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rs2253310 and rs4946936 common variants of *FOXO3* gene in octogenarians and cancer: a pilot study in north India

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Abstract

Background: Healthy aging perceives human longevity probably due to carrying the defensive genes. Forkhead box O (FOXO) transcription factors provide the most convincing example of a conserved genetic pathway at the point between aging and cancer. This pilot study was performed to examine the single nucleotide variants rs2253310 and rs4946936 of the Forkhead box O 3 (*FOXO3* gene) in octogenarians and gastrointestinal tract (GIT) cancer patients in the north Indian population.

Main body: In silico mutational analysis of the *FOXO3* gene in 25 participants. Two single nucleotide variants (SNVs) g.7556C>G (rs2253310) heterozygous and g.122284T>C (rs4946936) homozygous observed and reported previously. However, there is a common association of these SNVs in different ethnic groups. No significant differences in the genotype and allele frequencies for the study groups observed.

Short conclusion: This study observes two single nucleotide variants, g.7556C>G (rs2253310) and g.122284T>C (rs4946936), of the *FOXO3* gene in the study groups which influence human longevity. Longevity-associated *FOXO3* variants may be associated with GIT cancer in the north Indian population. As a result, looking for genes linked to longevity will lead to discovering new cancer targets. Further studies with a large population are necessary to elucidate the role of the *FOXO3* gene in octogenarians.

Keywords: *FOXO3*, Human longevity, Octogenarians, Gastrointestinal tract cancer, Single nucleotide variants

Background

Aging is characterized through the general decline in body function and the increased receptiveness to age-related pathologies. The Forkhead box O (FOXO) transcription factor family is a key player in an evolutionarily conserved pathway. It promotes longevity downstream of insulin and insulin-like growth factor receptors (IGFRs) in a variety of organisms. The accumulation of molecular damages, including DNA and mitochondria both within and outside the cell, is thought to cause aging. Overactivation of these pathways is the basis of cellular senescence, age-related disease, including cancer [1]. Aging and cancer are intimately linked. The frequency of most cancers increases with age following an accumulation of mutations. Errors in DNA replication cause mutations exacerbated by intracellular reactive oxygen species (ROS) that rise during cellular stress [2, 3]. The correlation between aging and cancer raises the opportunity that genes that extend lifespan may also be part of a molecular system. An example of such genes is FOXO transcription factors, which play a crucial role in the line between longevity and cancer [4].

In humans, *FOXO3* inactivation correlates with a poor prognosis of breast cancers [5]. Thus, *FOXO3* may be an imperative part of a regulatory network that controls aging and cancer [6]. As the worldwide human population ages, the ability to avoid and treat cancer becomes essential to allow people to live longer, healthier life [7].

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In light of the above-mentioned facts, the *FOXO3* gene is selected to screen SNVs in octogenarians and gastrointestinal cancer patients in the north Indian population.

Main text

Materials and methods

A total of 25 participants were included in this pilot study. The study groups were divided into groups: Group 1: octogenarians, i.e., ≥80 years healthy aged individuals (age range 80–102 years) *n*=16 without any major chronic illness. Group 2 is composed of gastrointestinal tract cancer *n*=9 (age range 45–75). All of the participants were recruited from the Department of General Medicine and Department of Surgical Oncology, Institute of Medical Sciences, Varanasi, Uttar Pradesh, India. Written consent was obtained from the participants to use their samples and clinical details for the study.

