Variability of contribution of vitamin D receptor gene polymorphisms to outcome of HLA-matched sibling allogeneic bone marrow transplantation


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Variability of contribution of vitamin D receptor gene polymorphisms to outcome of HLA-matched sibling allogeneic bone marrow transplantation


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ABSTRACT
Graft-versus-host disease (GVHD) remains one of the major complications of hematopoietic stem cell transplantation (HSCT). Several etiological factors were investigated. Among these, vitamin D and hence its receptor (VDR) gene polymorphisms have gained much interest; however, the results are still controversial. Using PCR-RFLP, we genotyped VDR polymorphisms FokI (rs10735810), ApaI (rs7975232), and Taq1 (rs731236) in 80 patient/donor pairs according to DNA availability. No association was encountered between VDR polymorphisms and GVHD. Neither was there any impact on survival. Only grade II–IV acute GVHD was associated with inferior overall (p = .01), but not disease-free survival. The controversy between our results and the literature may be attributed to marked variability in the relative distribution of VDR genotypes in different populations. Also different environmental factors, including exposure to sun, may ensure vitamin D sufficiency nullifying the impact of VDR polymorphisms.

Introduction
Hematopoietic stem cell transplantation (HSCT) is a well-established therapeutic modality for several hematological disorders [1]. However, graft-versus-host disease (GVHD) still constitutes a major challenge. Though the pathogenesis is understood to a great extent, the predisposing factors are far from settled and prediction of GVHD, occurrence or severity, is still a long way from achieving. Hence a plethora of literature is addressing the potential contributing factors. Among these, vitamin D and, hence, its receptor (VDR) have gained attention. Unlike highly variable environmental exposures leading to seasonal and regional variations in vitamin D levels [2], genetic variants are constant, thereby avoiding reverse causation concerns [3].

VDR is present on a wide variety of tissues outside the intestine, bones, and kidneys, which are the organs most involved in the classical role of vitamin D. In the hematopoietic system, the VDR is expressed on various hematopoietic precursors as well as monocytes, some thymocytes, as well as activated B and T lymphocytes [4].

In addition to the role of VDR in normal hematopoietic development and leukocyte differentiation [5], the presence of the VDR on activated lymphocytes suggests a role for vitamin D and VDR in immune modulation. Thus, genetic alterations of the VDR gene could lead to important effects on gene activation affecting calcium metabolism, cell proliferation, immune function, etc., which could be explained by changes in the protein sequence. In general, stimulation of the VDR has been shown to favor the Th2 response by suppressing IFN-γ. The VDR also seems to be crucial for proper development of invariant natural killer (iNK) cells, a subset of lymphoid cells involved in the most basic immune responses, and also in restricting autoimmunity; reduced iNKT may promote autoimmunity [6].

Though the exact ways in which various polymorphisms change VDR activity are often unknown, these genetic variations have been associated with variability in immune function and other vitamin D activities such as growth, bone formation, and susceptibility to infectious diseases [7]. In view of these immune-modulatory actions, it was hypothesized that vitamin D and its VDR may be potential players in occurrence of GVHD after HSCT and may affect the outcome of the transplant. Several studies have investigated the...
association of VDR gene polymorphisms with HSCT outcome with controversial results [7–10].

In this work, we tested three of the common polymorphisms identified, namely FokI (rs10735810), Apal (rs7975232), and TaqI (rs731236) [11], in a cohort of Egyptian patients who received allogeneic peripheral blood stem cell (PBSC) transplantation to verify potential association with transplant complications, mainly GVHD as well as transplant outcome in the form of survival.

