Frequency of rs731236 (Taql), rs2228570 (Fok1) of Vitamin-D Receptor (VDR) gene in Emirati healthy population

Enas Osman a, Fatme Al Anouti b, Gehad El ghazali c, Afrozul Haq d, Rajaa Mirdani e, Habiba Al Safar a,⁎,†

a Khalifa University of Science, Technology & Research, Biomedical Department, Abu Dhabi, United Arab Emirates
b Zayed University, Abu Dhabi, United Arab Emirates
c Institute of Laboratory Medicine, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates
d VPS Healthcare, Abu Dhabi, United Arab Emirates
e Fatima College for Health Sciences, Abu Dhabi, United Arab Emirates
f Khalifa University Center of Biotechnology, Abu Dhabi, United Arab Emirates

A R T I C L E   I N F O

Article history:
Received 30 May 2015
Revised 16 August 2015
Accepted 2 September 2015
Available online xxxx

Keywords:
Vitamin D
VDR
UAE
Genetic polymorphism
rs731236 (Taql) and rs2228570 (Fok1)

A B S T R A C T

Vitamin D is getting more attention everyday due to its importance in maintaining bone and calcium homeostasis, cellular proliferation, differentiation and immune response. Vitamin D is derived from diet or elicited in the skin by the activation of 7-dehydrocholesterol, which is an inert molecule that must be activated by ultraviolet light to form pre-vitamin D3. Recent studies connected the gene encoding for vitamin D (VDR) to the genetic control of bone mass and other diseases. As VDR SNPs have been associated with several disorders and diseases, it’s important to investigate the allelic and genotypic distribution among populations. The aim of this study is to determine the frequency of rs731236 (Taq1) and rs2228570 (Fok1) variants in healthy Emirati individuals and compare their genotype and allele distribution with other populations. In this study 282 (female, 187; male, 95) unrelated healthy UAE nationals were involved. Two hundreds and eight two DNA samples been collected to genotype rs731236 (Taq1) and rs2228570 (Fok1) VDR SNPs. Our results indicate that the distribution of the alleles and genotypes of rs731236 (Taq1) and rs2228570 (Fok1) vary considerably in different populations. In the Emirati population the distribution of rs731236 (Taq1) and rs2228570 (Fok1) were AA 38%, AG 42%, GG 20% and AA 27%, AG 42%, GG 31% respectively. The Emirati population genotype and allele distribution of rs731236 (Taq1) and rs2228570 (Fok1) had no difference with Caucasians from USA and France. However, there was significant difference with Asian populations.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Vitamin D has gained a lot of interest in recent years; due to its importance in maintaining bone and calcium homeostasis, cellular proliferation, differentiation and immune response (Wang et al., 2012). Furthermore, it has been associated with prostate cancer, skin cancer, obesity, Metabolism syndrome, Type 2 Diabetes Mellitus (T2DM), and Cardiovascular diseases (CVD) (Rezende et al., 2007). Vitamin D is derived from diet or elicited in the skin by the activation of 7-dehydrocholesterol which is an inert molecule that must be activated by ultraviolet light to form pre-vitamin D3 (Holick et al., 1977). The nuclear Vitamin D Receptor (VDR) mediates most of the biological actions of Vitamin D (Tuoresmaki et al., 2014). VDR is a member of the steroid hormone receptor family which is located in chromosome 12q13.1 (Uitterlinden et al., 2004). It has eight exons and six alternatively spliced regions positioned in genetically active areas, containing the promoter region (Tuoresmaki et al., 2014).

Genetic polymorphism in VDR has been reported and more than 470 VDR single nucleotide polymorphisms (SNPs) have been identified. The two most common (SNPs) in VDR gene in Caucasian subjects are rs22828570 (Fok-1) and rs731236 (Taq-1) (Davis, 2008). These two RFLPs in the VDR gene were described by using Fok-1 and Taq-1 restriction enzymes. The Taq-1 RFLP is located between the 8 and 9 axon in an are of unknown function, while the Fok-1 RFLP is located in the exon 2 and cause by T to C nucleotide substitution (Bhanushali et al., 2009).

