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ORIGINAL ARTICLE

Impact of vitamin D receptor polymorphisms in centenarians

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Abstract Vitamin D is a seco-sterol produced endogenously in the skin or obtained from certain foods. It exerts its action through binding to intracellular vitamin D receptor (VDR). Lately, the role of vitamin D has been revised regarding its potential advantage on delaying the process of aging. The aim of this study was to assess the contribution of VDR gene polymorphisms in healthy aging and longevity. We evaluated the frequency of four polymorphisms of the VDR gene (*Fok*I, *Bsm*I, *Apa*I, and *Taq*I) in centenarians (102 subjects, mean age: 102.3 ± 0.3 years), compared to septuagenarians (163 subjects, mean age: 73.0 ± 0.6 years) and we analyzed a variety of pathophysiologically relevant functions in centenarians. *Bsm*I and *Apa*I provided a significant association with longevity: there was a highly significant difference in the frequency of *Bsm*I genotypes ($p = 0.037$), *Apa*I genotypes ($p = 0.022$), and *Apa*I alleles ($p = 0.050$) in centenarians versus septuagenarians. Furthermore, we found a significant correlation of all the VDR gene polymorphisms in centenarians with some measured variables such as hand grip strength, body mass index, blood pressure, HDL cholesterol, and mini-mental state examination. We also found a correlation with the prevalence of medical history of hypertension, acute myocardial infarction, angina, venous insufficiency, dementia, chronic

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obstructive pulmonary disease, and arthrosis. In conclusion, this study proposes a new scenario in which the variability of the VDR gene is relevant in the aging process and emphasizes the role of VDR genetic background in determining healthy aging.

Keywords Biology of aging · Centenarian · Co-morbidity · Longevity

Introduction

Vitamin D is a seco-sterol primarily produced in the skin through the action of UV-B light on 7-dehydrocholesterol. The active form of vitamin D is 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). It is formed by the hydroxylation of the not biologically active form, namely 25-hydroxyvitamin D_3 ($25\text{-}OHD_3$) [1, 2]. The effects of vitamin D are mediated through its interaction with a high-affinity nuclear vitamin D receptor (VDR), a member of the nuclear receptors' superfamily of ligand-activated transcription factors [3]. The VDR binds to DNA as VDR/VDR homodimers or VDR/RXR (retinoid X receptor) heterodimers in order to regulate gene expression of thousands of genes [4]. The VDR has been located in many cell types [5, 6] and the interaction between vitamin D and VDR sites in various organs of the body produces numerous biological effects unveiling the potential development of many diseases like multiple autoimmune diseases, upper respiratory tract infections, heart disease, cancer, depression, and dementia [7–9]. Recently, the role of vitamin D has been revised regarding its potential advantage on delaying the aging process [10]. Human studies show an inverse relationship between serum vitamin D and age-related diseases as well as mortality [11]. Elderly populations suffer from vitamin D deficiency that leads to impaired bone mineralization [12]. A recent study reported a positive correlation between the concentration of serum vitamin D and telomere length which emphasizes the potential advantages of vitamin D for delaying aging [13]. Since vitamin D in cooperation with its receptor could improve longevity [14, 15], we hypothesize that surviving to extreme old age might be influenced by VDR genotypes. A great number of VDR single nucleotide polymorphisms (SNPs) have been described, but only a few of them were related to a phenotypic effect such as an altered level of gene expression [16]. We focused on four polymorphisms named *Fok*I (rs10735810), located in the coding part, at the start codon, whose polymorphic form gives rise to a shorter protein with 3 amino acids; *Bsm*I (rs1544410) and *Apa*I (rs7975232) in intron 8; and *Taq*I (rs731236) in exon 9, probably these last three are responsible for the stability of the resulting mRNA [17].

Several studies reveal that VDR polymorphisms have been linked to changes in cognitive function and depressive symptoms in old age and are also associated with life-limiting diseases such as cancer, cardiovascular diseases, and immune system disturbances [18]; moreover, some studies show differences in genotype and allele distribution in elderly population compared to young adults [19, 20]

In the present study, we mainly focused on VDR polymorphisms in centenarians, an extreme phenotype that escaped the major age-related diseases compared to cross-sectional cohorts.