Material and methods

Subjects

The study was performed on 80 patients who received PBSC transplantation at Nasser Institute, MOH, Cairo, Egypt, from January 2014 to March 2015. The study was performed according to the latest Declaration of Helsinki for studies performed on humans, 2008. It was approved by the Institutional Review Board of Nasser Institute and a written informed consent was obtained from patients and their donors. Patients included 59 males and 21 females with an age range of 18–56, median 31 years. Donors included 52 males and 28 females with an age range of 18–58, median 29 years. Patients’ diagnoses included 36 acute myeloid leukemia (AML), 21 severe aplastic anemia (SAA), 11 acute lymphoblastic leukemia (ALL), 5 chronic myeloid leukemia (CML), 3 myelodysplastic syndrome (MDS), and 1 case each of biphenotypic leukemia, chronic lymphocytic leukemia (CLL), primary myelofibrosis (PMF), and paroxysmal nocturnal hemoglobinuria (PNH).

All patients received stem cells from human leukocyte antigen (HLA)-matched siblings and in all patients, the stem cell source was granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood. Gender matching was achieved in 61 cases (40 male and 21 female pairs); gender mismatch male to female was present in 12, and female to male in 7 cases.

Patients were followed-up for 1 year at least.

Time to neutrophil engraftment was defined as the first of three consecutive days with neutrophil count $\geq 0.5 \times 10^9/L$. Time to platelet engraftment was defined as the first of three consecutive days with a count of $\geq 20 \times 10^9/L$ unsupported by platelet transfusion.

Disease-free survival (DFS) was defined as the time from stem cell infusion to progression or death from any cause. Overall survival (OS) was defined as the time from stem cell infusion to death from any cause.

Genotyping of VDR

Genotyping of VDR polymorphisms FokI (rs10735810), Apal (rs7975232), and TaqI (rs731236) was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was extracted from peripheral blood leukocytes by salting out technique [12]. Primers were obtained from Metabione International AG (Planegg, Germany) and restriction enzymes from Thermo Fisher Scientific Inc. (IBM Inc., Waltham, MA, USA).

Amplification was performed in 25 $\mu$L reaction. Genomic DNA (300 ng) was used together with 5 $\mu$L BioLineMyTaq TM RedMix (BioLine, Eveleigh NSW, Australia), in addition to 0.5 $\mu$L of 10 Pico mole specific primers.

DNA amplification was performed using thermal cycler Biometra T3000 (Biometra, Göttingen, Germany). The amplified digested products were separated on 2% agarose gel at 100 V for 30 min. A DNA marker was included in each run, 50 bp for FokI and 100 bp for Apal and TaqI.

Primer sequences and the digestion patterns of the different genotypes are presented in Supplementary Table (SM1).

FokI (rs10735810) genotyping was performed according to Arjumand et al. [13]. The amplification program consisted of denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 1 min and one final cycle of extension at 72 °C for 7 min. Five microliters of PCR product were digested with 10 units of FokI restriction enzyme for 1 h at 37 °C; digestion of the amplified 265-bp PCR product yielded two fragments: 169 and 96 bp.

Apal and TaqI genotyping was performed according to Sainz et al. [14]. The amplification program consisted of denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min and one final cycle of extension at 72 °C for 7 min. Digestion was performed for 1 h at 37 °C using 10 units Apal or TaqI. The PCR product for the Apal and TaqI polymorphisms was 2000 bp long; Apal digestion generates 1700 and 300 bp bands while TaqI generates 1800 and 200 bp bands. Absence of the restriction site is considered wild.

The genotypes of the three VDR gene polymorphisms, FokI, Apal, and TaqI, are expressed as wild ‘W’ (W = FF, AA, TT, respectively), heterozygous ‘H’ (H = Ff, Aa, Tt, respectively), and homozygous/mutant ‘M’ (M = ff, aa, tt, respectively); Supplementary Figures (SM1, SM2 and SM3).

Statistical analysis

Data were analyzed by SPSS version 21 (IBM Inc., Waltham, MA, USA). Quantitative data were summarized as mean ± standard deviations (SD), if it is normally distributed and as median (range) if it is not.
Qualitative data were described as frequencies and percentages. Chi-square test was used for Hardy–Weinberg equilibrium.

Student t-test, Mann–Whitney U test, ANOVA F-test, and Kruskal–Wallis H tests were used to determine significance of difference for quantitative data.

Relations of qualitative data were determined using Chi-square test.

