ORIGINAL REPORT

Association of Vitamin D Receptor with Longevity and Healthy Aging

Farnaz Najmi Varzaneh[1], Farshad Sharifi[2], Arash Hossein-Nezhad[3], Mojde Mirarefin[4], Zhila Maghbooli[1], Maryam Ghaderpanahi[4], Bagher Larijani[5], and Hossein Fakhrzadeh[6]

[1]*Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Elderly Health Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Department of Genetics, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran*

[4]*Master of Nutrition, Elderly Health Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

[5] *Endocrinology & Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran*

[6]*Elderly Health Research Center, Endocrinology & Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

Received: 21 May 2012; Received in revised form: 30 Dec. 2012; Accepted: 5 Jan. 2013

Abstract- Longevity is a multifaceted trait in which variety of genes and environmental factors are involved. Newly, the role of vitamin D has been revived regarding its potential advantage on delaying the aging process. Vitamin D exerts its effect through vitamin D receptor (VDR). VDR-FokI is the only polymorphism which alters the VDR length. We examined the frequency of FokI genotypes in old age population as compared to young adults to determine the discerning genotype of FokI polymorphism leading to longer living. In addition, to highlight the position of FokI polymorphism in quality of life; a cognitive function assessment was performed. 728 participants participated in this study of which 166 individuals were elderly residents of Kahrizak Charity Foundation. The rest were participants of Iranian Multicenter Osteoporosis Study (IMOS). Genomic DNA was extracted from peripheral blood and VDR genotype was detected by the polymerase chain reaction. The participants in the elderly group underwent a cognitive function assessment. Cognitive function was measured with the mini mental state examination (MMSE). Data were analyzed by SPSS 16.5. The prevalence of ff genotype showed 48% decrease in elderly population as compared to young adults (P=0.06). In addition, F allele was over-represented in the elderly group as compared to controls (P=0.05). Also, "FF" participants of elderly group had higher MMSE as compared to "ff" genotype (18.16Vs17.12). Our data suggest that single nucleotide polymorphisms (SNPs) in FokI may be possibly involved in longevity and cognitive function.

Acta Medica Iranica, 2013; 51(4): 236-241.

Keywords: Cognition; Longevity; Single Nucleotide Polymorphisms (SNPs); Polymorphism; Receptors; Vitamin D

Introduction

Aging is associated with several age-related disorders which can potentially deteriorate health status and worsen quality of life. Longevity which is usually accompanied by healthy aging is a multifaceted trait in which a variety of genes, environmental factors, social and stochastic issues are involved (1). During the last century longevity has been extended due to socioeconomic improvement and prevention of life-threatening diseases. In the few past years, the role of vitamin D has been revived as an anti-inflammatory and immune-modulating hormone (2).

In addition, vitamin D as a great aid for reducing inflammation has been related to telomere and its stabilizer length enzyme, telomerase (3). Telomeres, the termini of linear chromosomes which shorten in each cell division, have been stated as the primary cause of human senescence and telomere

Corresponding Author: Hossein Fakhrzadeh
Elderly Health Research Center, Endocrinology & Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
4th floor, Number4, Dr Nejatollahi Street, Enghelab Avenue, Tehran, Iran. Postal Code : 1599666615
Tel: +98 21 88808082, Fax: +98 21 88808082, E-mail: fakhrzad@tums.ac.ir

length has been determined as a biological marker of aging (4). Besides, the telomere attrition rate is enhanced by inflammation and oxidative stress (5). In this view, recent study reported a positive correlation between serum vitamin D concentration and telomere length which emphasizes the potential advantages of vitamin D for delaying the aging process (6).

Vitamin D exerts its effect by binding to the widely expressed vitamin D receptor (VDR) (1). Depends on variation in the VDR activity; vitamin D effects may alter among individuals. A number of VDR polymorphisms have been identified as BsmI, TaqI, ApaI and FokI. FokI variants in the VDR first start codon are the only polymorphisms which alter the VDR protein length; producing the truncated F allele and the lengthened f allele (7).

Since vitamin D in cooperation with its receptor could improve longevity (8), we hypothesize that surviving to extreme old age might be influenced by VDR-Fok1 genotypes. In this respect, genome wide association (GWA) studies have identified several polymorphisms associated with increased risk of many common diseases which potentially limit the life span (9).

In addition, it has been shown that VDR binding sites are significantly enriched near autoimmune and cancer associated genes (10). Therefore, GWA studies may lead to insight on longevity by providing new potential goals that are important to the aging process.

