Vitamin D Receptor FokI polymorphism is a determinant of both maternal and neonatal Vitamin D concentrations at birth

Spyridon N. Karras[1], Theocharis Koufakis[1], Vasiliki Antonopoulou[1], Dimitrios G. Goulis[2], Merve Alaylıoğlu[3], Erdinc Dursun[3,4], Diugy Gezen-Ak[3], Cedric Annweiler[5,6], Stefan Pilz[7], Hana Fakhoury[8], Fatme Al Anouti[9], Vikentia Harizopoulou[2], Declan P. Naughton[10], Pantelis Zebekakis[1], Kalliopi Kotsa[1]

[1]Division of Endocrinology and Metabolism, First Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA University Hospital, Thessaloniki, Greece

[2]Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3]Brain and Neurodegenerative Disorders Research Laboratory, Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey

[4]Department of Neuroscience, Institute of Neurological Sciences, Istanbul University-Cerrahpasa, Istanbul, Turkey

[5]Department of Neuroscience, Division of Geriatric Medicine, Angers University Hospital, Angers, France; University Memory Clinic, UPRES EA 4638, University of Angers, UNAM, Angers, France

[6]Robarts Research Institute, Department of Medical Biophysics, Schulich School of Medicine and Dentistry, the University of Western Ontario, London, Ontario, Canada

[7]Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University Graz, Graz, Austria

[8]Department of Biochemistry and Molecular Biology, College of Medicine, AlFaisal University, Riyadh, KSA

[9]Zayed University, College of Natural and Health Sciences, Abu Dhabi, United Arab Emirates

[10] School of Life Sciences, Kingston University London, UK

Correspondence: Dr. Spyridon N. Karras, Division of Endocrinology and Metabolism, First Department of Internal Medicine, AHEPA Hospital, S. Kiriakidi 1 str., Thessaloniki, Greece, karraspiros@yahoo.gr; Tel.: +30 2310324863.

Abstract

Maternal vitamin D deficiency is considered to be the key determinant of the development of neonatal vitamin D deficiency at birth and during early infancy. Specific vitamin D receptor (*VDR*) gene polymorphisms have been associated with adverse pregnancy and offspring outcomes. The aim of this study was to evaluate the effect of maternal and neonatal *VDR* polymorphisms (ApaI, TaqI, BsmI, FokI, Tru9I) on maternal and neonatal vitamin D status. *VDR* polymorphisms were genotyped in 70 mother-neonate pairs of Greek origin, and classified according to international thresholds for Vitamin D status. Mean neonatal and maternal 25-hydroxy-vitamin D [25(OH)D] concentrations were 35 ± 20 and 47 ± 26 nmol/l, respectively. Neonatal *VDR* polymorphisms were not associated with neonatal 25(OH)D concentrations. In contrast, mothers with the FokI FF polymorphism had a 70% lower risk of vitamin D deficiency [25(OH)D <30 nmol/l] compared with ff ones, after adjustment for several confounders. They were also in 73% and 88% lower risk of giving birth to vitamin D deficient [25(OH)D <30 nmol/l] neonates compared with Ff and ff mothers, respectively. These results suggest a protective role of maternal FokI FF genotype against both maternal and neonatal vitamin D deficiency. Further studies are needed to clarify the complex gene-gene and gene-environment interactions that determine vitamin D status at birth.

Keywords: Vitamin D; pregnancy; neonatal health; calcium; rickets.

1. Introduction

The teleological purpose of an ongoing pregnancy is to fulfil its fundamental role in a successful, uncomplicated delivery, along with an optimal intrauterine environment for the developing fetus [1]. Vitamin D homeostasis during pregnancy is adapted to meet both those demands, first, by stimulation of calcium (Ca) absorption for adequate intrauterine bone mineral accrual of the fetus and second, by enhancing systemic and local maternal tolerance to paternal and fetal alloantigens [1]. On that basis, data from observational studies during the last decade have suggested a potential adverse effect of maternal hypovitaminosis D during pregnancy on maternal and offspring health outcomes. Randomized trials of moderate quality indicate that vitamin D supplementation during pregnancy might reduce the risk of pre-eclampsia, gestational diabetes, low birth weight and severe postpartum hemorrhage [1]. Maternal vitamin D deficiency is also considered to be the key determinant of the development of neonatal vitamin D deficiency at birth and during early infancy.

Maternal 25-hydroxy-vitamin D [25(OH)D] crosses the placental barrier and represents the main pool of vitamin D for the fetus [2]. Serum fetal (cord blood) 25(OH)D concentrations correlate strongly with maternal 25(OH)D concentrations, being on average 25% lower compared with the latter [3,4].

Guidelines suggest a maternal vitamin D intake of >600 IU/day, to prevent elevated cord blood alkaline phosphatase, increased fontanelle size, neonatal hypocalcemia and congenital rickets [2] and to ensure the adequacy of maternal vitamin D status, especially in women at risk of deficiency [5]. However, there is an ongoing controversy among experts worldwide about the definition of maternal vitamin D deficiency during pregnancy, especially about the optimal thresholds of maternal 25(OH)D concentrations (≥50 nmol/l vs. ≥75 nmol/l) [6,7]. On the other hand,

different criteria are used to define optimal neonatal vitamin D status (sufficiency >50 nmol/l, insufficiency 30-50 nmol/l, deficiency <30 nmol/l) [5].