Survival analysis was done using Kaplan–Meier method to determine OS and DFS. Log rank (Mantel–Cox) test was used to examine difference between survivals of different groups. *p* value < .05 was considered significant.

**Results**

The study was performed on 80 patient/donor pairs. All patients received G-CSF-mobilized peripheral blood stem cells from HLA-matched siblings.

Conditioning regimen consisted of Bu/Cy in 41 (50.6%) patients, Flu/Cy in 23 (28.4%), TBI/Cy in 10 (12.3%), and 6 patients (7.4%) received other regimens such as Flu/Alk, Flu/Bu, or Flu/Bu/ATG.

GVHD prophylaxis consisted of CSA + MTX in 77 and CSA + MMF in 3 patients.

The majority of patients (78/80) were CMV IgG positive. Hepatitis C virus by PCR was positive in 11 patients and Hepatitis B virus (HBV) sAg was positive in one patient only.

Time to granulocyte recovery (ANC ≥ 500/μL) ranged from 8 to 22 with a median of 13 days and time to platelet recovery (≥20,000/μL unsupported by platelet transfusion) ranged from 8 to 26 with a median of 15 days.

There were eight relapses (10.0%) and 17 (21.25%) deaths.

**Genotype frequencies of VDR polymorphisms**

Genotypes for the three VDR restriction sites for both patients and donors are presented in Table 1. The distribution of donor’s genotypes was in Hardy–Weinberg equilibrium. Genotype frequencies for the three sites Apa1, Taq1 and FOK1 were comparable between patients and donors.

**Association between VDR gene polymorphism and acute GVHD**

Grade II–IV acute GVHD was encountered in 27 patients (33.75%); skin was the predominant site (20 cases, 74.1%) followed by gastrointestinal tract (GIT) (18 cases, 66.67%) and last was liver (6 cases, 22.2%).

No association was encountered between the three studied VDR gene polymorphisms and Grade II–IV acute GVHD (Table 2).

**Other risk factors for the development of Grade II–IV acute GVHD**

Various risk factors were evaluated including age, diagnosis, gender mismatch, CD34 count, and conditioning regimen; none was significantly associated with Grade II–IV acute GVHD.

**Association between VDR gene polymorphism and chronic GVHD**

Chronic GVHD was encountered in 23 patients (28.75%), 14 with extensive and 9 with limited disease.

No association was encountered between the three studied VDR gene polymorphisms and chronic GVHD (Table 3).

Other risk factors were evaluated including patients’ age, diagnosis, prior acute GVHD, the use of a female donor for a male recipient, CD34 count and the conditioning regimens; none of these factors was associated with an increased risk of chronic GVHD.

Table 1. Frequencies of VDR gene polymorphisms in patients receiving allogeneic PBSC transplantation and their sibling donors.

<table>
<thead>
<tr>
<th>Restriction site</th>
<th>Genotype</th>
<th>Patient No.</th>
<th>Frequency</th>
<th>Donor No.</th>
<th>Frequency</th>
<th>HW donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apa1 (rs7975232)</td>
<td>FF</td>
<td>80</td>
<td>50 (62.5%)</td>
<td>75</td>
<td>40 (53.3%)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Ff</td>
<td>21</td>
<td>26 (36.1%)</td>
<td>28</td>
<td>37 (37.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ff</td>
<td>9</td>
<td>11 (13.1%)</td>
<td>7</td>
<td>9 (9.3%)</td>
<td></td>
</tr>
<tr>
<td>Taq1 (rs731236)</td>
<td>TT</td>
<td>68</td>
<td>31 (45.6%)</td>
<td>65</td>
<td>26 (40%)</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>Tt</td>
<td>37</td>
<td>37 (54.4%)</td>
<td>34</td>
<td>52 (52.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tt</td>
<td>0</td>
<td>0 (0%)</td>
<td>5</td>
<td>7.5%</td>
<td></td>
</tr>
</tbody>
</table>

VDR: vitamin D receptor; PBSC: peripheral blood stem cell.
Impact of VDR gene polymorphism on survival

There was no statistically significant association between OS and any of the three VDR genotypes for either patients or donors (Figure 1).

