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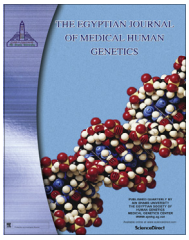

ORIGINAL ARTICLE

Does FVL have an effect on longevity?

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KEYWORDS

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Abstract *Background:* Different genetic and non-genetic factors have been reported to play a role in human longevity. Longevity has been associated with genetically favourable conditions which protected humans from cardiovascular disease (CVD). We have tried to confirm this statement in Turkish young and old aged groups.

Aim of the study: We aimed to investigate selected genetic polymorphisms (Factor V 1691 G-A, Prothrombin 20210 G-A and methylenetetrahydrofolate reductase 677 C-T) in CVD and controls to analyse their effects on longevity.

Patients and methods: The case control study included 362 persons aged 0–18 years and 209 persons aged 70 and above with diagnosis of thrombosis. Genetic polymorphisms were detected with Light Cycler Real Time PCR.

Results: The results were compared to those of 332 and 266 healthy persons of same age groups who served as controls. MTHFR 677 C-T and PT 20210 G-A genotype frequencies in the old and young study groups were similar for all polymorphisms, but MTHFR 677T may have synergy with FVL (Factor V Leiden) imparting a risk of thromboembolism.

Conclusion: This study concludes that common variations in genes associated with cardiovascular risk do not contribute significantly to longevity.

Abbreviations: CVD, cardiovascular disease; FV-FVL, factor V-factor V leiden; PT, prothrombin; MTHFR, methylenetetrahydrofolate reducatese; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; LC, Light Cycler; DNA, deoxyribose nucleic acid; EDTA, ethylenediaminetetraacetic acid; OR, odd ratio.

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1. Introduction

The search for factors involved in aging and longevity has progressed extensively in recent years because of increased human life expectancy and elevation of the number of elderly people which in turn results in increased prevalence of age-related illnesses [1]. There is large variation in the maximum age which human reach before they die. The age of demise is determined by environmental factors as well as genetic factors [2–10].

In today's world a major cause of mortality is cardiovascular disease which is a multifactorial disease such as stroke, thrombosis, and acute myocardial infarction. Several variations in genes involved in haemostasis and blood pressure regulation have been associated with the evolution of CVD, either directly or through their association with blood levels of CVD risk factors [2-4-5-6-7-11]. Venous thromboembolism is the most prevalent significant CVD after ischaemic heart disease and stroke, and 1 in 1000 individuals is affected annually [11].

The aetiology of thromboembolism is thought to be multifactorial and caused by congenital and acquired risk factors. Among inherited factors are FV 1691 G-A, PT 20210 G-A, and MTHFR 677 C-T single nucleotide polymorphisms.

FVL is the leading cause of constitutional thrombophilia [11]. This gene defects include G-A transition at nucleotide 1691 in exon 10 of the FV gene causing APC (Activated Protein C) resistance [12]. The PT 20210 G-A polymorphism is the second most common inherited risk factor for thrombophilia. The polymorphism is located at position 20210 in the 3′ untranslated region of the prothrombin gene and is caused by single base change G-A. Carriers of the rare A allele have higher prothrombin levels than carriers of the G allele and a higher risk of venous thrombosis and myocardial infarction. The hyperhomocysteinemia is also a risk factor for cerebrovascular, peripheral vascular, coronary heart disease and thromboembolism. The polymorphism is located in nucleotide 677 in the MTHFR gene and is caused by single base change C-T leading to an amino acid replacement of alanine (A) to valine (V) at MTHFR enzyme. Carriers of the 677T allele have thermolabile MTHFR enzyme and higher plasma homocysteine concentration than carriers of the 677A allele and have been associated with an increased cardiovascular risk [1-3-4-6].

Comparison of genotype frequencies in very old people and in young people may be a useful tool to study the effect of genetic polymorphisms on CVD causing premature death [2].

The aim of this study was to evaluate the effect of some cardiovascular, thrombotic risk factors on longevity such as Factor V (FV) 1691 G-A, Prothrombin (PT) 20210 G-A and methylenetetrahydrofolate reductase (MTHFR) 677 C-T to determine the role of combination of mutations in Turkish young and old groups. We have studied selected genetic polymorphisms in CVD and controls to analyse the effect of these Single nucleotide polymorphisms (SNPs) on longevity.

2. Patients and methods

2.1. Study populations

Our case control study included patients with clinical thrombosis aged between 0 and 18 (362 individuals), and 70 years and over (209) as well as 332 individuals aged 0–18 years and 266 individuals aged 70 years and above and healthy individuals as control group. An informed written consent was obtained from all the patients' parents. The study is carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. DNA Isolation and FV 1691 G-A, PT 20210 G-A, MTHFR 677C-T Mutations Light Cycler Real Time PCR Screening

Blood samples were collected with Ethylenediaminetetraacetic acid (EDTA) containing tubes and deoxyribose nucleic acid (DNA) was extracted from peripheral blood leukocytes according to MagNA Pure DNA isolation system (Roche Diagnostics, Manheim, Germany). Genotyping of FV 1691 G-A, PT 20210 G-A, and MTHFR 677C-T polymorphisms was performed by real time PCR (polymerase chain reaction) using fluorescence melting curve detection analysis by means of the Light Cycler (LC) System (Roche Diagnostics, Manheim, Germany). For FV; forward primer: 5′-TGCCCAG TGCTTAACAAGACCA-3′; reverse

Table 1 Genotype distributions and frequencies in elderly and young subjects.