Well-designed clinical trials indicate that maternal 25(OH)D concentrations >100 nmol/l (40 ng/ml) during pregnancy are associated with a 60 % reduction in preterm birth risk in the daily clinical obstetric care [8]. In this setting, prenatal screening programs for optimizing 25(OH)D concentrations have been demonstrated as an effective approach to detect deficient women and prevent pregnancy complications [9]. Previously published randomized controlled trials demonstrated that a daily vitamin D dose of 4000 IU safely elevated circulating 25(OH)D concentrations and normalized vitamin D metabolism and Ca homeostasis in pregnant women, regardless of race [10,11]. More specifically, a circulating 25(OH)D level of about 100 nmol/l was found to be the required concentration to optimize production of $1,25(OH)_2D$ during pregnancy through renal and/or placental production of the hormone [12].

Based on these findings, a target of maternal 25(OH)D >100 nmol/l seems to be biologically and scientifically sound [13]. The fact that the aforementioned data was not incorporated into previous systematic reviews [1], might lie in the fact that most of those systematic analyses did not include trials in which any amount of the investigated agent was given to the control group, including the study by Hollis et al. [12], in which the authors considered unethical not to supplement with minimal dose the control group.

Despite the differences in definitions of maternal and neonatal vitamin D status and the lack of uniform results on the association between maternal thresholds and neonatal outcomes, the appliance of criteria for vitamin D status resulted in an improvement of the management of maternal hypovitaminosis D in the daily clinical

111 setting [5-7]. On the other hand, the clinical aspects of this controversy are largely
112 reflected in the conflicting results between observational and supplementation studies
113 [14], also affected by country-specific dietary patterns, public health policies and
114 variation in ultraviolet B (UVB) exposure, due to cultural and life-style reasons [15].
115 In this context, the effects of genetic variations of vitamin D receptor (*VDR*) gene on
116 maternal and neonatal vitamin D status are gaining increasing interest. Specific *VDR*
117 polymorphisms have been associated with adverse pregnancy and offspring outcomes
118 [16-18]. However, robust evidence of such an association is currently unavailable,
119 given that various studies present significant heterogeneity in terms of maternal and
120 neonatal criteria for vitamin D status, study design, sample size and racial descent of
121 the included subjects.
122 The aim of this study was to evaluate the effect of maternal and neonatal *VDR*
123 polymorphisms (ApaI, TaqI, BsmI, FokI, Tru9I) on maternal and neonatal vitamin D
124 status, by applying internationally-adopted criteria for maternal and neonatal vitamin
125 D deficiency.
126
127 **2. Methods**
128 **2.1. Inclusion and exclusion criteria**
129 This study included data and samples from a cohort of mother-child pairs at birth that
130 has been previously described [3]. Pregnant women on regular follow-up were
131 recruited from the Maternity Unit of the 1st Department of Obstetrics and Gynecology,
132 Aristotle University, Thessaloniki, Greece. The inclusion criterion was full-term
133 pregnancy (gestational week 37-42). Maternal exclusion criteria were primary
134 hyperparathyroidism, secondary osteoporosis, heavy alcohol use (≥7 alcohol units per
135 week or ≥6 units at any time during pregnancy), hyperthyroidism, nephritic syndrome,

136 inflammatory bowel disease, rheumatoid arthritis, osteomalacia, obesity [body mass
137 index (BMI) >30 kg/m^2], gestational diabetes and use of medications affecting Ca or
138 vitamin D status (e.g. corticosteroids), except for Ca and vitamin D supplements.
139 Neonatal exclusion criteria were being small-for-gestational age (SGA) and presence
140 of severe congenital anomalies. Informed consent was obtained from all mothers. The
141 study was conducted from January 2018 to September 2018. The protocol received
142 approval from the Bioethics Committee of the Aristotle University of Thessaloniki,
143 Greece (approval number 1/19-12-2011).
144
145 **2.2. Demographics and dietary assessment**
146 At enrolment, maternal demographic and social characteristics, as well as dietary
147 habits, were recorded. Ca and vitamin D dietary intake during the last month of
148 pregnancy were assessed through a validated, semi-quantitative, food frequency
149 questionnaire that includes 150 foods and beverages [19-21]. For each dietary item,
150 participants were asked to report their frequency of dairy products consumption and
151 portion size. From these data, calculations were made for estimations of consumed
152 quantities (in g per day) based on a food composition database, modified to
153 accommodate the particularities of the Greek diet [22] for estimating daily dietary
154 calcium and vitamin D intake. Maternal education was classified as elementary
155 (primary), standard (secondary) and higher (tertiary and holding of academic
156 degrees). Maternal alcohol use during pregnancy was treated as a dichotomous
157 variable, defined either as none (subdivided in never drinking alcohol or drinking
158 alcohol but not during pregnancy) or light (1-2 units per week or at any one time
159 during pregnancy) / moderate (3-6 units per week or at any one time during
160 pregnancy) [23].