Neither was there an association between DFS and any of the three VDR genotypes for either patients or donors (Figure 2).

Impact of acute GVHD on survival

The occurrence of Grade II–IV acute GVHD was associated with lower OS compared to the absence of or Grade 1 acute GVHD (12.67 months vs. 19.41 months, respectively, \( p = .010 \); Figure 3(A)). DFS was not affected by acute GVHD \( (p = .488; \) Figure 3(B)).

Effect of chronic GVHD on OS

Chronic GVHD did not affect either OS \( (p = .783; \) Figure 3(C)) or DFS \( (p = .483; \) Figure 3(D)).

Relation between VDR genotypes and engraftment (granulocyte and platelet)

There was no association between engraftment and any of the three VDR genotypes of either patients or donors (Supplementary Table SM2).

Discussion

HSCT, a potential curative measure for many hematological disorders, is still hindered by the post-transplant complications especially GVHD \([15,16]\). The pathogenesis of GVHD is multi-factorial with the immune system at the core of events. Apart from its well-known role in bone metabolism and calcium homeostasis, vitamin D has a significant role in the regulation of host immune responses and the prevention of autoimmunity; its action is mediated by the nuclear VDR \([10]\). Polymorphisms of VDR gene are associated with change in VDR activity and hence effect of vitamin D. For instance, homozygosity for ‘a’ allele of Apal polymorphism and ‘I’ allele of FokI RFLP are associated with higher VDR activity \([17]\).

Polymorphism of the VDR gene has been associated with diseases of immune dysfunction \([7]\) and association between VDR polymorphism, in recipient and/or donor, and clinical outcome of sibling HLA-matched HSCT has been reported \([7–10]\).

In this study, we evaluated the relation between VDR polymorphism (FokI, Apal, and TaqI) in Egyptian HSCT recipients and their sibling donors and the risk of development of acute and chronic GVHD (within 1 year from the transplantation) as primary end points while the secondary end points were OS and DFS.

In our study, Grade II–IV acute GVHD was reported in 33.75% of patients. This is comparable to a 31% previously reported by our group \([18]\) and others \([19]\) but lower than the 51% reported by Kanda et al. \([20]\). Variability in frequency of acute GVHD is well documented \([21]\). In our series, acute GVHD primarily affected the skin, GIT, and liver in 74.1%, 66.7% and 22.2%. This is comparable to our previous report with Grade II–IV acute GVHD reported in 34% of patients affecting the skin, GIT, and liver in 69.7%, 57.6% and 21.2%, respectively \([22]\).

In the current study, Grade II–IV acute GVHD was associated with lower OS \( (p = .010) \). This is consistent

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### Table 2. VDR gene polymorphism in relation to acute GVHD after allogeneic peripheral stem cell transplantation from a sibling.

<table>
<thead>
<tr>
<th>Restriction site</th>
<th>Patients genotype</th>
<th>Donor’s genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No GVHD (%)</td>
<td>GVHD (%)</td>
</tr>
<tr>
<td>FokI</td>
<td>FF</td>
<td>32 (62.1)</td>
</tr>
<tr>
<td></td>
<td>Ff</td>
<td>13 (25.4)</td>
</tr>
<tr>
<td></td>
<td>ff</td>
<td>6 (11.7)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100)</td>
<td>29 (100)</td>
</tr>
<tr>
<td>Apal</td>
<td>AA</td>
<td>23 (51.1)</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>19 (42.2)</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>3 (6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (100)</td>
<td>27 (100)</td>
</tr>
<tr>
<td>TaqI</td>
<td>TT</td>
<td>22 (52.4)</td>
</tr>
<tr>
<td></td>
<td>Tt</td>
<td>20 (47.6)</td>
</tr>
<tr>
<td></td>
<td>tt</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>42 (100)</td>
<td>26 (100)</td>
</tr>
</tbody>
</table>

VDR: vitamin D receptor; GVHD: graft-versus-host disease.