Polymeric chain reaction

Genomic DNA was extracted from 3-ml peripheral blood lymphocytes using a standard phenol–chloroform extraction method. DNA quantification was assessed by UV absorbance using a Nanodrop (BioSpec-nano SHIMADZU BIOTECH). DNA fragments of 321 bp and 224 bp containing intron 2 and 3′UTR (untranslated region) were amplified through PCR from 10 ng of genomic DNA with the primers. Primers used in this study were chosen from the previous work (Li et al. [8]). The first primer sequences used in this study were *FOXO3* 5′ GAGCTTGCTTTGGAGATGCA 3′ (forward primer) and *FOXO3* 5′CCCAGTCACTCACATAGTCCT 3′ (reverse primer). PCR conditions were set according to the following protocol for primer 1: initiation heat activation at 95 °C for 3 min, DNA denaturation at 95 °C for 30 s followed by annealing at 58 °C for 40 s, and extension at 72 °C for 45 s and final extension 5 min. The second primer sequences were *FOXO3* 5′ GGGTCCTGAGAACT TCTGAGT 3′ (forward primer) and *FOXO3* 5′ GACA TTCTGTAAGACATTCTGCCT 3′ (reverse primer). The PCR conditions for primer 2 were initiation heat activation at 95 °C for 5 min, DNA denaturation at 95 °C for 30 s followed by annealing at 60 °C for 40 s, and extension at 72 °C for 45 s and 5 min. The amplified DNA fragments were purified, and the Sanger sequencing analysis was performed by the ABI3730XL genetic analyzer (Applied Biosystem). Two single nucleotide variants (SNVs) were detected in intron 2 of *FOXO3* genomic sequence (*Homo sapiens* chromosome 6, GRCh38.p12 primary assembly NCBI (National Center for Biotechnology Information) Accession: NC_000006.12). Sequencing data were analyzed through in silico analysis tools as mentioned. The effect of single nucleotide polymorphism (SNP) was predicted by using in silico analysis using Mutation Taster and other prediction tools such as the database of single nucleotide polymorphism (dbSNP) (www.ncbi.nlm.nih.gov/SNP), 1000 Genomes (http://internationalgenome.org), and Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org) used to determine the frequency of sequence alteration in the population.

Statistical analysis

The data were analyzed with SPSS version 16.0. Hardy–Weinberg equilibrium (HWE) was evaluated using the χ^2 test to estimate the study quality. Genotype and allele frequencies were calculated for the described SNPs. The groups were compared using the χ^2 test to analyze the statistical significance of the difference in allelic distribution of various polymorphisms in the study groups. Statistical significance was considered when $P = 0.05$.

Results

Characterization of population

The mean age of octogenarians and GIT cancer patients was 86.12±8.7 years and 61.22±10.22 years respectively which shows a statistically significant difference. No statistically significant differences between the two groups were observed in terms of BMI, SBP, and DBP (Table 1).

In silico analysis of mutational effects

In the sequencing of the *FOXO3* gene, two single nucleotide variants (SNVs) were identified, and both were reported previously (Table 2), and their electropherograms are shown in Fig. 1. In in silico analysis of Mutation Taster, FOXO3, g.7556C>G (rs2253310) variant present in intronic region and g.122284T>C (rs4946936) present on (3′UTR) untranslated region were found in both octogenarians and GIT cancer patients.

Table 1 The characteristics of the studied groups

	Octogenarians (*n*=16)	Gastrointestinal tract cancer patients (*n*=09)	*P* value
Age (years)	86.12±8.7	61.22±10.2	<0.001
BMI (kg/m^2)	19.43±2.58	21.88±2.71	0.035
SBP (mmHg)	118.12±9.97	115.78±11.11	0.593
DBP (mmHg)	76.50±5.34	75.33±6.24	0.626

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure

Table 2 Genotype and allelic frequencies of the FOXO3 gene variants compared between octogenarians and gastrointestinal tract cancer patients

Reference sequence	Genomic position	Genotype	Octogenarians *N*=16	GIT cancer patients *N*=09	χ^2	OR (95% CI)	*P* value
rs2253310	g.7556C>G (108567390)	CC	10 (0.625)	6 (0.666)	12	0.93 (0.516–1.705)	0.213
		CG	6 (0.375)	3 (0.333)		1.12 (0.367–3.447)	
		GG	0	0			
		Allele frequency					
		C	26 (0.812)	15 (0.833)	6.5	0.975 (.748–1.27)	0.159
		G	6 (0.187)	3 (0.166)		1.12 (.319–3.96)	
rs4946936	g.122284T>C (9108682118)	TT	9 (0.562)	4 (0.444)	6.5	1.26 (0.542–2.957)	0.159
		TC	7 (0.437)	5 (0.556)		0.788 (0.352–1.764)	
		CC	0	0			
		Allele frequency					
		T	25 (0.781)	13 (0.722)	6	0.788 (.292–2.12)	0.199
		C	7 (0.218)	5 (0.278)		1.08 (.77–1.52)	