In this study, we evaluated the frequency of VDR-Fok1 polymorphism genotypes in old age population as compared to young adult individuals to determine the discerning genotype of FokI polymorphism leading to longer living.

In addition, to highlight the role of FokI genotype polymorphisms in quality of life and healthy aging; we performed a cognitive function assessment with mini mental status examination (MMSE) in old age population to investigate any significant difference in cognition among various genotypes of FokI polymorphism.

Conclusively, based on the facts describing about VDR polymorphism relationship as a complex with longevity and healthy aging effects, the present hypothesis is to discriminate any alteration in frequency of FokI genotype polymorphisms in elderly population for detecting a novel pattern of FokI polymorphism distribution.

Materials and Methods

Study population

A total of 728 participants were studied of which 166 Individuals were elderly residents of Kahrizak Charity Foundation in Tehran, Iran, restricted to age 65 years and older as a representative of elderly population. The control group were 562 young adults with the mean age of 30.98 years from the participants of Iranian Multicenter Osteoporosis Study (IMOS) as a representative of young adult population (11). In terms of ethnicity, there was no difference between the Kahrizak and IMOS participants. In addition, the study population of both studies was selected from Iranian population that was residing in Tehran for at least ten years.

The participants of Kahrizak and IMOS were both selected by a randomized sampling based on unique code, if they fulfilled the criteria. The exclusion criteria for each population were as described before (12).

Informed written consent was obtained from each subject before data collection. The study was approved by the Research Ethics Committee of the Tehran University of Medical Sciences and conforms to the Declaration of Helsinki.

Single nucleotide polymorphisms (SNP) genotyping

About 4 ml aliquots of peripheral blood samples were collected from the participants and stored in EDTA coated vacutainers. Genomic DNA was isolated from peripheral blood leukocytes according to standard methods previously described(13). The FokI polymorphism of the VDR gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR was performed to amplify target regions of DNA. The amplification conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 35 amplification cycles (denaturation at 94°C for 15 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec) and a final extension at 72°C for 10 min. PCR products were digested with FokI (Boehringer Mannheim) for 1.5–2 h at 37°C.

The digested PCR products were resolved on 2.0% agarose gels. For FokI polymorphism sited in exon 2, the f genotype was indicated by the presence of the restriction site that generates two fragments of 196 bp and 69 bp and the F genotype by a single uncleaved 265-bp fragment. To confirm the accuracy of genotyping, partial samples (20% of all samples) were examined by DNA sequencing.

Cognitive function

The participants in the elderly group underwent a cognitive function assessment. Overall cognitive function was measured with the MMSE. It consists of 11 questions assessing a broad range of cognitive functions including orientation, recall, attention, calculation, language manipulation, and constructional praxis. The validity of MMSE in Persian language has been tested by Iranian experts (14).

Statistical analysis

Data were analyzed by means of a personal computer implemented with dedicated software (SPSS 16.5), to obtain mean ± SD values, correlation matrix, Student's t-test, analysis of variance and/or Chi-square tests, as appropriate.

A Chi-square test was used to confirm that the SNPs were in Hardy Weinberg equilibrium. The level of significance was settled at < 5%, as usual.

Results

In total, 166 elderly participants and 562 sex-matched young adults were recruited in this study. Their demographic characteristics are summarized in table 1. Mean age of the elderly and control groups were 77.49 and 30.98 years respectively. In the elderly group, 60.8% were females and 39.2% were males. Mean age and BMI in elderly females were higher as compared to elderly males. All study participants were genotyped for FokI in the VDR gene. Allele and genotype frequencies for the Fok1 polymorphism are shown in table 2. The genotype frequencies of the SNPs (Table 2) were in agreement with Hardy–Weinberg equilibrium in both young adult and elderly population.

Overall prevalence of the VDR-FokI genotypes in young adult population was 55.16 %, 36.12 % and 8.71% respectively in FF, Ff and ff genotype (Figure 1). Overall prevalence of the VDR-FokI genotypes in the elderly population was 61.44%, 21.68% and 4.21 respectively in FF, Ff and ff (Figure 1). The prevalence of ff genotype showed 48% decrease in the elderly population as compared to young adult controls (p=0.06). In addition, F allele was over-represented in elderly group as compared to controls [*P*=0.05, odds ratio= 1.344 (1.002 to 1.804)].