The genetic control of bone mass as well as bone disease has been associated to genetic polymorphism in VDR (Bhanushali et al., 2009). Studies have shown that low vitamin D or VDR polymorphisms have been associated with autoimmune diseases and liver cancer (Zuniga et al., 2011). Moreover, it has been reported that vitamin D and VDR provide Reno-protection against diabetic nephropathy (Gross et al., 1996). Numerous reports have presented the frequency of VDR SNPs and their association to different diseases in different ethnic groups,

http://dx.doi.org/10.1016/j.mgene.2015.09.001
2214-5400/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
nevertheless studies on this part of the world are scarce. Most of the VDR polymorphisms studies have been conducted on the Caucasian populations. In this study we present the genotype allele frequencies of rs731236 (Taq-I), rs2228570 (Fok-I) of Vitamin-D Receptor (VDR) gene in Emirati healthy population.

2. Materials and methods

2.1. Study population and design

For determining the sample size, we conducted the Power Calculator for Genetic Studies developed by Skol and his team from their website http://www.sph.umich.edu/csg/abecasis/CaTS/index.html. Using Vita-

2.2. DNA extraction and quantification

The extraction of genomic DNA was done using QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. The DNA samples were diluted with free nuclease water to 5–20 ng/μL at room temperature for the subsequent PCR reactions.

2.3. Genotyping of Taq-I (rs731236) and Fok-I (rs2228570) polymorphisms

All molecular genetic studies were performed in the laboratory of Biotechnology Center at Khalifa University. Two SNPs (Taq-I (rs731236) and Fok-I (rs2228570)) in VDR been selected from Hap Map database phase III, to study the allelic and genotypic distribution of both VDR variants among Emirati population and compare it with other ethnic groups. The genotyping for the two SNPs Taq-I (rs731236) and Fok-I (rs2228570) was performed using TaqMan SNP genotyping assay which consists of a predesigned mix of unlabeled polymerase chain reaction (PCR) primers and the TaqMan® minor groove binding group (MGB) probe (FAM™ and VIC® dye-labeled). All TaqMan SNP Genotyping Assays are designed to work with TaqMan® Universal PCR MasterMix which contains DNA polymerase, dNTPs and optimized mix components and uses the same thermal conditions. These assays were purchased from ABI (Applied Biosystems, USA) and they were applied using The ViiA™ 7 Real-Time PCR System (Applied Biosystems, USA). The real time PCR experiments were performed according to the manufacturer’s instructions with a final reaction volume of 10 μL that contained 1 μL of genomic DNA (20 ng), 8.5 μL of TaqMan Genotyping Master Mix (2×) (Applied Biosystems, USA) and 0.5 μL of assay mix (20×). The Real Time PCR thermal conditions were as follows: Initial denaturing at 95 °C for 20 s; 40 cycles of 96 °C for 3 s (denaturing) and 60 °C for 30 s (annealing/extension). Results assessment was carried out using the ViiA™ 7 Software (Applied Biosystems, USA). Samples were run in duplicates, with positive, negative controls and blanks. SNPs call rate was 99.65%.

2.4. Data analysis

The Hardy–Weinberg equation was used for the assessment of the predicted genotype frequencies of the VDR gene polymorphism in healthy controls in UAE population. Two-tailed Fisher’s exact test and chi square test was done to compare the allele and genotype frequencies of Emiratis to different population using NCSS 10.0 software program and data were analyzed by using the statistical program Stata (StataCorp. College Station, Texas, USA) (version 13). Haplotype analy-

3. Results

The allele frequencies of VDR SNPs Taq-I (rs731236) and Fok-I (rs2228570) and genotype frequencies in the Emirati population are shown in Tables 1 and 2. Out of the 282 subjects analyzed for Taq-I (rs731236) and Fok-I (rs2228570), the following genotypic frequencies were obtained: AA 38%, AG 42%, GG 20% and AA 27%, AG 42%, GG 31% respectively.