161

162 **2.3. Biochemical and hormonal assays**

163 Blood samples were obtained from mothers by antecubital venipuncture 30-60 min
164 before delivery. Umbilical cord blood was collected immediately after clamping, from
165 the umbilical vein. Serum and umbilical cord specimens were stored at -20°C prior to
166 analysis for the following parameters: Ca, phosphorus (P), parathyroid hormone
167 (PTH), 25-hydroxyvitamin D_2 [25(OH)D_2] and 25(OH)D. Serum Ca and P
168 determinations were performed using the Cobas INTEGRA clinical chemistry system
169 (D-68298; Roche Diagnostics, Mannheim, Germany). The inter- and intra-assay
170 coefficients of variation (CVs) were 1.0% and 3.5% for Ca, and 1.3% and 2.5% for P,
171 respectively. PTH determinations were performed using the electro-
172 chemiluminescence immunoassay ECLIA (Roche Diagnostics GmbA, Mannheim,
173 Germany). Reference range for PTH was 15-65 pg/ml, functional sensitivity 6.0
174 pg/ml, within-run precision 0.6-2.8% and total precision 1.6-3.4%. Concentrations of
175 25(OH)D_2 and 25(OH)D were determined using novel assay, liquid chromatography-
176 tandem mass spectrometry (LC-MS/MS), with lower limits of quantification (LLOQ):
177 25(OH)D_2(0.5 ng/ml), 25(OH)D (0.5 ng/ml). Briefly, the assay involves analyte
178 purification using liquid-liquid extraction followed by chromatographical separation
179 using a chiral column in tandem with a rapid resolution microbore column. Full
180 method validation parameters have been previously reported [24,25].

181

182 **2.4. Neonatal and maternal vitamin D status**

183 Differences in the frequency of neonatal *VDR* polymorphisms were determined
184 between three groups of neonates, according to their vitamin D status at birth:
185 25(OH)D <30 nmol/l (deficiency), 30≤ 25(OH)D ≤50 nmol/l (insufficiency) and

25(OH)D >50 nmol/l (sufficiency) [2]. Differences in the genotype distribution of maternal *VDR* polymorphisms were evaluated between different groups of Vitamin D status, defined by using two thresholds for maternal 25(OH)D concentrations: [25(OH)D <30 nmol/l)] vs. [25(OH)D ≥30 nmol/l] and [25(OH)D <50 nmol/l] vs. [25(OH)D ≥50 nmol/l] [26].

2.5. *VDR* analysis

DNA was isolated from peripheral blood samples by QIAamp DNA Blood Mini Kit (Cat. No. 51304, QIAGEN, Hilden, Germany) according to manufacturer's protocol. In order to determine the genotypes of rs7975232 (ApaI), rs7731236 (TaqI), rs757343 (Tru9I) and rs1544410 (BsmI) SNPs within VDR gene, Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) methods were performed as previously described [27]. Real-Time PCR (RT-PCR) method was used for determining genotypes of rs2228570 (FokI) SNP by using Simple Probe (LightSNiP, TibMolBiol, Berlin, Germany) and LightCycler Fast Start DNA Master HybProbe Kit (Cat. No. 12239272001, Roche Diagnostics, Mannheim, Germany) with LightCycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). Melting curve analysis were performed for genotyping as previously described [28]. Each SNP allele named after as follows: for rs7731236 (TaqI), "t" represents C, "T" represents T nucleotide; for rs7975232 (ApaI), "a" represents C, "A" represents A nucleotide, for rs757343 (Tru9I), "u" represents A, "U" represents G nucleotide, for rs1544410 (BsmI), "b" represents G, "B" represents A nucleotide, and for rs2228570 (FokI), "f" represents T, "F" represents C nucleotide.

2.6. Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk analyses were carried out on the data to test for normality. Group differences were tested using the chi-square test, whereas Fisher's exact test was applied, in case the expected values were less than five. Categorical data were presented as absolute numbers and frequencies (percentages). Univariate logistic regression was performed to identify independent associations of maternal and neonatal polymorphisms with both neonatal and maternal vitamin D status, after adjusting for confounders, such as age, smoking status, education level, alcohol consumption, Ca supplementation, dietary daily Ca and vitamin D intake during the third trimester, pre-pregnancy BMI and delivery BMI. The genotype frequencies were tested for the Hardy-Weinberg equilibrium using the https://ihg.gsf.de/ihg/index_engl.html for cases and controls through the Pearson chi-square (χ^2) test. ORs and *p*-values, were all adjusted for confounders and tested for co-dominant, dominant and additive genetic models.

3. Results

Seventy mother-neonate pairs were included in the study. Demographic and laboratory data of mothers and neonates are presented in **Table 1**. Mean neonatal 25(OH)D concentrations were 35 ± 20 nmol/l. Overall, 52% (n=36) of the neonates were vitamin D deficient, 27% (n=19) insufficient and 21% (n=15) sufficient. Mean maternal age and 25(OH)D concentrations were 33 ± 6 years and 47 ± 26 nmol/l, respectively. Overall, 34% (n=24) of the mothers were vitamin D deficient, 30% (n=21) insufficient and 36% (n=25) sufficient. No deviations from HWE were observed.