We evaluated the frequency of these four polymorphisms of the VDR gene in centenarians compared to septuagenarians and we analyzed a variety of pathophysiologically relevant functions in centenarians to highlight the role of VDR in quality of life and healthy aging.

Materials and methods

From 2007 to 2014, we contacted 46 registry offices in Northern Italy in order to collect the demographical data (name, date of birth, living place) of people $\geq$100 years at the time of enrolment. A letter explaining methods and aims of the study was sent to each eligible individual. Each centenarian willing to participate (85 % of contacted subjects representative of the whole population of centenarians [21]) was enrolled (102 subjects, mean age 102.3 $\pm$ 0.3 years), the control subjects (163 subjects, mean age 73.0 $\pm$ 0.6 years) were Caucasians living in Northern Italy who were prospectively enrolled from a larger population of outpatients attending the Geriatric Unit of the Ospedale Maggiore Policlinico IRCCS Ca' Granda, University of Milan, Italy. The study protocol received approval from the local Ethical Committee. All subjects gave their informed consent to participate in the study. A trained multidisciplinary staff went to each centenarian's house or nursing home to administer a standard structured questionnaire including health status information, cognitive and functional tests, physical and performance measures, and to collect blood samples. Medical history was gained by a physician through patients' medical reports, and classified according to the "International Statistical Classification of Diseases and Related Health Problems 10th Revision" (ICD-10). Cognitive status was assessed by standardized Mini-Mental State Examination (MMSE) test while functional status was measured by both the Lawton Instrumental Activities of Daily Living (IADL) scale and the Katz index of independence in Activities of Daily Living (ADL) scale [22, 23]. Whole blood was collected by the means of a venipuncture into Vacutainers (Becton–Dickinson Co., Rutherford, NJ, USA). Standard sampling of glycemia, total cholesterol, HDL, triglycerides, and uric acid were

performed by commercially available kits. Genomic DNA was extracted using a previously described salting-out method [24] and its concentration and purity were determined by the means of spectrophotometric analysis. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed for genotyping of SNPs at positions rs10735810 (*Fok*I), rs1544410 (*Bsm*I), rs7975232 (*Apa*I), and rs731236 (*Taq*I) on VDR gene (Fig. 1). The sequences of primers and the conditions for the identification of VDR gene polymorphisms are shown in Table 1. The PCR amplifications were carried out in a total volume of 25 μL including 1 μg genomic DNA, 2 nM of each primer (Eurofins Genomics, Ebersberg, Bayern, Germany), and 14 μL PCR mix (MegaMix-Royal, Microzone Ltd, Haywards Heath, UK). The PCR products were analyzed on 2 % agarose gels containing ethidium bromide and visualized under a UV transilluminator. The amplified products were digested using restriction enzymes *Fok*I, *Bsm*I, *Apa*I, and *Taq*I (New England Biolabs, Ipswich, MA, USA), according to the manufacturer's instructions. Briefly, 5 μL of each related PCR product was mixed with 1 μL of each restriction enzyme, 2 μL of 10× buffers, and 12 μL H_2O. Digested samples were run on 2 % agarose gels containing ethidium bromide and visualized under a UV transilluminator [25]. Genotypes were determined according to the presence or absence of an appropriate restriction site. The usual nomenclature for restriction fragment length polymorphism alleles was used in this study. The lowercase allele represents the presence of the restriction site (f, b, a, or t) and the uppercase allele portrays the absence of the restriction site (F, B, A, or T) [26, 27].

Statistical analysis was performed with SPSS statistical package (SPSS version 22, Chicago, IL, USA).

Data were expressed as mean values ± standard error since the distribution was normal and/or as percentage of distribution. Difference in continuous variables was examined using the analysis of variance (ANOVA), with a Bonferroni post-test. Student's *t* test was used to paired comparisons. Categorical data were compared by using the Chi-square test. We used SHEsis software to perform statistical inference of haplotypes from our sample data. A p value ≤ 0.05 was defined as statistically significant.

Results

The genotype distribution conform to the Hardy–Weinberg equilibrium was observed for all SNPs in the control samples except for *Bsm*I; maybe there is a selective pressure on this SNP in our sample.