The most frequently identified allele of Taq-I (rs731236) is A (333/ 562, 59.25%) while the most frequently identified allele of Fok-I (rs2228570) is G (292/562, 51.95%). Our data shows that the frequency of ‘A’ vs ‘A’ in Taq-I (rs731236) and Fok-I (rs2228570) were 59.25% vs 48.04% respectively. Comparison of the genotype and allele frequencies of Taq-I (rs731236) and Fok-I (rs2228570) between the Emirati population and other populations are illustrated in Tables 1 and 2. In this study, no significant differences in the distribution of genotypes and alleles of Taq-I (rs731236) were found between Emirati individuals and the Caucasians of USA Minnesota and France and the African Americans of Pennsylvania (Bid et al., 2005; Zmuda et al., 1997). Taq-I (rs731236) genotypic and allelic distribution in Emiratis was statistically significant from those in Asians from Jordan (Karasneh et al., 2013), Japan (Tokita et al., 1996), India and North India (Bhanushali et al., 2009; Bid et al., 2005). The Emirati population Taq-I (rs731236) genotype distribution was significantly different from the Syrian population, however allelic frequency was not significantly different between the two populations. Likewise, Fok-I (rs2228570) allele and genotype frequencies in Emiratis were significantly different from the Asians populations of Jordan, Japan, India and North India. A significant difference in the genotype frequency of Fok-I (rs2228570) between Emiratis and Syrians was observed, while no significant difference was found in the allele frequencies of Fok-I (rs2228570) between these two populations.

Table 3 demonstrating the four haplotypes from the two SNPs (rs731236 and rs2228570) were analyzed among healthy Emirates. The haplotype having both alleles AA exhibited a frequency of 29% while the highest frequency of 31% was presented by the AG haplotype. However, the GA and GG haplotypes occurred at lower frequencies of 20% and 21% respectively.

4. Discussion

Ethnic differences in VDR polymorphism have been reported in literature by using Restriction Fragment Length Polymorphism (RFLP) assay (Smolders et al., 2009). The minor allele of Fok-I (rs2228570) has been shown to be present in a substantially lower frequency in Africans compared to Caucasians or Asians while the minor allele of Taq-I (rs731236) was found to be present in much lower frequency in Asians compared to Caucasians and African (Smolders et al., 2009).
Table 1
Comparing genotypes and allele frequency of VDR gene polymorphism (Taq-I) between Emirati and different populations.

| Ethnicity          | No.  | Genotype [%] | P-value | Alleles [%] | P-value | Reference       |
|--------------------|------|--------------|---------|-------------|---------|-----------------|
|                    |      | Taq-I (rs731236) |         | Major | Minor |                        |
|                    |      | AA AG GG     |         | A | G |                        |
| Caucasian          |      |              |         |       |       |                         |
| USA, white Minnesota | 130  | 41 44 15 | NS      | 63 37 | NS | Bid et al. (2005) |
| French             | 189  | 33 49 18 | NS      | 57 43 | NS | Zmuda et al. (1997) |
| Asian              |      |              |         |       |       |                         |
| Japan              | 488  | 77 22 1  | ***     | 88 12 | *** | Tokita et al. (1996) |
| North Indian       | 346  | 49 40 11 | ***     | 66 34 | *  | Bhanushali et al. (2009) |
| India              | 143  | 49 44 7  | **       | 71 29 | *** | Bid et al. (2005) |
| African            |      |              |         |       |       |                         |
| USA, Black Pennsylvania | 101  | 32 53 15 | NS      | 58 42 | NS | Zmuda et al. (1997) |
| Middle East        |      |              |         |       |       |                         |
| Jordan             | 126  | 32.5 47.7 19.8 | ** | 56.4 43.6 | ** | Karazneh et al. (2013) |
| Syria              | 78   | 36 58 6  | **       | 65 35 | NS | Haddad (2014) |
| UAE                | 281  | 38 42 19.6 | 59.2 40.8 |          |       | Current study |

*p = 0.05, ** = p < 0.01, *** = p < 0.001, NS = not significant.

In our study we have genotyped two VDR polymorphism SNPs Taq-I (rs731236) and Fok-I (rs2228570) using a high-throughput genotyping technology (Real-time PCR) to study the allele distribution in UAE population. Our results showed that the allele and genotype distribution of the Taq-I (rs731236) and Fok-I (rs2228570) in the Emirati population is similar to the Caucasians, since there was no significant difference in neither the allele nor the genotype frequencies of these two VDR SNPs Taq-I (rs731236) and Fok-I (rs2228570) in the Emirati population.