Results are given as an *N* (%). No statistically significant difference χ^2 (chi-square test) was found in the frequency of either variants between octogenarians and GIT cancer patients; OR (odds ratio) with 95% confidence interval

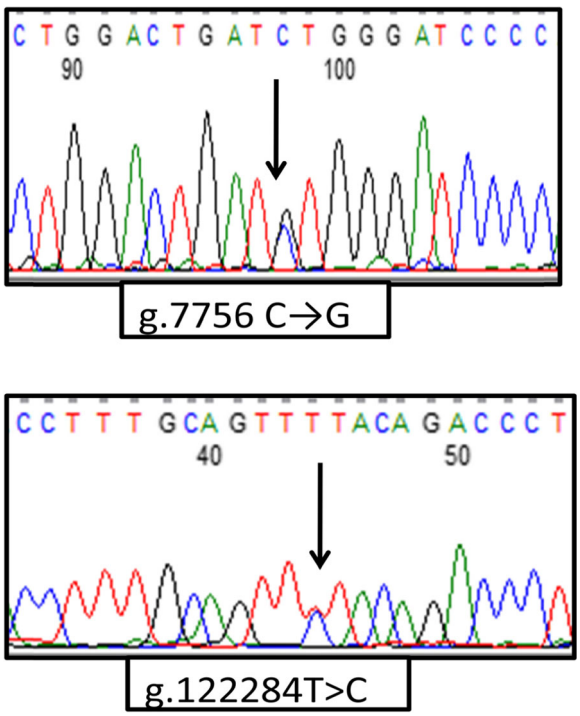

Fig. 1 Electropherograms of 2 variants observed during the screening of the FOXO3 gene in the study groups

Genotype and allelic frequency

The genotype and allele frequencies of the *FOXO3* gene variants in the octogenarians and the GIT cancer patients are shown in Table 2. Two single nucleotide variants g.7556C>G (rs2253310) heterozygous and g.122284T>C (rs4946936) homozygous genotype frequencies, in both the groups, were in agreement with the Hardy–Weinberg equilibrium (HWE) test. There were no significant differences in the genotype and allele frequencies for the *FOXO3* rs2253310 variants and rs4946936 between the octogenarians and GIT cancer patients.

This study demonstrated that the *FOXO3* gene might be associated with human longevity and cancer in the north Indian population. Interestingly, we found the *FOXO3* gene variants rs2253310 and rs4946936 were in both octogenarians and GIT cancer patients, responsible for human longevity and explore genetic contribution to the pathogenesis of cancer in the north Indian population.

FOXO3, g.7556C>G (rs2253310 C>G transversion) homozygous located at genomic position 108567390 of chromosome 6 (human reference genome GRCh 38), and the worldwide population frequency of the alternative alleles C and G is 0.49 and 0.47 (1000 Genomes), respectively. The SNV g.122284T>C (rs4946936 T>C) homozygous located at genomic position 109003321T>C of chromosome 6 (human reference genome GRCh 38), and the worldwide population frequency of the reference alleles

Table 3 Minor allele frequency of SNVs in different populations reported on ExAC browser

Population	rs2253310	rs4946936
African	0.2460	0.1641
East Asian	0.6946	0.7037
European (Finnish)	0.5664	0.6180
European (non-Finnish)	0.5922	0.6895
Latino	0.5674	0.6076
Others	0.5664	0.6275
Total minor allele frequency	0.4972	0.5327

T and alternative alleles C is 0.517 and 0.482, respectively (1000 Genomes). The frequency of the C-alleles rs2253310 and T-alleles rs4946936 varies among the different 1000 Genome population shown in Table 3. But these SNVs did not result in an amino acid change during translation and did not predict any specific effects for the SNVs. Thus, they are not considered for subsequent functional studies.