3.1. Association between neonatal polymorphisms and neonatal Vitamin D status

No differences in the genotype distribution of the *VDR* gene polymorphisms (ApaI, TaqI, BsmI, FokI, Tru9I) were detected among sufficient, insufficient and deficient neonates (**Table 2**). In the case of the FokI polymorphism, the difference in the frequency of the FF genotype between sufficient and deficient neonates approached, but did not reach, significance (67 vs. 36%, p=0.05).

3.2. Association between maternal polymorphisms and maternal Vitamin D status

FokI FF genotype was more frequent among mothers with 25(OH)D concentrations ≥30 nmol/l compared with those with <30 nmol/l (57 vs. 25%, p=0.02) (**Table 3**). Similar results were yielded when the threshold of 50 nmol/l was used to define groups of maternal Vitamin D status [64% in mothers with 25(OH)D ≥50 nmol/l vs. 36% in those <50 nmol/l, p=0.02] (**Table 4**). No differences in the genotype distribution of the other polymorphisms (ApaI, TaqI, BsmI, FokI, Tru9I) were detected between maternal groups of Vitamin D status, irrespectively of the Vitamin D threshold applied. The probability of maternal deficiency [25(OH)D <30 nmol/l] was 70% lower in FokI FF mothers compared with Ff ones [odds ratio (OR) 0.3, 95% confidence interval (CI) 0.09-0.92, p=0.03) and 88% lower in carriers of the FF genotype than those of the ff genotype (OR 0.12, 95% CI 0.02-0.78, p=0.03).

3.3. Association between maternal polymorphisms and neonatal Vitamin D status

Maternal FokI FF genotype was more frequent among mothers of non-deficient [25(OH)D ≥30 nmol/l] neonates compared with those of deficient ones [25(OH)D <30 nmol/l)] (62 vs. 31%, p<0.01) (**Table 5**). When the same analysis was performed

using a neonatal 25(OH)D threshold of 50 nmol/l [25(OH)D <50 nmol/l vs. 25(OH)D ≥50 nmol/l], no differences in the distribution of maternal *VDR* genotypes were observed between groups. Mothers with the FokI FF genotype presented a 73% lower risk of giving birth to vitamin D deficient neonates (logistic regression - OR 0.27, 95% CI 0.1-0.77, p=0.01) compared with carriers of the Ff genotype (**Table 6**).

4. Discussion

This study aimed to evaluate the effects of maternal and neonatal *VDR* polymorphisms on maternal and neonatal vitamin D status at birth, including a population from a sunny Mediterranean area in Northern Greece. Results from this maternal-neonatal pair cohort indicate that specific maternal genotypes might be protective against neonatal vitamin D deficiency, defined according to internationally applied criteria for vitamin D status [5], irrespective of neonatal *VDR* genetic variation. These findings are the first to be reported on the association between neonatal *VDR* polymorphisms and vitamin D status from this region.

A protective effect of maternal FokI FF genotype against the development of neonatal vitamin D deficiency [25(OH)D <30 nmol/l] was demonstrated. This effect was rationally mediated through the attainment of sufficient maternal vitamin status [25(OH)D ≥30 nmol/l and ≥50 nmol/l] [6,7], in mothers with the FF genotype. Neonatal 25(OH)D concentrations at birth roughly follow the maternal pattern in the deficient and insufficient mother groups, while follow the normal distribution in the group of mothers with sufficient vitamin D status [3,4]. The attainment of maternal vitamin D sufficiency during pregnancy is associated with a decreased prevalence of maternal and neonatal complications [29]. Still, there is a lack of consensus regarding specific maternal 25(OH)D thresholds that affect neonatal outcomes. Taking into

account that neonatal vitamin D status at birth is decreased by approximately 25% compared with the respective maternal vitamin D concentrations, a maternal 25(OH)D threshold of ≥50 nmol/l, could theoretically be able to prevent the development of neonatal vitamin D deficiency. However, the extent to which this phenomenon is affected by genetic variants and ethnic differences has not been elucidated.

The present study was conducted in an area with a high prevalence of maternal vitamin D deficiency during pregnancy, albeit abundant sunshine [30], mainly due to sartorial habits, lack of food fortification [31] and reduced sunshine exposure, especially during the hot summer months [32]. The identification of maternal FokI FF carriers could contribute to the overall improvement of prediction scores for management of maternal and neonatal vitamin D deficiency, yet to be developed in this region. In addition, such an approach could provide individualized management of vitamin D supplementation to the future mother.

The FokI FF genotype was associated with optimal maternal 25(OH)D concentrations (≥30 nmol/l and ≥50 nmol/l), still not with an increased probability of neonatal vitamin D sufficiency [25(OH)D ≥50 nmol/l]. Possible reasons for this observation might be related to the small study sample or other parameters implicated in the regulation of maternal-neonatal vitamin D equilibrium. Apart from low dietary vitamin D intake during pregnancy, additional factors such as sunlight / UVB exposure, dark skin pigmentation and maternal anthropometry may constitute gene-environment interactions that affect neonatal Vitamin D status.