The importance of this study arises from elucidating the genotype, allele distribution any haplotype of the two most commonly studied SNPs of VDR, namely Taq-I (rs731236) and Fok-I (rs2228570) in Emirati and understanding the differences from other ethnic groups. VDR haplotypes derived from Taq-I (rs731236) and Fok-I (rs2228570) polymorphisms were examined using combination of four genotypes. The distribution of haplotype frequencies in Emirati population is shown in Table 3. Results show that haplotype carrying A allele from Taq-I (rs731236) and G from Fok-I (rs2228570) are more frequent in Emirati population that’s important for risk prediction and prognosis for a number of clinically significant diseases such as cancer and type 2 Diabetes in the Emirati population.

In conclusion, we have determined the frequency of Taq-I (rs731236) and Fok-I (rs2228570) polymorphism in the VDR gene in the Emirati population. In addition, the highest haplotype frequency of Taq-I (rs731236) and Fok-I (rs2228570) in Emirati population was presented by the AG haplotype. Further studies needs to be conducted to structure the haplotype between VDR variants and chronic disease.

Table 2
Comparing genotypes and allele frequency of VDR gene polymorphism (Fokl) between Emirati and different populations.

| Ethnicity          | No.  | Genotype [%] | P-value | Alleles [%] | P-value | Reference       |
|--------------------|------|--------------|---------|-------------|---------|-----------------|
|                    |      | Fokl (rs2228570) |         | Major | Minor |                        |
|                    |      | AA AG GG     |         | A | G |                        |
| Caucasian          |      |              |         |       |       |                         |
| UK                 | 108  | 48 41 11 | NS      | 68.50 31.50 | NS | Bid et al. (2005) |
| French             | 100  | 43 47 10 | NS      | 66.50 33.50 | NS | Zmuda et al. (1997) |
| Asian              |      |              |         |       |       |                         |
| Japan              | 249  | 37 51 12 | NS      | 62.50 37.50 | NS | Tokita et al. (1996) |
| North Indian       | 346  | 44 49 7  | *        | 68.50 31.50 | *  | Bhanushali et al. (2009) |
| India              | 143  | 59 36 5  | ***      | 77.00 23.00 | *** | Bid et al. (2005) |
| Middle East        |      |              |         |       |       |                         |
| UAE                | 282  | 27 42 31 | 48.04 51.96 |       |       | Current study |

*p = 0.05, ** = p < 0.01, *** = p < 0.001, NS = not significant.
Table 3
Frequencies of haplotypes for two SNPs Taq-I (rs731236) and Fok-I (rs2228570) polymorphisms in Emirati population.

| Haplotype | Frequency |
|-----------|-----------|
| A/G       | 0.31      |
| A/A       | 0.29      |
| G/A       | 0.20      |
| G/G       | 0.21      |

Authors contribution

Drs. Alsafar, Al Anouti and Osman have designed the study, prepared the manuscript and performed all the data analyses with assistance from co-authors. Specifically, Ms. Enas has performed all laboratory work in Molecular Cell Biology laboratory at Khalifa University.

Conflict of interest

All the authors declare no conflict of interest.

Acknowledgment

We gratefully acknowledge the contribution of the study participants whose cooperation made this study possible. This study was supported by research funds from Zayed University granted to Al Anouti.

References

Bhanushali, A.A., Lajpal, N., Kulkarni, S.S., Chavan, S.S., Bagadi, S.S., Das, B.R., 2009. Frequency of fok I and taq I polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Indian J. Hum. Genet. 15 (3), 108–113. http://dx.doi.org/10.4103/0971-6866.60186.

Bid, H.K., Mishra, D.K., Mittal, R.D., 2009. Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population. Asian J. Clin. Nutr. 88 (2), 565s–569s.

Dvornyk, V., Long, J.R., Liu, P.Y., Shen, H., Recker, R.R., Deng, H.W., 2006. Polymorphisms of the vitamin D receptor gene predict the onset of surgical menopause in Caucasian females. Gynecol. Endocrinol. 22 (10), 552–556. http://dx.doi.org/10.1080/0951359060098258.

Gross, C., Eccleshall, T.R., Malloy, P.J., Villa, M.L., Marcus, R., Feldman, D., 1996. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J. Bone Miner. Res. 11 (12), 1850–1855. http://dx.doi.org/10.1002/jbmr.505011204.

Haddad, S., 2014. Vitamin-D receptor (VDR) gene polymorphisms (Taq-I & Apa-I) in Syrian healthy population. Meta Gene 2, 646–650. http://dx.doi.org/10.1016/j.magen.2014.08.005.