The following paragraphs briefly review data of rs2253310 and rs4946936 variants of the *FOXO3* gene from different populations in previous studies. Li et al. investigated *FOXO3* SNPs (rs2253310 and rs4946936) in southern China and northern China. They found that these SNPs were associated with longevity. In the Han Chinese population, rs2253310 and rs4946936 SNPs were found to be associated with longevity in both males and females [8]. Turkcu et al. indicate that rs4946936 of the FOXO3A gene may associate susceptibility of active vitiligo [9]. The *FOXO3* gene rs4946936 was correlated with early-onset psoriasis positively and enhanced keratinocyte proliferation in psoriasis pathogenesis [10]. Single nucleotide variant rs4946936 in the *FOXO3* gene was positively associated with an increased risk of childhood acute lymphoblastic leukemia (ALL) in a Chinese population [11].

Conclusions

FOXO3 belongs to the Forkhead box, class O subfamily of transcription factors characterized through an evolutionarily conserved DNA-binding domain. It regulates the expression of genes controlling a multitude of processes that could boost health and lifespan. The present study demonstrated that the FOXO3 gene might be associated with human longevity and susceptibility to GIT cancer in the north Indian population. Interestingly, we found the FOXO3 gene variants rs2253310 and rs4946936 found in both octogenarians and GIT cancer patients, and it may be responsible for human longevity in the north Indian population. Identification of longevity allied gene or loci is not only defining the underlying mechanism of human longevity, but also providing insights into the study of the pathogenesis of the age-related disease.

Abbreviations

FOXO: Forkhead box O; IGFR: Insulin-like growth factor receptor; SNVs: Single nucleotide variants; NCBI: National Center for Biotechnology Information; ExAC: Exome Aggregation Consortium; SNPs: Single nucleotide polymorphisms; GIT: Gastrointestinal tract; ALL: Acute lymphoblastic leukemia

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Authors' contributions

ISG and NT made the work design and sample collection. NT performed the experimental work and writing of the manuscripts. ML and NT analyzed and interpreted the data. All authors read and approved the final manuscripts.

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Availability of data and materials

All data are included in the manuscripts.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee Department of Medicine, Institute of Medical Sciences, Banaras Hindu University ECR/bhu/Inst/UP/2013/ Re-registration-2017 dt31.01.2017 (approval number Dean/2018/EC/336). Written informed consent for study participation was obtained from every patient before enrolment.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. Berman AE, Leontieva OV, Natarajan V, McCubrey JA, Demidenko ZN, Nikiforov MA (2012) Recent progress in genetics of aging, senescence and longevity: focusing on cancer-related genes. Oncotarget. 3(12):1522
2. Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC (2003) Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annual review of medicine. 54(1):131–152
3. Masoro EJ. (2005) Overview of caloric restriction and ageing. Mechanisms of ageing and development.126(9): 913-922.
4. Greer EL, Brunet A (2005) FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene. 24(50):7410–7425
5. Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY et al (2004) IκB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell. 117(2):225–237
6. Renault VM, Thekkat PU, Hoang KL, White JL, Brady CA, Broz DK et al (2011) The pro-longevity gene FoxO3 is a direct target of the p53 tumor suppressor. Oncogene. 30(29):3207–3221
7. Saunders LR, Verdin E (2007) Sirtuins: critical regulators at the crossroads between cancer and aging. Oncogene. 26(37):5489–5504
8. Li Y, Wang WJ, Cao H, Lu J, Wu C, Hu FY, et al. (2009) Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. Hum.Mo.l Genet. 18: 4897–904.
9. Turkcu UO, Tekin NS, Edgunlu TG, Karakas SÇ, Oner S (2013) The association of FOXO3A gene polymorphisms with serum FOXO3A levels and oxidative stress markers in vitiligo patients. Gene. 536:129–134

10. Pektas SD, Dogan G, Edgunlu TG, Karakas-Celik S, Ermis E, Tekin NS (2018) The role of forkhead box class O3A and SIRT1 gene variants in early-onset psoriasis. Indian journal of dermatology. 63(3):208
11. Wang Y, Zhou L, Chen J, Li J, He L, Wu P et al (2014) Association of the 3′ UTR FOXO3a polymorphism rs4946936 with an increased risk of childhood acute lymphoblastic leukemia in a Chinese population. Cellular Physiology and Biochemistry. 34(2):325–332

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