Although an association between *VDR* polymorphisms and adverse pregnancy outcomes, such as preterm birth and SGA neonates [18,33,34] has been suggested, relative evidence is still inconclusive. In a case-control study, maternal but not

311 placental, *VDR* FokI Ff genotype was found to be lower in preeclamptic women
312 compared with controls [35]. On the other hand, FokI *VDR* variant was associated
313 with a higher risk for preterm birth and recurrent pregnancy loss [36,37]. The
314 significant heterogeneity among the studies might be explained by the lack of
315 standardized thresholds for vitamin D status, which would enable a universal
316 stratification of mothers and neonates. The racial diversity of included populations
317 might also contribute to the inconsistency of the results, underlying the importance of
318 regionally-derived data in the implementation of national health policies for the
319 prevention of vitamin D-associated adverse maternal and neonatal outcomes [38, 39].
320 The main limitation of the present study is the relatively small sample size, which
321 attenuates its power to reveal gene-outcome associations. Consequently, the
322 probability that potentially significant associations may have been missed should be
323 considered. Furthermore, neonatal vitamin D status and adverse pregnancy outcomes
324 are dependent on complex gene-gene and gene-environment interactions, that
325 interplay at both maternal and neonatal levels. As a result, the variation in a single
326 gene cannot sufficiently explain the entire spectrum of the pathophysiology of vitamin
327 D deficiency at birth. On the other hand, the strengths of the present study are the
328 inclusion of an ethnically homogenous population of mothers and neonates and the
329 use of multiple thresholds to determine vitamin D status. Further studies with larger
330 sample sizes that will involve subjects of different ethnic origins are needed to
331 replicate the findings of the present study and clarify the complex underlying
332 mechanisms. To conclude, this study highlights the value of population-specific,
333 genetic profiling in understanding vitamin D deficiency among neonates and their
334 mothers.
335

Declaration of interest: Authors report no conflict of interest.

Source of funding: This work was supported by the Hellenic Endocrine Society.

Informed consent: Informed consent was obtained from all participants.

Ethical approval: The study protocol was approved by the Bioethics Committee of the Aristotle University of Thessaloniki.

References

1. Palacios C, Kostiuk LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev. 2019;7:CD008873.
2. Karras SN, Wagner CL, Castracane VD. Understanding vitamin D metabolism in pregnancy: From physiology to pathophysiology and clinical outcomes. Metabolism. 2018;86:112-23.
3. Karras SN, Shah I, Petroczi A, Goulis DG, Bili H, Papadopoulou F, Harizopoulou V, Tarlatzis BC, Naughton DP. An observational study reveals that neonatal vitamin D is primarily determined by maternal contributions: implications of a new assay on the roles of vitamin D forms. Nutr J. 2013;12:77.
4. Markestad T, Aksnes L, Ulstein M, Aarskog D. 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D of D2 and D3 origin in maternal and umbilical cord serum after vitamin D2 supplementation in human pregnancy. Am J Clin Nutr. 1984;40:1057-63.
5. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Mäkitie O, Ramos-Abad L, Ward L, DiMeglio LA, Atapattu N, Cassinelli H, Braegger C, Pettifor JM, Seth A, Idris HW, Bhatia V, Fu J, Goldberg G, Sävendahl L, Khadgawat R, Pludowski P, Maddock J, Hyppönen E, Oduwole A, Frew E, Aguiar M, Tulchinsky T, Butler G, Högler W. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab. 2016;101:394-415.
6. Pludowski P, Holick MF, Grant WB, Konstantynowicz J, Mascarenhas MR, Haq A, Povoroznyuk V, Balatska N, Barbosa AP, Karonova T, Rudenka E, Misiorowski W, Zakharova I, Rudenka A, Łukaszkiewicz J, Marcinowska-Suchowierska E, Łaszcz N, Abramowicz P, Bhattoa HP, Wimalawansa SJ

Vitamin D supplementation guidelines. J Steroid Biochem Mol Biol. 2018;175:125-35.

7. Pilz S, Trummer C, Pandis M, Schwetz V, Aberer F, Grübler M, Verheyen N, Tomaschitz A, März W. Vitamin D: Current Guidelines and Future Outlook. Anticancer Res. 2018;38:1145-51.

8. McDonnell SL, Baggerly KA, Baggerly CA, Aliano JL, French CB, Baggerly LL, Ebeling MD, Rittenberg CS, Goodier CG, Mateus Niño JF, Wineland RJ, Newman RB, Hollis BW, Wagner CL. Maternal 25(OH)D concentrations ≥40 ng/mL associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. PLoS One. 2017 ;12:e0180483.

9. Rostami M, Tehrani FR, Simbar M, Bidhendi Yarandi R, Minooee S, Hollis BW, Hosseinpanah F. Effectiveness of Prenatal Vitamin D Deficiency Screening and Treatment Program: A Stratified Randomized Field Trial. J Clin Endocrinol Metab. 2018;103:2936-48.

10. Motamed S, Nikooyeh B, Kashanian M, Hollis BW, Neyestani TR. Efficacy of two different doses of oral vitamin D supplementation on inflammatory biomarkers and maternal and neonatal outcomes. Matern Child Nutr. 2019;15:e12867.

11. Dawodu A, Saadi HF, Bekdache G, Javed Y, Altaye M, Hollis BW. Randomized controlled trial (RCT) of vitamin D supplementation in pregnancy in a population with endemic vitamin D deficiency. J Clin Endocrinol Metab. 2013;98:2337-46.

12. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res. 2011;26:2341–57.

394 **13.** Wagner CL, Hollis BW. The Implications of Vitamin D Status During Pregnancy
395 on Mother and her Developing Child. Front Endocrinol (Lausanne). 2018;9:500.
396 **14.** Karras SN, Anagnostis P, Naughton D, Annweiler C, Petroczi A, Goulis DG.
397 Vitamin D during pregnancy: why observational studies suggest deficiency and
398 interventional studies show no improvement in clinical outcomes? A narrative
399 review. J Endocrinol Invest. 2015;38:1265-75.
400 **15.** Wagner CL, Hollis BW, Kotsa K, Fakhoury H, Karras SN. Vitamin D
401 administration during pregnancy as prevention for pregnancy, neonatal and
402 postnatal complications. Rev Endocr Metab Disord. 2017;18:307-22.
403 **16.** Suarez F, Zeghoud F, Rossignol C, Walrant O, Garabédian M. Association
404 between vitamin D receptor gene polymorphism and sex-dependent growth during
405 the first two years of life. J Clin Endocrinol Metab. 1997;82:2966-70.
406 **17.** Lorentzon M, Lorentzon R, Nordström P. Vitamin D receptor gene polymorphism
407 is associated with birth height, growth to adolescence, and adult stature in healthy
408 caucasian men: a cross-sectional and longitudinal study. J Clin Endocrinol Metab.
409 2000;85:1666-70.
410 **18.** Workalemahu T, Badon SE, Dishi-Galitzky M, Qiu C, Williams MA, Sorensen T,
411 Enquobahrie DA. Placental genetic variations in vitamin D metabolism and
412 birthweight. Placenta. 2017;50:78-83.
413 **19.** Crowe FL, Steur M, Allen NE, Appleby PN, Travis RC, Key TJ. Plasma
414 concentrations of 25-hydroxyvitamin D in meat eaters, fish eaters, vegetarians and
415 vegans: results from the EPIC-Oxford study. Public Health Nutr. 2011;14:340-6.
416 **20.** Liu J, Arcot J, Cunningham J, Greenfield H, Hsu J, Padula D, Strobel N, Fraser
417 DR. New data for vitamin D in Australian foods of animal origin: impact on

418 estimates of national adult vitamin D intakes in 1995 and 2011-13. Asia Pac J Clin
419 Nutr. 2015;24:464-71.
420 **21.** Dunlop E, Cunningham J, Sherriff JL, Lucas RM, Greenfield H, Arcot J, Strobel
421 N, Black LJ. Vitamin D_3 and 25-Hydroxyvitamin D_3 Content of Retail White Fish
422 and Eggs in Australia. Nutrients. 2017;9:E647.
423 **22.** Trichopoulou A, Georga K. Composition tables of foods and Greek dishes. Athens
424 Greece: Parisianos, 2004.
425 **23.** Connelly R, Platt L. Cohort profile: UK Millennium Cohort Study (MCS). Int J
426 Epidemiol. 2014;43:1719-25.
427 **24.** Shah I, James R, BarkerJ, Petroczi A, Naughton DP. Misleading measures in
428 Vitamin D analysis: a novel LC-MS/MS assay to account for epimers and isobars.
429 Nutr J. 2011;10:46.
430 **25.** Shah I, Petroczi A, Naughton DP. Method for simultaneous analysis of eight
431 analogues of vitamin D using liquid chromatography tandem mass spectrometry.
432 Chem Central J. 2012;6:112.
433 **26.** Ross AC, Taylor CL, Yaktine AL and Del Valle HB (eds). Dietary Reference
434 Intakes for Calcium and Vitamin D. Washington (DC): The National Academies
435 Press, 2011, p. 98.
436 **27.** Gezen-Ak D, Alaylıoğlu M, Genç G, Gündüz A, Candaş E, Bilgiç B, Atasoy İL,
437 Apaydın H, Kızıltan G, Gürvit H, Hanağası H, Ertan S, Yılmazer S, Dursun E. GC
438 and VDR SNPs and Vitamin D Levels in Parkinson's Disease: The Relevance to
439 Clinical Features. Neuromolecular Med. 2017;19(1):24-40.
440 **28.** Gezen-Ak D, Dursun E, Bilgiç B, Hanağasi H, Ertan T, Gürvit H, Emre M, Eker
441 E, Ulutin T, Uysal O, Yilmazer S. Vitamin D receptor gene haplotype is

associated with late-onset Alzheimer's disease. Tohoku J Exp Med. 2012;228(3):189-96

29. Karras SN, Fakhoury H, Muscogiuri G, Grant WB, van den Ouweland JM, Colao AM, Kotsa K. Maternal vitamin D levels during pregnancy and neonatal health: evidence to date and clinical implications. Ther Adv Musculoskelet Dis. 2016;8:124-35.

30. Karras S, Paschou SA, Kandaraki E, Anagnostis P, Annweiler C, Tarlatzis BC, Hollis BW, Grant WB, Goulis DG. Hypovitaminosis D in pregnancy in the Mediterranean region: a systematic review. Eur J Clin Nutr. 2016;70:979-86.