### Table 3. VDR gene polymorphism in relation to chronic GVHD after allogeneic peripheral stem cell transplantation from a sibling.

<table>
<thead>
<tr>
<th>Restriction site</th>
<th>Patients genotype</th>
<th>Donor’s genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No GVHD (%)</td>
<td>GVHD (%)</td>
</tr>
<tr>
<td>FokI</td>
<td>FF</td>
<td>36 (62.1)</td>
</tr>
<tr>
<td></td>
<td>Ff</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td></td>
<td>ff</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100)</td>
<td>22 (100)</td>
</tr>
<tr>
<td>Apal</td>
<td>AA</td>
<td>30 (55.5)</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>19 (35.2)</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>5 (9.3)</td>
</tr>
<tr>
<td>Total</td>
<td>54 (100)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>TaqI</td>
<td>TT</td>
<td>23 (46.0)</td>
</tr>
<tr>
<td></td>
<td>Tt</td>
<td>27 (54.0)</td>
</tr>
<tr>
<td></td>
<td>tt</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100)</td>
<td>18 (100)</td>
</tr>
</tbody>
</table>

VDR: vitamin D receptor; GVHD: graft-versus-host disease.
Figure 1. Impact of VDR genotypes on overall survival in recipients of hematopoietic cell transplantation from an identical sibling: (A) patient’s Apal 72 cases ($p = .880$), (B) patient’s Taql 68 cases ($p = .288$), (C) patient’s FokI 80 cases ($p = .686$), (D) donor’s Apal 66 cases ($p = .608$), (E) donor’s Taql 65 cases ($p = .873$), (F) donor’s FokI 75 cases ($p = .877$).
Figure 2. Impact of VDR genotypes on disease-free survival in recipients of hematopoietic cell transplantation from an identical sibling: (A) patient’s ApaI 72 cases ($p = .792$), (B) patient’s TaqI 68 cases ($p = .899$), (C) patient’s FokI 75 cases ($p = .0783$), (D) donor’s ApaI 66 cases ($p = .742$), (E) donor’s TaqI 65 cases ($p = .577$), (F) donor’s FokI 75 cases ($p = .230$).
with our previous study \((p = 0.021)\) [22] and other previous reports [23,24]. However, the impact of acute GVHD on survival was denied by others [25].

Unlike OS, acute GVHD showed no impact on DFS \((p = 0.488)\). This is consistent with one report [24] and inconsistent with another [26].

In our study, chronic GVHD was reported in 28.75% with 11.1% limited and 17.3% extensive. An incidence of 33% chronic GVHD was previously reported [27] with 16% limited and 34% extensive [20]. The relatively low incidence of chronic GVHD in our series, in spite of using PBSC as a graft, may be attributed to several factors. These include the young age of most recipients (median 31 years), all are receiving a graft from a fully matched sibling, the limitation of CD34+ dose to a maximum of \(7 \times 10^6/kg\), and the relatively low incidence of acute GVHD which is a major risk factor for chronic GVHD.

In the current study, no impact of chronic GVHD on OS or DFS was demonstrated. This is consistent with a
previous study involving 183 patients [20]. Other previous reports demonstrated significant association of chronic GVHD with OS (p = .009), but only a trend with DFS [25]; a deleterious effect of extensive chronic GVHD on OS was also reported [28].

Our results demonstrated that there was no statistically significant association between any of the three VDR genotypes in either patients or donors, and the development of acute GVHD.

Comparable results for FokI, TaqI, and BsmI were reported; only the VDR patients’ genotype ‘a’ alleles of Apal showed a trend of association with increased acute GVHD (p = .058) in a U.K. population [7]. In contrast to our results, Bogunia et al. [8] evaluated VDR polymorphisms for FokI, Apal, and TaqI in Polish allo-HSCT. Patients’ genotype ‘aa’ of Apal was associated with Grade II–IV acute GVHD (p = .025) with a trend retained in multivariate analysis (OR = 3.233, p = .069); however, donors’ genotype ‘AA’ was the genotype associated with a higher incidence of acute GVHD (p = .033). Both patients’ and donors’ genotype ‘FF’ of FokI was associated with increased risk for acute GVHD that was significant for patient’s (p = .0149) but not for donor’s genotype (11/37 vs. 14/80, NS).