There was a highly significant difference in the frequency of *Bsm*I genotypes ($p = 0.037$), *Apa*I genotypes ($p = 0.022$), and *Apa*I alleles ($p = 0.050$) in centenarians versus control subjects (Table 2). No statistical differences were observed for haplotype distribution between controls and septuagenarians.

Moreover we considered, in centenarians, the association between these SNPs and measured variables and furthermore between SNPs and pathologies.

Regarding *Fok*I (Supplementary Table 1), the FF carriers show significantly higher hand grip strength ($p = 0.021$) and MMSE score ($p = 0.029$), indicating greater strength and better cognitive status of these subjects compared to Ff&ff carriers. Considering comorbidities the prevalence of hypertension was higher in FF than Ff and ff ($p = 0.015$) and Ff&ff carriers ($p = 0.005$) while the prevalence of dementia was lower than Ff&ff carriers ($p = 0.024$).

Regarding *Bsm*I (Supplementary Table 2), Bb carriers show significantly lower systolic blood pressure than BB ($p = 0.050$). bb carriers show significantly lower BMI

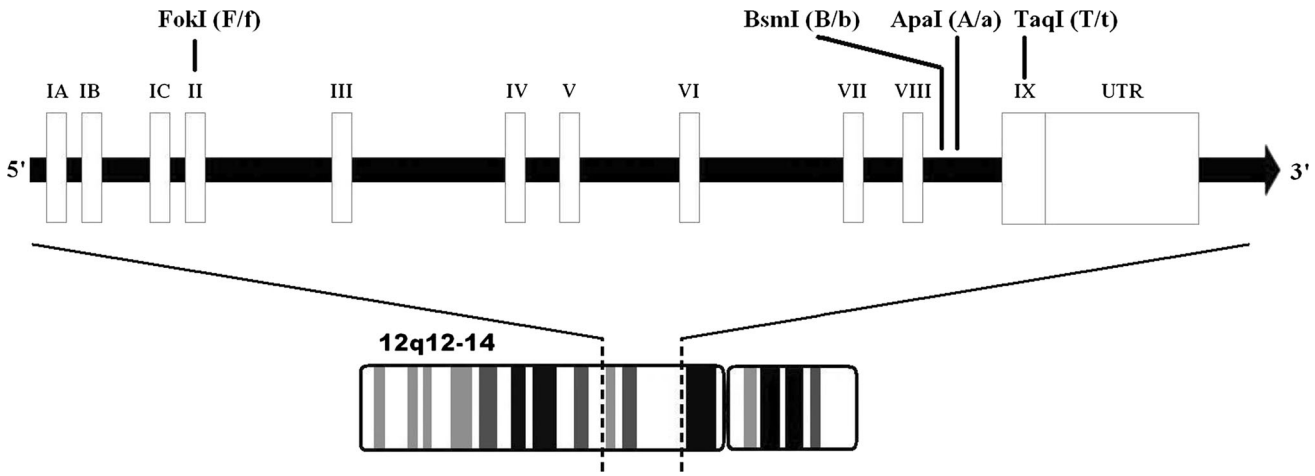

Fig. 1 Human vitamin D receptor gene polymorphic sites

Table 1 Primers sequences and restriction fragment length polymorphism conditions for the identification of vitamin D receptor gene polymorphisms

Polymorphic site	PCR primer sequences (5′–3′)	PCR conditions	RFLP conditions	Restriction fragment lengths (bp)
*Fok*I	F: AGCTGGCCCTGGCACTGACTCTGCTCT	94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and a final step at 72 °C for 5 min	55 °C 3 h	F: 265 bp f: 196 bp, 69 bp
rs10735810 (C>T) recognition site: 5′..GGATG(N)$_9$..3′	R: ATGGAA ACACCTTGCTTCTTCTCCCTC			
*Bsm*I	F: CAACCAAGACTACAAGTACCGCGTCAGTGA	94 °C for 5 min, followed by 30 cycles of 94 °C for 5 min, 65 °C for 1 min, and 72 °C for 1 min, and a final step at 72 °C for 5 min	37 °C overnight	B: 825 bp b: 650 bp,175 bp
rs1544410 (C>T) recognition site: 5′..GAATGCN..3′	R: AACCAGCGGGAAGAGGTCAAGGG			
*Apa*I	F: CAGAGCATGGACAGGGAGCAA	94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min, and a final step at 72 °C for 5 min	37 °C overnight	A: 740 bp a: 530 bp, 210 bp
rs7975232 (A>C) recognition site: 5′..GGGCCC..3′	R: GCAACTCCTCATGGCTGAGGTCCTC			
*Taq*I	F: CAGAGCATGGACAGGGAGCAA	94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min, and a final step at 72 °C for 5 min	65 °C 3 h	T: 495 bp, 245 bp t: 290 bp, 245 bp and 205 bp
rs731236 (A>G) recognition site: 5′..TCGA..3′	R: GCAACTCCTCATGGCTGAGGTCCTC			