Polymorphisms	0–18 Age controls n = 332 (%)	70 and above controls n = 266 (%)	0–18 Age patients n = 362 (%)	70 and above patients n = 209 (%)
FV 1691 G-A				
G/G	294 (88.5)	237 (89)	304 (84)	173 (83)
G/A	35 (10.5)	29 (11)	53 (15)	33 (16)
A/A	3 (0.9)	_	5 (1)	3 (1)
PT 20210 G-A				
G/G	314 (94.5)	254 (95)	348 (96)	197 (94)
G/A	18 (5)	11 (4)	14 (4)	12 (6)
A/A	_	1 (0.3)	_	_
MTHFR677 C/T				
C/C	174 (52)	138 (52)	173 (48)	91 (43,5)
C/T	126 (38)	108 (41)	157 (43)	106 (51)
T/T	32 (10)	20 (7.5)	32 (9)	12 (6)

Table 2 Combined risk assessment of two gene polymorphisms (FV 1691 G-A and MTHFR 677 C-T) between 70 and above age groups in patients and controls.

FV 1691 G-A	MTHFR 677 C-T	70 and above controls $n = 266$ (%)	70 and above patients $n = 209$ (%)	OR	CI (%95)	*P*
G/G	C/C	137 (51.5)	88 (42)	1		
G/G	C/T	92 (34.5)	73 (35)	1.2	0.8–1.8	0.3
G/G	T/T	18 (6.8)	10 (4.8)	0.8	0.3–1.9	0.7
G/A	C/C	11 (4)	14 (6.7)	1.9	0.8–4.5	0.09
G/A	C/T	15 (5.6)	18 (8.6)	1.8	0.8–3.8	0.08
G/A	T/T	2 (0.7)	1 (0.4)	0.7	0.06–8.7	0.6
A/A	C/C	–	2 (0.95)	6.2	0.2–139	0.01
A/A	C/T	–	1 (0.4)	3.1	0.1–93.7	0.5
A/A	T/T	–	–	–	–	–

Table 3 Combined risk assessment of two gene polymorphisms (FV 1691 G-A and MTHFR 677 C-T) between 0 and 18 age, groups in patients and controls.

FV 1691 G-A	MTHFR 677 C-T	0–18 Age controls $n = 332$ (%)	0–18 Age patients $n = 362$ (%)	OR	CI (%95)	*P*
G/G	C/C	148 (44.5)	143 (39.5)	1		
G/G	C/T	113 (34)	132 (36)	1.2	0.8–1.6	0.2
G/G	T/T	28 (8)	21 (5.8)	0.7	0.4–1.4	0.5
G/A	C/C	21 (6)	25 (7)	1.2	0,6–2.2	0.5
G/A	C/T	11 (3)	20 (5.5)	1.8	0.8–4.0	0.09
G/A	T/T	3 (0.9)	5 (1.3)	1.7	0.4–7,3	0.7
A/A	C/C	2 (0.6)	4 (1.1)	2.0	0.3–11,4	0.6
A/A	C/T	–	1 (0.2)	2.0	0.06–62	0.2
A/A	T/T	1 (0.3)	–	0.5	0.01–15	0.2

Table 4 Combined risk assessment of two gene polymorphisms (FV 1691 G-A and PT 20210 G-A) between 70 and above age groups in patients and controls.

FV 1691 G-A	PT 20210 G-A	70 and above controls $n = 266$ (%)	70 and above patients $n = 209$ (%)	OR	CI (%95)	*P*
G/G	G/A	9	8	1		
G/A	G/A	2	2	1.1	0.12–9.9	0.6
G/A	A/A	1	–	0.5	0.16–19.1	0.2
A/A	G/A	–	2	4.5	0.17–115	0.7

primer: 5′-CTTGAAGGAAATG CCCCATTA-3′; anchor hybridization probe: 5′-LC-Red705-TGTCCTTGAAGTAA CCTTTCAGAAATTCT G-3′-PHO; Mutation probe: 5′-GG CGAGGAATACAGGTA T-3′-floresan (Roche Diagnostics, Mahheim, Germany) For Prothrombin; forward primer: 5′-CCGCTGGTATCAAATG GGG-3′; reverse primer: 5′-CCA GTAGTATTACTGGCTCT TCCTG-3′: anchor hybridization probe: 5′-LC-Red640-TCCCAGTGCTATTCATGGGC-3′PHO; Mutation probe: 5′-CTC AGCGAGCCTCAATG-3′ floresan (Roche Diagnostics, Manheim, Germany). For MTHFR; forward primer: 5′-TGG CAG GTT ACC CCA AAG G-3′; reverse primer: 5′-TGA TGC CCA TGT CGG TGC-3′; anchor hybridization probe: 5′-LC–640-CGG GAG CCG ATT TCA TCA T-3′-PHO; Mutation probe: 5′-TGA GGC TGA CCT GAA GCA CTT GAA GGA GAA GGT GTC T-3′-Flu.) were used (TIB MOLBION, Berlin, Germany). Melting point analysis was performed according to the instruction manual [13,14].

