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].
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 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′ GAGCTTGCTTGTGATGCA 3′ (forward primer) and FOXO3 5′ CCCAGCTCCTCATATGCTCT 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′ GGGTCTGAGAATT TCTGAGT 3′ (forward primer) and FOXO3 5′ GACA TTCTGTAAGACATTCTGCTT 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 χ² test to estimate the study quality. Genotype and allele frequencies were calculated for the described SNPs. The groups were compared using the χ² 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 86.1±8.7 years and 61.2±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=9)** | **P value** |
| Age (years) | 86.1±8.7 | 61.2±10.2 | <0.001 |
| BMI (kg/m²) | 19.43±2.58 | 21.8±2.71 | 0.035 |
| SBP (mmHg) | 118.1±9.97 | 115.7±11.11 | 0.593 |
| DBP (mmHg) | 76.5±5.34 | 75.3±6.24 | 0.626 |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure
The genotype and allelic frequency 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.

### 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 | $\chi^2$ | 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 (0.748–1.27) | 0.159   |
|                    |                  | G        | 6 (0.187)            | 3 (0.166)                 | 1.12   | (0.319–3.96)      |         |
| 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 (0.292–2.12) | 0.199   |
|                    |                  | C        | 7 (0.218)            | 5 (0.278)                 | 1.08   | (0.77–1.52)       |         |

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

**Genotype and allelic frequency**

The genotype and allelic 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.

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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 109003321 T>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    |

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 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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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].
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