Table 2 Vitamin D receptor (VDR) *Fok*I, *Bsm*I, *Apa*I, and *Taq*I genotypes and alleles frequency distributions observed in centenarians and controls

VDR polymorphic sites		Centenarians	Controls	Significance
*Fok*I *n* (%)	FF	45 (47.4 %)	79 (48.4 %)	$\chi^2 = 0.587$
	Ff	40 (42.1 %)	63 (38.7 %)	$p = 0.746$
	ff	10 (10.5 %)	21 (12.9 %)	
	F	130 (68.4 %)	221 (67.8 %)	$\chi^2 = 0.001$
	f	60 (31.6 %)	105 (32.2 %)	$p = 0.995$
*Bsm*I *n* (%)	bb	22 (24.7 %)	65 (39.9 %)	$\chi^2 = 6.580$
	Bb	44 (49.5 %)	56 (34.3 %)	$p = 0.037$
	BB	23 (25.8 %)	42 (25.8 %)	
	b	88 (49.4 %)	186 (57.1 %)	$\chi^2 = 2.390$
	B	90 (50.6 %)	140 (42.9 %)	$p = 0.1218$
*Apa*I *n* (%)	AA	30 (31.9 %)	43 (26.7 %)	$\chi^2 = 7.661$
	Aa	57 (60.6 %)	85 (52.8 %)	$p = 0.022$
	aa	7 (7.5 %)	33 (20.5 %)	
	A	117 (62.2 %)	171 (53.1 %)	$\chi^2 = 3.660$
	a	71 (37.8 %)	151 (46.9 %)	$p = 0.050$
*Taq*I *n* (%)	TT	25 (31.7 %)	63 (40.9 %)	$\chi^2 = 2.522$
	Tt	43 (54.4 %)	77 (50.0 %)	$p = 0.283$
	tt	11 (13.9 %)	14 (9.1 %)	
	T	93 (58.8 %)	203 (65.9 %)	$\chi^2 = 1.951$
	t	65 (41.2 %)	105 (34.1 %)	$p = 0.163$

($p = 0.050$), and diastolic blood pressure ($p = 0.050$) than BB and lower MMSE score than Bb&BB ($p = 0.032$). bb carriers also show high frequency of acute myocardial infarction ($p = 0.041$) and angina ($p = 0.022$) compared to Bb&BB and high frequency of venous insufficiency than Bb and BB ($p = 0.050$).

For *Apa*I (Supplementary Table 3), AA carriers show significantly higher systolic blood pressure compared to Aa ($p = 0.050$) and significantly higher systolic ($p = 0.011$) and diastolic blood pressure ($p = 0.037$) compared to Aa&aa carriers. Furthermore, the AA carriers have lower frequency of chronic obstructive pulmonary disease (COPD) ($p = 0.050$) compared to Aa&aa carriers.

Regarding *Taq*I (Supplementary Table 4), Tt carriers show significantly higher HDL cholesterol compared to tt ($p = 0.050$) and TT carriers show significantly lower diastolic blood pressure ($p = 0.040$) and frequency of arthrosis ($p = 0.037$) compared to Tt&tt carriers.