3. Results

The genotype frequencies of the FV 1691 G-A, PT 20210 G-A and MTHFR 677 C-T polymorphisms in old and young subjects are shown in Table 1. There were no significant differences in the allele frequency for PT 20210 G-A and MTHFR 677 C-T polymorphisms between the 0–18 age and 70 and above groups. Possible effect of the FV 1691 G-A with other polymorphisms (PT 20210 G-A, MTHFR 677 C-T) are given in Tables 2–5. The frequencies of these mutation alleles in the patient and control groups were similar.

Heterozygosities of FV 1691 G-A and MTHFR 677 C-T substitutions' state brought a risk of thrombosis in the patients and controls aged 70 and above and 0 to 18 respectively [(Odd Ratio (OR): 1.8/0.8–3.8 CI%95), (OR:1.8/0.8–4.0 CI%95)] (Tables 2 and 3). Allelic distributions of FV 1691 G-A, PT 20210 G-A and MTHFR 677 C-T are given in Table 6 respectively.

Table 5 Combined risk assessment of two gene polymorphisms (FV 1691 G-A and PT 20210 G-A) between 0 and 18 age groups in patients and controls.

FV 1691 G-A	PT 20210 G-A	0 to 18 age controls $n = 332$ (%)	0 to 18 age patients $n = 362$ (%)	OR	CI (%95)	*P*
G/G	G/A	16	12	1		
G/A	G/A	1	1	1.3	0.07–23.5	0.5

Table 6 The allele frequencies of all study genes in all the groups.

Polymorphisms	0–18 Age controls $n = 332$ (%)	70 and above controls $n = 266$ (%)	0–18 Age patients $n = 362$ (%)	70 and above patients $n = 209$ (%)
FV 1691 G-A				
G	93	95	91	91
A	0.06	0.05	0.08	0.09
PT 20210 G-A				
G	97	97	98	97
A	0.02	0.02	0.01	0.02
MTHFR677 C/T				
C	71	72	69	68
T	28	27	30	31

4. Discussion

Different genetic markers related with CVD and longevity, have been investigated between young and elderly subjects in various studies. In the present study, no significant differences were observed in genotype frequency between younger and elderly subjects for polymorphisms in selected haemostasis and cardiovascular risk indicators reported to be associated with the CVD. In accordance with our results other studies have reported a lack of relationship between single genetic polymorphisms and longevity.

The hypothesis of MTHFR involvement in longevity is based upon that the MTHFR TT genotype is associated with higher levels of homocysteine and premature death; its prevalence should decrease with advancing age [15]. This has been supported by a study of Masushita et al. who found a significant difference in frequency between younger and older groups. The TT genotype frequency decreased from 19% to 7% in healthy Japanese individuals aged <55 versus >80 years [16]. These findings were replicated by Todesco et al. [17]. In a study, the MTHFR 677C-T polymorphism was analysed in the elderly and young populations. Similar proportions of all populations were reported for this polymorphism [16].

The meta analysis by Brattsröm revealed no significant difference in genotype between young and elderly [2]. Bladbjerg et al. studied several genetic polymorphisms linked to vascular risk, in addition to MTHFR 677C-T and PT 20210 G-A in 187 unselected Danish centenarians and 201 healthy Danish blood donor (aged 20–64 years). The frequencies of the reported high-vascular risk alleles of all these polymorphisms were comparable in centenarians to the blood donors, suggesting a lack of association between these vascular risk factors and longevity in Danish population [4–19]. On the other hand Sabino De et al. reported that the present FV1691 G-A polymorphism posed a significant risk of thrombotic diseases, but PT20210 G-A and MTHFR 677 C-T polymorphisms were not significant risk factors for the same disease in Brazil young population [19]. The potential role in several investigators showed an increased frequency of MTHFR 677 C-T mutation in patients in a wide range of vascular diseases. In our study, comparison of healthy populations, young patients and elderly populations revealed no statistically significant difference in the 677 C-T genotype distribution. Our data suggested that MTHFR 677 C-T (3.1–2.0) and PT 20210 G-A (OR 4.5–1.3) may have little effect on CVD and longevity.

The Turkish population is a model for researching the effects of genes since the establishment of the republic, there had been neither a war causing the deaths nor a big natural disaster, famine and also her economic development was stable. Thus, the comparison of genotype frequencies in young and old individuals may be a useful method/tool to study the effects of genetic polymorphisms on CVD causing premature deaths.

We conclude that polymorphisms of haemostasis and blood pressure regulation genes do not predict longevity because the allele distributions were similar in Turkish elderly and younger individuals. MTHFR 677 C-T and PT 20210 G-A genotype frequencies in the old and young study groups were similar for all polymorphisms, but MTHFR 677T may have synergy with FVL imparting a risk of thromboembolism.

Competing interests

The author(s) declare that they have no competing interests.

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