Holick, M.F., Frommer, J.E., McNeill, S.C., Richtand, N.M., Henley, J.W., Potts Jr., J.T., 1977. Photometabolism of 7-dehydrocholesterol to previtamin D3 in skin. Biochem. Biophys. Res. Commun. 76 (1), 107–114.

Karasneh, J.A., Ababneh, K.T., Taha, A.H., Al-Abbadi, M.S., Marzouka, N., Jaradat, S.M., Thornhill, M.J., 2013. Association of vitamin D receptor gene polymorphisms with chronic and aggressive periodontitis in Jordanian patients. Eur. J. Oral Sci. 121 (6), 551–558. http://dx.doi.org/10.1111/eos.12085.

Muhairi, S.J., Mehairi, A.E., Khouri, A.A., Naqbi, M.M., Maskari, F.A., Al Kaabi, J., Al Dhaeri, A.S., Nagelkerke, N., Shah, S.M., 2013. Vitamin D deficiency among healthy adolescents in Al Ain, United Arab Emirates. BMC Public Health 13, 33.

Panierakis, C., Goulieinos, G., Mamoulakis, D., Petraki, E., Papavasiliou, E., Galanakis, E., 2009. Vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Crete, Greece. Clin. Immunol. 131 (2), 276–281. http://dx.doi.org/10.1016/j.clim.2009.08.004.

Rezende, V.B., Barbosa Jr., F., Montenegro, M.F., Sandrim, V.C., Gerlach, R.F., Tanus-Santos, J.E., 2007. An interethnic comparison of the distribution of vitamin D receptor genotypes and haplotypes. Clin. Chim. Acta 384 (1–2), 155–159. http://dx.doi.org/10.1016/j.cca.2007.05.010.

Rizzo, A., Domineaux, J., Menheere, P., Tervaert, J.W., Hupperts, R., 2009. Association study on two vitamin D receptor gene polymorphisms and vitamin D metabolites in multiple sclerosis. Ann. N. Y. Acad. Sci. 1173, 515–520. http://dx.doi.org/10.1111/j.1749-6632.2009.04656.x.

Tokuoka, A., Matsumoto, H., Tsuruta, T., Murao, Y., Fukushima, K., Eisman, J.A., 1996. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J. Bone Miner. Res. 11 (7), 1003–1009. http://dx.doi.org/10.1002/jbmr.1997.12.9.1446.

Uitterlinden, A.G., Fang, Y., van Meurs, J.B., Pols, H.A., 2004. Vitamin D receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. J. Bone Miner. Res. 19 (5), 187–193. http://dx.doi.org/10.1016/j.jbmr.2003.10.011.

Tokita, A., Matsumoto, H., Morrison, N.A., Tawa, T., Miura, Y., Fukamauchi, K., Eisman, J.A., 1996. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J. Bone Miner. Res. 11 (7), 1003–1009. http://dx.doi.org/10.1002/jbmr.5650110718.

Tuomesraki, P., Vaisanen, S., Neme, A., Heikkinen, S., Carlberg, C., 2014. Patterns of genome-wide VDR locations. PLoS ONE 9 (4), e96105. http://dx.doi.org/10.1371/journal.pone.0096105.

Uitterlinden, A.G., Fang, Y., van Meurs, J.B., van Leeuwen, H., Pols, H.A., 2004. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. J. Steroid Biochem. Mol. Biol. 89–90 (1–5), 187–193. http://dx.doi.org/10.1016/j.jsbmb.2004.03.083.

Wang, Y., Zhu, J., Deluca, H.F., 2012. Where is the vitamin D receptor? Arch. Biochem. Biophys. 523 (1), 123–133. http://dx.doi.org/10.1016/j.abb.2012.04.001.

Zmuda, J.M., Cauley, J.A., Danielson, M.E., Wolf, R.L., Ferrell, R.E., 1997. Vitamin D receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. J. Bone Miner. Res. 12 (9), 1446–1452. http://dx.doi.org/10.1038/jbmr.1997.12.9.1446.

Zuniga, S., Firrincieli, D., Houssuet, C., Chignard, N., 2011. Vitamin D and the vitamin D receptor in liver pathophysiology. Clin. Res. Hepatol. Gastroenterol. 35 (4), 295–302. http://dx.doi.org/10.1016/j.clinre.2011.02.003.