Discussion

In the present study, we compared allele, genotype, and haplotype distribution of four VDR gene variants between healthy-living centenarians and septuagenarians to assess VDR as a candidate gene affecting aging and longevity. Our study provides evidence of an association between VDR gene polymorphisms and longevity, as revealed by the differential distribution of VDR genotypes and alleles that we have observed. We found a highly significant association between *Bsm*I and *Apa*I polymorphisms and the survival to extreme old age. As a result, most centenarians are characterized by a specific genetic profile. The influence of VDR gene polymorphisms on VDR protein function and signaling is largely unknown. *Fok*I is the only genotype which alters the length of VDR, leading to potential differences in VDR function. The functional impact of VDR could mimic the functional consequences of vitamin D deficiency or excess and/or denaturated formation of the active form of vitamin D [28].

Moreover, the present study reveals a significant impact of the VDR gene polymorphisms in an oldest old population on some measured variables such as hand grip strength, BMI, blood pressure, HDL cholesterol, and MMSE, and on the prevalence of hypertension, acute myocardial infarction, angina, venous insufficiency, dementia, COPD, and arthrosis. Although we did not find a different distribution of *Fok*I and *Taq*I polymorphisms in centenarians compared to septuagenarians, all VDR polymorphisms appear to play a role in the measured variables and pathologies investigated suggesting that all four SNPs seem to be involved in the successful aging. Interestingly, within the group of centenarians, those subjects having a better health status show a particular genetic profile mainly associated with metabolic and cognitive status.

Indeed, our data show an important impact of all VDR polymorphisms on blood pressure and on the medical history of hypertension, probably due to vitamin D effects on endothelial function, inflammation and renin–angiotensin system activity. Observational data show a relationship between vitamin D levels and both current blood pressure and incident hypertension [29]. The importance of these data is due to the fact that higher blood pressure in centenarians seems to be associated with higher cognition and functionality [30], as well as reduced AD risk [31]. Moreover, VDR polymorphisms are known to have an impact on BMI and HDL triglycerides and on the prevalence of medical history of acute myocardial infarction, angina, and venous insufficiency. Although observational studies suggest that low vitamin D levels may represent a marker of metabolic imbalance, clinical benefit of vitamin D supplementation in this case remains less clear [32].

Moreover, our study shows an association of VDR SNPs and MMSE and the frequency of dementia. Broad data provide evidence that vitamin D is involved in brain function including neuroprotection, immunomodulation, and detoxification, and in neurodegenerative diseases [33–35]. In particular, we can assume that *Fok*I and *Bsm*I SNPs may possibly be involved in cognitive function. Our findings are consistent with a previous study in which Najmi Varzaneh et al. showed that subjects with FF genotype had higher MMSE compared to ff genotype [20]. Interestingly, a recent study describes significant reduction in MMSE after a follow-up of 3–4 years in those elderly that had low 25-OHD_3 levels at baseline [36].

In relation to the well-known implication of vitamin D in the calcium homeostasis [37], our study shows the involvement of VDR TaqI polymorphism in arthrosis. Vitamin D plays a key role in the regulation of calcium metabolism and in the maintenance of bone mass [38]. A recent paper [39], shows that VDR polymorphisms influence bone mineral density in patients with normocalcemic hyperparathyroidism. Recent meta-analysis confirms a significant association between VDR gene polymorphisms and the risk of autoimmune thyroid diseases and the susceptibility to osteoarthritis [40, 41].

Vitamin D-binding protein gene polymorphisms and vitamin D deficiency have been associated with COPD and severe lung pathologies [42, 43]. In agreement with these observations, we show an association between ApaI polymorphisms and COPD.

Finally, *Fok*I polymorphism seems to have an impact on hand grip strength, a good indicator of overall health [44]. VDR variability is associated with low hand grip strength also in elderly people in Taiwan [45], as well as low 25-OHD_3 concentrations are associated with poor grip strength in American centenarians [46]. The association between VDR polymorphisms and hand grip strength confirms the involvement of VDR in the better health status of our centenarians.

In conclusion, our study provides evidence of an association between VDR gene polymorphisms and successful

aging. This work supports the implication of the vitamin D metabolism in a broad spectrum of diseases and shows that the protective profiles of VDR gene, associated with a better health status, are prevailing in centenarians. The study results, open to a new scenario in which the variability of the VDR gene is relevant in the aging process and emphasize the role of VDR genetic background in determining healthy aging.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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