31. Pilz S, März W, Cashman KD, Kiely ME, Whiting SJ, Holick MF, Grant WB, Pludowski P, Hiligsmann M, Trummer C, Schwetz V, Lerchbaum E, Pandis M, Tomaschitz A, Grübler MR, Gaksch M, Verheyen N, Hollis BW, Rejnmark L, Karras SN, Hahn A, Bischoff-Ferrari HA, Reichrath J, Jorde R, Elmadfa I, Vieth R, Scragg R, Calvo MS, van Schoor NM, Bouillon R, Lips P, Itkonen ST, Martineau AR, Lamberg-Allardt C, Zittermann A. Rationale and Plan for Vitamin D Food Fortification: A Review and Guidance Paper. Front Endocrinol (Lausanne). 2018;9:373.

32. Anastasiou A, Karras SN, Bais A, Grant WB, Kotsa K, Goulis DG. Ultraviolet radiation and effects on humans: the paradigm of maternal vitamin D production during pregnancy. Eur J Clin Nutr. 2017;71:1268-72.

33. Javorski N, Lima CAD, Silva LVC, Crovella S, de Azêvedo Silva J. Vitamin D receptor (VDR) polymorphisms are associated to spontaneous preterm birth and maternal aspects. Gene. 2018;642:58–63.

34. Rosenfeld T, Salem H, Altarescu G, Grisaru-Granovsky S, Tevet A, Birk R. Maternal-fetal vitamin D receptor polymorphisms significantly associated with preterm birth. Arch Gynecol Obstet. 2017;296:215–22.

35. Farajian-Mashhadi F, Eskandari F, Rezaei M, Eskandari F, Najafi D, Teimoori B, Moradi-Sharbabak M, Salimi S. The possible role of maternal and placental vitamin D receptor polymorphisms and haplotypes in pathogenesis of preeclampsia. Clin Exp Hypertens. 2019. Epub ahead of print. doi:10.1080/10641963.2019.1601203.

36. Rezavand N, Tabarok S, Rahimi Z, Vaisi-Raygani A, Mohammadi E, Rahimi Z. The effect of VDR gene polymorphisms and vitamin D level on blood pressure, risk of preeclampsia, gestational age, and body mass index. J Cell Biochem. 2019;120:6441-448.

37. Barišić A, Pereza N, Hodžić A, Krpina MG, Ostojić S,Peterlin B. Genetic variation in the maternal vitamin D receptor FokI gene as a risk factor for recurrent pregnancy loss. J Matern Fetal Neonatal Med. 2019. Epub ahead of print. doi: 10.1080/14767058.2019.1660768.

38. Pereira-Santos M, Carvalho GQ, Louro ID, Dos Santos DB, Oliveira AM. Polymorphism in the vitamin D receptor gene is associated with maternal vitamin D concentration and neonatal outcomes: A Brazilian cohort study. Am J Hum Biol. 2019;31:e23250.

39. Rezende VB, Sandrim VC, Palei AC, Machado L, Cavalli RC, Duarte G, Tanus-Santos JE. Vitamin D receptor polymorphisms in hypertensive disorders of pregnancy. Mol Biol Rep. 2012;39:10903-6.

489 **Table 1.** Demographic and laboratory features of mothers and neonates.

Variable	**Value**
Mothers' age (years)	33 ± 6
BMI pre-pregnancy (kg/m^2)	25 ± 5
BMI term (kg/m^2)	30 ± 6
Weeks of gestation	39 ± 2
Smoking (%)	11 (15)
Alcohol consumption (%)	None: 51 (73) / Light: 12 (17) / Moderate: 7 (10)
Higher education (%)	Primary: 13 (19) / Secondary: 42 (60) / Higher: 15 (21)
Calcium supplementation (%)	20 (29)
Maternal 25(OH)D (nmol/l)	47 ± 26
Neonatal 25(OH)D (nmol/l)	35 ± 20
Calcium (mg/dl)	9 ± 1
PTH (pg/ml)	27 ± 13

490

491 Data are presented as mean ± standard deviation or absolute value (percentage).

492 Abbreviations: BMI: body mass index; PTH: parathyroid hormone; 25(OH)D: 25-

493 hydroxy-vitamin D.

494

Table 2. Genotype distribution of neonatal VDR polymorphisms according to neonatal vitamin D status.

Polymorphism	Genotype	Deficient n=36 (52%)	Insufficient n=19 (27%)	Sufficient n=15 (21%)	p-value
APAI	AA	12 (33)	8 (42)	3 (20)	0.57
	Aa	20 (56)	10 (53)	9 (60)	
	aa	4 (11)	1 (5)	3 (20)	
TAQI	TT	15 (42)	4 (21)	8 (53)	0.39
	Tt	16 (44)	11 (58)	5 (33)	
	tt	5 (14)	4(21)	2 (13)	
BSMI	BB	9 (25)	7 (37)	3 (20)	0.84
	Bb	14 (39)	7 (37)	6 (40)	
	bb	13 (36)	5 (26)	6 (40)	
FOKI	FF	13 (36)	10 (53)	10 (67)	0.05
	Ff	19 (53)	8 (42)	5 (33)	
	ff	4 (11)	1 (5)	0 (0)	
TRU9I	UU	24 (66)	11 (58)	11 (73)	0.70
	Uu	10 (28)	8 (42)	4 (27)	
	uu	2 (6)	0 (0)	0 (0)	

Deficient: 25(OH)D <30 nmol/l, Insufficient: 30≤ 25(OH)D ≤50 nmol/l, Sufficient: 25(OH)D >50nmol/l. Data are presented as absolute value (percentage).