In the elderly group, participants with "FF" genotype were older than those with "ff" genotype. Mean age of the participants with "ff" genotype was 73.80 as compared to 77.39 in "FF" and 76.78 in "Ff". Also, "FF" participants of the elderly group had higher MMSE as compared to those with "ff" genotype (18.16 Vs17.12). It should be noted that in "ff" genotype 42.85% participants were hospitalized due to physical causes compared with only 19.6% hospitalization rate in the "FF" and 13.88% in the "Ff" genotypes.

Table1. Characteristic of population under study.

Characteristic	Elderly group	Young adult	*P*-value
Age	77.49 ± 7.43	30.98 ± 6.05	<0.01
Weight	60.42 ± 14.39	69.90 ± 13.88	0.20
Height	154.04 ± 10.49	163.77 ± 10.54	0.40
BMI	25.42 ± 5.34	26.06 ± 5.67	0.10

BMI: Body Mass Index

Table 2. Genotype and allele distribution.

Genotypes alleles	Elderly population n (%)	Young adult n (%)	Odds ratio (95% CI)	*P*-value
FF	102 (61.44)	310 (55.16)	0.29 (0.9-1.84)	0.11
Ff	57 (21.68)	203 (36.12)	0.9 (0.64-1.33)	0.70
ff	7 (4.21)	49 (8.71)	0.46 (0.2-1.03)	0.06
F	261 (78.61)	823 (73.22)	1.34 (1.00-1.80)	0.05
f	71 (21.38)	301 (26.77)	0.74 (0.55-0.99)	0.05

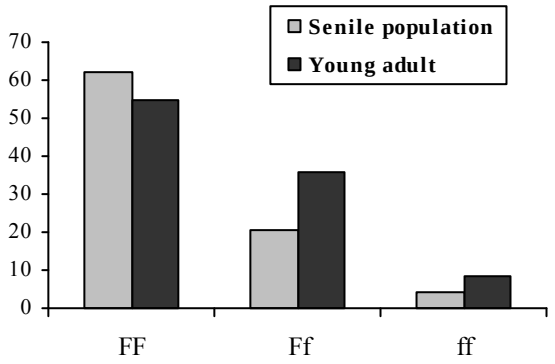

Figure 1. FokI polymorphism genotypes distribution in elderly and young adult.

Discussion

Life expectancy is influenced by genetic and environmental factors. For this reason, most studies of extended longevity have been performed through these two components. It is remarkable that vitamin D as an important environmental factor plays a significant role in conferring exceptional longevity and delaying the aging process (15). The positive role of vitamin D in slowing the aging process, as an anti-tumor agent and prohibitory of different malignancies has been supported by additional evidence (16). VDR as a nuclear receptor for vitamin-D regulates vitamin D-response genes and is responsible for its genomic action (1).

The gene encoding the VDR protein is known to display polymorphic variation. In this study, we selected FokI genotype with its polymorphisms FF, Ff and ff. The reason is that FokI is less likely to be in linkage disequilibrium (LD) with other genotypes and is the only genotype which alters the length of VDR, leading to potential differences in VDR function (7). Some evidences have represented F allele as an advantage factor (17). Arai *et al.* have shown that F-allele is approximately 1.7-fold greater in transactivation experiment than did the f-allele (7). Besides, our previous study detected a greater amount of vitamin D3 in FF genotype compared to other polymorphisms of FokI despite equivalent consumption of vitamin D supplements (17). In this regard, some evidence support that FokI polymorphism associate with various life-limiting disorders such as cancers (18-20), cardiovascular disease (21), neurologic disease (22) and auto immune disorders (23).

A meta-analysis of 67 independent studies revealed that the risk of breast, skin and prostate cancers may possibly be modulated by FokI polymorphisms (24). Some studies unveiled that the risk of breast and prostate cancer were higher in ff homozygote compare to the common FF genotype (20, 25). Also it has been mentioned that the carriers of ff genotype with less than median levels of circulating vitamin D are at higher risk of total and aggressive cancers (25).

Therefore, the effect of VDR polymorphisms on some cancer risk modulation has highlighted its crucial role as a valuable factor in modifying life expectancy and longevity.

In a recent study of Laplana *et al.*, a new scenario has been suggested in which VDR as a candidate gene is involved in human longevity and healthy aging (1). In our study, the ff genotype showed a marked decrease in prevalence in long-lived individuals as compared to young adult group and F allele was significantly over-represented in elderly group in comparison with e controls.

This is so explained that the genes which are resistant against life-limiting diseases are transferred to the next generation. Hence, individuals who carry these genes, have the chance to live longer. As a result, most of the octogenarians which have escaped from the age-related diseases are characterized by these resistant genes. This issue bolsters our findings which show that participants in the elderly group with FF (resistance) genotype are older than those with other genotypes.

