominant	C/C	37 (35.6%)	47 (55.3%)	0.55	0.57 (0.23-1.38)	0.218
			C/T - T/T	67 (64.4%)	38 (44.7%)	1		
		Recessive	C/C - C/T	80 (77%)	81 (95.3%)	1		
			T/T	24 (23%)	4 (4.7%)	0.66	1.95 (0.43-8.82)	0.386
		Overdominant	C/C - T/T	61 (58.7%)	51 (60%)	1		
			C/T	43 (41.3%	34 (40%)	0.28	1.33 (0.53-3.32)	0.536
	rs11568820 (Cdx-2)	Dominant	G/G	32 (30.8%)	31 (36.5%)	1		
			G/A - A/A	72(69.2%)	54 (63.5%)	0.39	1.48 (0.55-3.96)	0.434
		Recessive	G/G - G/A	78 (75%)	77 (90.6%)	1		
			A/A	26 (25%)	8 (9.4%)	1.28	3.62 (1.41-9.27)	0.007*
		Overdominant	G/G - A/A	58 (55.8%)	39 (45.9%)	1		
			G/A	46 (44.2%)	46 (54.1%)	0.65	1.92 (0.77-4.77)	0.160

VDR *versus* Neonate weight								
Gene	**SNP_ID**	**Model**	**Genotype**	**Case (%)**	**Control (%)**	**B**	**OR (95%IC)**	**P***
VDR	rs2228570 (FokI)	Dominant	C/C	37 (35.6%)	47 (55.3%)	0.18	1.20 (0.85-1.69)	0.293
			C/T - T/T	67 (64.4%)	38 (44.7%)	1		
		Recessive	C/C - C/T	80 (77%)	81 (95.3%)	1		
			T/T	24 (23%)	4 (4.7%)	- 0.27	0.76 (0.48-1.17)	0.220
		Overdominant	C/C - T/T	61 (58.7%)	51 (60%)	1		
			C/T	43 (41.3%	34 (40%)	- 0.03	0.96 (0.69-1.36)	0.854
	rs11568820 (Cdx-2)	Dominant	G/G	32 (30.8%)	31 (36.5%)	0.15	1.16 (0.80-1.67)	0.423
			G/A - A/A	72(69.2%)	54 (63.5%)	1		
		Recessive	G/G - G/A	78 (75%)	77 (90.6%)	1		
			A/A	26 (25%)	8 (9.4%)	0.40	1.50 (1.07-2.24)	0.046*
		Overdominant	G/G - A/A	58 (55.8%)	39 (45.9%)	1		
			G/A	46 (44.2%)	46 (54.1%)	0.13	1.15 (0.82-1.61)	0.418
VDR *versus* Presence of infection* during pregnancy								
Gene	**SNP_ID**	**Model**	**Genotype**	**Case (%)**	**Control (%)**	**B**	**OR (95%IC)**	**P***

							1.84	
	rs2228570			37	47		(0.78-	
VDR	(FokI)	Dominant	C/C	(35.6%)	(55.3%)	0.61	4.31)	0.160
				67	38			
			C/T - T/T	(64.4%)	(44.7%)	1		
					81			
		Recessive	C/C - C/T	80 (77%)	(95.3%)	1		
							0.76	
					4	-	(0.26-	
			T/T	24 (23%)	(4.7%)	0.26	2.22)	0.624
				61	51			
		Overdominant	C/C - T/T	(58.7%)	(60%)	1		
							0.64	
					34	-	(0.28-	
			C/T	43 (41.3%	(40%)	0.44	1.43)	0.282
							0.88	
	rs11568820			32	31	-	(0.38-	
	(Cdx-2)	Dominant	G/G	(30.8%)	(36.5%)	0.12	2.04)	0.772
			G/A -		54			
			A/A	72(69.2%)	(63.5%)	1		
			G/G -		77			
		Recessive	G/A	78 (75%)	(90.6%)	1		
							3.17	
					8		(1.30-	
			A/A	26 (25%)	(9.4%)	1.15	7.69)	0.011*
			G/G -	58	39			
		Overdominant	A/A	(55.8%)	(45.9%)	1		
							2.72	
				46	46		(1.13-	
			G/A	(44.2%)	(54.1%)	1.00	6.55)	0.025*

VDR *versus* Gestational diabetes								
Gene	**SNP_ID**	**Model**	**Genotype**	**Case (%)**	**Control**	**B**	**OR**	**P***

					(%)		(95%IC)	
VDR	rs2228570 (FokI)	Dominant	C/C	37 (35.6%)	47 (55.3%)	0.71	2.05 (0.38-10.42)	0.398
			C/T - T/T	67 (64.4%)	38 (44.7%)	1		
		Recessive	C/C - C/T	80 (77%)	81 (95.3%)	1		
			T/T	24 (23%)	4 (4.7%)	1.55	4.71 (1.1-22.30)	0.044
		Overdominant	C/C - T/T	61 (58.7%)	51 (60%)	1		
			C/T	43 (41.3%	34 (40%)	0.56	1.75 (0.33-9.27)	0.509
	rs11568820 (Cdx-2)	Dominant	G/G	32 (30.8%)	31 (36.5%)	0.23	1.26 (0.23-6.68)	0.786
			G/A - A/A	72(69.2%)	54 (63.5%)	1		
		Recessive	G/G - G/A	78 (75%)	77 (90.6%)	1		
			A/A	26 (25%)	8 (9.4%)	0.28	1.32 (0.15-11.41)	0.796
		Overdominant	G/G - A/A	58 (55.8%)	39 (45.9%)	1		
			G/A	46 (44.2%)	46 (54.1%)	- 0.35	0.70 (0.15-3.22)	0.702

VDR *versus* Premature birth among multiparous

Gene	SNP_ID	Model	Genotype	Case (%)	Control (%)	B	OR (95%IC)	P*
VDR	rs2228570 (FokI)	Dominant	C/C	37 (35.6%)	47 (55.3%)	- 0.19	0.82 (0.31-	0.697

						2.17)	
			C/T - T/T	67 (64.4%)	38 (44.7%)	1	
		Recessive	C/C - C/T	80 (77%)	81 (95.3%)	1	
			T/T	24 (23%)	4 (4.7%)	0.19	1.21 (0.22-6.49)	0.823
		Overdominant	C/C - T/T	61 (58.7%)	51 (60%)	1	
			C/T	43 (41.3%	34 (40%)	0.13	1.14 (0.41-3.14)	0.791
	rs11568820 (Cdx-2)	Dominant	G/G	32 (30.8%)	31 (36.5%)	0.40	1.49 (0.50-4.37)	0.467
			G/A - A/A	72(69.2%)	54 (63.5%)	1	
		Recessive	G/G - G/A	78 (75%)	77 (90.6%)	1	
			A/A	26 (25%)	8 (9.4%)	1.39	4.01 (1.30-12.33)	0.015*
		Overdominant	G/G - A/A	58 (55.8%)	39 (45.9%)	1	
			G/A	46 (44.2%)	46 (54.1%)	0.69	2.00 (0.73-5.46)	0.172

** Lower genital tract infection. Zika virus was introduced in Brazil after this sample collection (2014) and therefore testing the virus was not performed.*

Highlights

- This is a new report relating polymorphisms within *VDR* gene and prematurity's outcomes.
- It is also the first study correlating clinical characteristics and spontaneous preterm birth, according to the maternal genotype for the VDR gene.
- The binary logistic regression detected a direct relationship between the recessive genotype of VDR polymorphisms, found among the women with spontaneous premature birth, and some clinical characteristics identified in those women.