Abbreviations: VDR: Vitamin D receptor; 25(OH)D: 25-hydroxy-vitamin D.

Table 3. Genotype distribution of maternal VDR polymorphisms according to maternal vitamin D status [25(OH)D <30 nmol/l vs. 25(OH)D ≥30 nmol/l].

Polymorphism	Genotype	Maternal vitamin D status		p-value
		25(OH)D <30 nmol/l n=24 (34%)	**25(OH)D ≥30 nmol/l n=46 (66%)**	
APAl	AA	10 (42)	19 (43)	0.98
	Aa	11 (46)	22 (46)	
	aa	3 (12)	5 (11)	
TAQl	TT	7 (29)	18 (39)	0.60
	Tt	12 (50)	21 (46)	
	tt	5 (21)	7 (15)	
BSMl	BB	10 (42)	16 (35)	0.63
	Bb	8 (33)	13 (28)	
	Bb	6 (25)	17 (37)	
FOKI	FF	**6 (25)**	**26 (57)**	**0.02**
	Ff	14 (58)	18 (39)	
	ff	4 (17)	2 (4)	
TRU9l	UU	16 (67)	25 (54)	0.30
	Uu	8 (33)	18 (39)	
	Uu	0 (0)	3 (7)	

Data are presented as absolute value (percentage). Significant differences are presented in bold.

Abbreviations: VDR: Vitamin D receptor; 25(OH)D: 25-hydroxy-vitamin D

Table 4. Genotype distribution of maternal VDR polymorphisms according to maternal Vitamin D status [25(OH)D <50 nmol/l vs. 25(OH)D ≥50 nmol/l].

		Maternal vitamin D status		
Polymorphism	**Genotype**	**25(OH)D <50 nmol/l n=45 (64%)**	**25(OH)D ≥50 nmol/l n=25 (36%)**	**p-value**
APAI	AA	19 (42)	10 (40)	0.68
	Aa	22 (49)	11 (44)	
	aa	4 (9)	4 (16)	
TAQI	TT	14 (31)	11 (44)	0.60
	Tt	23 (51)	10 (40)	
	tt	8 (18)	4 (16)	
BSMI	BB	17 (38)	9 (36)	0.57
	Bb	15 (33)	6 (24)	
	bb	13 (29)	10 (40)	
FOKI	FF	**16 (36)**	**16 (64)**	**0.02**
	Ff	23 (51)	9 (36)	
	ff	6 (13)	0 (0)	
TRU9I	UU	27 (60)	14 (56)	0.91
	Uu	16 (36)	10 (40)	
	uu	2 (4)	1 (4)	

Data are presented as absolute value (percentage). Significant differences are presented in bold.

Abbreviations: VDR: Vitamin D receptor; 25(OH)D: 25-hydroxy-vitamin D.

Table 5. Genotype distribution of maternal VDR polymorphisms according to neonatal Vitamin D status [Vitamin D deficient: 25(OH)D <30 nmol/l, Vitamin D non-deficient: 25(OH)D ≥30 nmol/l].

		Neonatal vitamin D status		
Polymorphism	**Genotype**	**25(OH)D <30 nmol/l n=36 (51%)**	**25(OH)D ≥30 nmol/l n=34 (49%)**	**p-value**
APAI	AA	15 (42)	14 (41)	1.00
	Aa	17 (47)	16 (47)	
	aa	4 (11)	4 (12)	
TAQI	TT	11 (31)	14 (41)	0.69
	Tt	18 (50)	15 (44)	
	Tt	7 (19)	5 (15)	
BSMI	BB	14 (39)	12 (36)	0.63
	Bb	12 (33)	9 (26)	
	bb	10 (28)	13 (38)	
FOKI	FF	**11 (31)**	**21 (62)**	**<0.01**
	Ff	21 (58)	11(32)	
	ff	4 (11)	2 (6)	
TRU9I	UU	23 (64)	18 (53)	0.59
	Uu	12 (33)	14 (41)	
	uu	1 (3)	2 (6)	

Data are presented as absolute value (percentage). Significant differences are presented in bold.

Abbreviations: VDR: Vitamin D receptor; 25(OH)D: 25-hydroxy-vitamin D.

Table 6. Association between maternal FokI genotypes and risk of maternal and neonatal Vitamin D deficiency [25(OH)D <30 nmol/l].

Outcome	**Maternal genotypes**	**OR**	**CI**	**p-value**
Neonatal deficiency	FF vs. Ff	0.27	0.10 - 0.77	0.01
Maternal deficiency	FF vs. Ff	0.30	0.09 - 0.92	0.03
Maternal deficiency	FF vs. ff	0.12	0.02 - 0.78	0.03

Abbreviations: OR: odds ratio, CI: confidence interval