To elucidate the relationship between genetic variants of FokI genotype and healthy aging, we performed MMSE to determine any cognitive impairment in elderly society.

In the present study, participants with "FF" genotype in elderly group achieved 5.72% higher MMSE scores as compared to those with "ff" genotype (18.16 vs. 17.12). So it can be assumed that SNPs in FokI may possibly be involved in cognitive function. The current assumption is in agreement with the result of a prospective study on 563 participants which revealed substantial variability in cognition among different polymorphisms of VDR gene (26).

Cognitive impairment as the salient feature of dementia is one of the most common age-related disorders which are responsible for not attaining successful aging. The role of inflammation in early onset dementia has been revealed recently (27).; *i.e.* inflammation is associated with cognitive impairment. So, it would be rational that vitamin D can retard early onset by its anti-inflammatory action via VDR. In this regard, there is some evidence which provides the association between low levels of vitamin D and worse cognitive function (28). In addition, it has been previously shown that vitamin D has a potential neuro-

protective effect through its nuclear receptors which are broadly distributed in human cortex and hippocampus (26,29).

Our study is consistent with the mentioned studies which showed that polymorphisms of VDR influence cognitive function. Regarding association between dementia and life expectancy, it has been shown that median survival of individuals with dementia is much lower than normal population (9). Since cognitive impairment was more prevalent in "ff" genotype in this study, it could be concluded that this genotype would play a role in decreasing longevity through its effects on cognitive function.

The reason for admission in about half of individuals of the elderly group with "ff" genotype was due to physical causes which represent the high prevalence of physical co-morbidity in this genotype as compared the two other genotypes.

The drop of ff genotype in the elderly group has two probable implications. First, the longer f allele in first start codon is less functional than the truncated F allele (7). So the "ff" genotype is associated with reduced level of vitamin D and less profits from its role as a down-regulator of telomerase which could inhibit the growth of cancer (3) and its immunosuppressive and anti-inflammatory actions. So "ff" genotype is associated with much more malignancies and other life-limiting disorders. Second, the high prevalence of cognitive impairment in "ff" genotype as compared to "FF" may be a factor leading to shorter life expectancy in this genotype.

There are two types of limitation in this study. First, it was better to select the participants of both groups from one society, but there was a lack of data for old age population in IMOS study and we could not selected young adult population from Kahrizak due to other confounder issues. Second, elderly residents of Kahrizak charity foundation may not be an exact prototype of Iranian old people, despite it is the center where more than 1050 old people live and receive health care.

This research reveals recent advance with regard to contribution of VDR-FokI polymorphism on life expectancy and cognition in old age population. Therefore, we suggest that more attention to be paid to people with "ff" genotype to help protect them from cognitive impairment and other life limited disorders.

Acknowledgment

This study was fully supported by research grants from Endocrinology and metabolism research center of Medical Science University of Tehran and the authors gratefully acknowledge EMRC for necessary facilities provided to carry the research work.

References

1. Laplana M, Sánchez-de-la-Torre M, Aguiló A, Casado I, Flores M, Sánchez-Pellicer R, Fibla J. Tagging long-lived individuals through vitamin-D receptor (VDR) haplotypes. Biogerontology 2010;11(4):437-46.
2. Raman M, Milestone AN, Walters JR, Hart AL, Ghosh S. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. Therap Adv Gastroenterol 2011;4(1):49-62.
3. Jiang F, Bao J, Li P, Nicosia SV, Bai W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J Biol Chem 2004;279(51):53213-21.
4. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci U S A 2004;101(49):17312-5.
5. Carrero JJ, Stenvinkel P, Fellström B, Qureshi AR, Lamb K, Heimbürger O,Barany P, Radhakrishnan K, Lindholm B, Soveri I, Nordfors L, Shiels PG. Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. J Intern Med. 2008;263(3):302-12.
6. Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, Lu X, Surdulescu GL, Swaminathan R, Spector TD, Aviv A. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. Am J Clin Nutr 2007;86(5):1420-5.
7. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 1997;12(6):915-21.
8. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. Exp Biol Med (Maywood) 2010;235:1034-45.
9. Newman AB, Walter S, Lunetta KL, Garcia ME, Slagboom PE, Christensen K, Arnold AM, Aspelund T, Aulchenko YS, Benjamin EJ, Christiansen L, D'Agostino RB Sr, Fitzpatrick AL, Franceschini N, Glazer NL, et al. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. J Gerontol A Biol Sci Med Sci 2010;65(5):478-87.

10. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, Handunnetthi L, Handel AE, Disanto G, Orton SM, Watson CT, Morahan JM, Giovannoni G, Ponting CP, Ebers GC, Knight JC. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 2010;20(10):1352-60.
11. Khashayar P, Meybodi HR, Homami MR, Amini MR, Mohajeri-Tehrani MR, Heshmat R, Larijani B. The discriminative value of various biochemical parameters in detecting varying degrees of vitamin D deficiency in the Iranian population. Clin Lab 2011;57(3-4):163-70.
12. Larijani B, Moayyeri A, Keshtkar AA, Hossein-Nezhad A, Soltani A, Bahrami A, Omrani GH, Rajabian R, Nabipour I. Peak bone mass of Iranian population: the Iranian Multicenter Osteoporosis Study. J Clin Densitom 2006; 9(3):367-74.
13. Ahangari G, Hossein-Nezhad A, Behzadi H, Maghbooli Z, Larijani B. Vitamin D Receptor gene polymorphism may predict response to vitamin D intake and bone turnover. DARU Journal of Pharmaceutical Sciences 2010;17(Suppl 1):13-19.
14. Ansari NN, Naghdi S, Hasson S, Valizadeh L, Jalaie S. Validation of a Mini-Mental State Examination (MMSE) for the Persian population: a pilot study. Appl Neuropsychol 2010;17(3):190-5.
15. Lanske B, Razzaque MS. Vitamin D and aging: old concepts and new insights. J Nutr Biochem 2007;18(12):771-7.
16. Speer G. [The role of vitamin D in the prevention and the additional therapy of cancers]. Magy Onkol 2010;54(4):303-14.
17. Hossein-Nezhad A, Ahangari G BH, Maghbooli Z, Larijani B. Vitamin D Receptor gene polymorphism may predict response to vitamin D intake and bone turnover. DARU,Suppl 1. 2009;17:13-9.
18. Park K, Woo M, Nam J, Kim JC. Start codon polymorphisms in the vitamin D receptor and colorectal cancer risk. Cancer Lett 2006;237(2):199-206.
19. Kostner K, Denzer N, Muller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009;29(9):3511-36.
20. Sinotte M, Rousseau F, Ayotte P, Dewailly E, Diorio C, Giguere Y, Bérubé S, Brisson J. Vitamin D receptor polymorphisms (FokI, BsmI) and breast cancer risk: association replication in two case-control studies within French Canadian population. Endocr Relat Cancer. 2008;15(4):975-83.
21. Amato M, Pacini S, Aterini S, Punzi T, Gulisano M, Ruggiero M. Iron indices and vitamin D receptor polymorphisms in hemodialysis patients. Adv Chronic Kidney Dis 2008;15(2):186-90.
22. Simon KC, Munger KL, Xing Y, Ascherio A. Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis. Mult Scler 2010;16(2):133-8. PMCID: 2819633.
23. Chen RH, Chang CT, Chen HY, Chen WC, Tsai CH, Tsai FJ. Association between vitamin-D receptor gene FokI polymorphism and Graves' disease among Taiwanese Chinese. J Clin Lab Anal 2007;21(3):173-7.
24. Raimondi S, Johansson H, Maisonneuve P, Gandini S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Carcinogenesis 2009;30(7):1170-80.
25. Li H, Stampfer MJ, Hollis JB, Mucci LA, Gaziano JM, Hunter D, Giovannucci EL, Ma J. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med. 2007;4(3):e103. PMCID: 1831738.
26. Kuningas M, Mooijaart SP, Jolles J, Slagboom PE, Westendorp RG, van Heemst D. VDR gene variants associate with cognitive function and depressive symptoms in old age. Neurobiol Aging 2009;30(3):466-73.
27. Marchesi VT. Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy. FASEB J 2011;25(1):5-13.
28. Slinin Y, Paudel ML, Taylor BC, Fink HA, Ishani A, Canales MT, Canales MT, Yaffe K, Barrett-Connor E, Orwoll ES, Shikany JM, Leblanc ES, Cauley JA, Ensrud KE. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology 2010;74(1):33-41.
29. Annweiler C, Allali G, Allain P, Bridenbaugh S, Schott AM, Kressig RW, Beauchet O. Vitamin D and cognitive performance in adults: a systematic review. Eur J Neurol 2009;16(10):1083-9.