Also, Cho et al. [9] evaluated VDR polymorphisms for BsmI, Apal, and TaqI in Korean patients; Grade II–IV acute GVHD developed less frequently in patients with the Apal ‘TT’ (equal to ‘AA’ in other studies) genotype than those with the non-‘TT’ genotypes (7.7% vs. 35.3%; p = .059).

The variability between various studies may be attributed, at least partly, to the difference in allele frequency among the studied populations and ours [7–10]. For instance, the ‘A’ allele of the Apal polymorphism is found in only 5% of Chinese population [29].

Our results demonstrated that there was no significant association between any of the three VDR genotypes in either patients or donors, and the development of chronic GVHD. Previous studies documented no association of VDR polymorphism with either incidence [8] or severity of chronic GVHD [9]. Only a trend (p = .057) of association with donor’s Apa ‘aa’ was reported [7].

In our study, there was no association between any of the three VDR genotypes in either patients or donors and OS or DFS.

In contrast to our results, Middleton et al. [7] revealed that the donor ‘A’ allele associates with increased likelihood of death (AA vs. Aa or aa, hazard ratio 2.03, p = .0232). Also, Bogunia et al. [8], demonstrated that patients with genotype ‘aa’ were twice as likely to die as patients with ‘AA’ or ‘Aa’ genotypes (p = .0228).

In contrast, Cho et al. [9] demonstrated that patients with Apal ‘TT’ genotype (equal to ‘AA’ in other studies) showed a trend of improved OS compared to those with the Apal non-‘TT’ genotypes (p = .062), and patients with the TaqI ‘TC’ genotype (equal to ‘t’ in other studies) showed longer OS than those with the ‘TT’ genotype (p = .022). The same trend with OS was also, partly, observed with DFS; patients with the TaqI ‘TC’ genotype showed longer DFS than their counterparts (p = .038) [9].

The association of VDR polymorphism with the risk of post-transplant complications may vary due to environmental factors, personal habits, and diet while undergoing HSCT or vitamin D support [8]. For instance, the majority of our patients were vitamin D sufficient at the time of transplantation (unpublished data), a situation that may diminish or even abolish the impact of VDR polymorphisms.

Therefore, the use of VDR polymorphisms as markers for the outcome of HSCT is still a matter of debate. Further effort must be placed in understanding the molecular and cellular variations affected by the polymorphisms and in performing observational studies in bigger populations. In these studies, special attention should be paid to the effects of environmental contributions for the better understanding of the role of VDR polymorphisms. Until then, the role of VDR polymorphisms will still be a topic for discussion and further studies.

Lack of impact of other risk factors, including age, conditioning regimen, CD34 dose, diagnoses, etc., on development of acute and/or chronic GVHD should be cautiously interpreted in view of the relatively small sample size.

Conclusions

Our results revealed that none of the three VDR genotypes in either patients or donors had an impact on the occurrence of GVHD, DFS, or OS. Apart from the relatively small sample size in some studies including ours, the controversy in the results may be attributed to various factors. First, the marked variability in the relative distribution of VDR genotypes in different populations might influence the impact of the polymorphisms on HSCT outcome. Second, the different environmental factors including exposure to sun which may ensure vitamin D sufficiency and decrease the impact of VDR polymorphisms. The impact of VDR polymorphism and vitamin D level on HSCT outcome must be evaluated in each population to be able to
decide the necessity and schedule of potential vitamin D supplementation in the HSCT setup. In spite of the relatively small sample size, the current study may pave the way for a larger observational study which may better reveal the impact of gene-environmental interaction. In some areas of the world with a sunny weather, the impact of VDR polymorphisms may be overcome by the predominance of vitamin D sufficiency.

**Potential conflict of interest:** Disclosure forms provided by the authors are available with the full text of this article online at https://doi.org/10.1080/10428194.2018.1459608.

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