Does *Foxo3a* Gene Affect Life Span in Pakistani Population?

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ABSTRACT

Background and Objective: *Variations in FOXO3A gene have been associated with longevity and healthy aging. Present study was designed to determine the frequency of FOXO3ASNP rs2802288 and its association with longevity and co-morbidities in a sample of local population exceeding life expectancy.*

Methods: *The study was carried out on 91 samples collected from individuals of ages 70 years and above from the local population of Lahore. After DNA extraction and quantification, rs2802288 was assayed by Restriction Fragment Length Polymorphism. Results were analyzed using SPSS version 20.*

Results: *Study population comprised of 91 subjects. 45 (49.5%) being males and 46 (50.5%) females. Mean age of the sample group was 72.4 ± 3.8 years, mean height was 5.3 ± 0.37 feet and mean weight was 65.3 ± 13.8 kg. The major allele of rs2802288 was "G" with a frequency of (59.9%) and the minor allele was "A" and its minor allelic frequency (MAF) was 0.4. Prevalence of co-morbidities was higher in the sedentary group (85%) as compared to the athletic group (56%) (P=0.109). An insignificant association was found between genetic variations of rs2802288and longevity (P=0.98) or age related comorbidities (P= 0.379).*

Conclusion: *This pioneer study on local population shows minor allele of FOXO3 SNP rs2802288 as "A" with an MAF of 0.40. A weak association of genetic variations of rs2802288 and susceptibility to co-morbidities and longer life span occurs in our local population. Nevertheless, evidence from literature suggests that a link between FOXO3 gene and longevity may occur, which warrants further exploration.*

KEYWORDS: *FOXO3, Longevity, Allele frequency, Genetics, Aging*.

INTRODUCTION

To surpass expected life span is called longevity and all individuals surpassing this age are considered to be long lived. The expected lifespan of Pakistani population according to a survey carried out by World Bank is expectancy in Pakistan is 65.7 years for males and 67.4 years for females, with an average of 66.5 years.[1] Longevity is a multifactorial trait and various genes are thought to play an important role among which *FOXO* gene is of particular significance.[2] FOXO proteins are a member of the Fork head Family of transcription fac-tors which are common integration point for many important cellular processes.[3] Four mammalian FOXOs – FOXO1, FOXO3, FOXO4 and FOXO6 have been documented in mammals.[3] FOXO3, a key regulator in insulin/IGF-1 signaling pathway (IIS), is known to have a protective role against biological and environmental stress factors thereby resulting in longer lifespan.[4] FO-XO3 controls the expression of multiple genes regulating cell survival, autophagy, cell proliferation and metabolism which ultimately results in stress resistance, nutrient sensing and tumor suppression in various cells and tissues.[5] FOXO3 also plays an essential role in preventing various age-related diseases such as type 2 diabetes mellitus, cardiovascular diseases, neurodegenerative disorders and cancers.[3] Various single nucleotide polymorphisms (SNPs) of *FOXO3* gene have been observed to be associated with multiple age-related diseases and longevity.[6] Among these SNPs, the minor allele "A" of rs2802288 is particularly found to be associated with longevity and healthy aging in individuals.[7,8] The far reaching effects of FOXO3 warrant detailed study of this gene. No previous study on *FOXO3* and its polymorphism could be found in the local Pakistani population. The present study is a preliminary frequency study of *FOXO* and its SNP rs2802288 in our population.

METHODS

This descriptive cross-sectional study was conducted on general population of Lahore from January, 2017 to June, 2018. After informed consent, ninety-one healthy individuals of both genders, above the age of 70 and residents of Lahore were included through non-probability consecutive sampling. Individuals not native of Lahore were excluded. Prior to collection of samples, the study was approved by the Ethical Review

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Committee for Medical and Biomedical Research, University of Health Sciences, Lahore. Informed consent was taken in written from all the participants of the study. Comprehensive demographic information, such as age, height, weight, gender, caste and presence of any co-morbid conditions, was taken along with detailed relevant history. After aseptic measures, 5ml venous blood was drawn into an EDTA vial, from each participant. The samples were stored at -40°C prior to DNA extraction. Favor Prep™ Blood DNA extraction kit was used for DNA extraction. Extracted DNA was quantified using Nano Drop Microvolume Spectrophotometer. SNP rs2802288 was analyzed by Restriction Fragment Length Polymorphism. "Primer 3" software was used to design oligonucleotide primers for SNP detection.

Left Primer: 5'-GGATAATGTCCAGAGGATAGACTGA-3'.
Right Primer: 5'-GGGAAGGTAAGCAGGAGGC-3'.

PCR was performed at 59°C after temperature optimization. Restriction enzyme "PstI" was used for restriction digestion of PCR product on presence of A nucleotide. Restricted Bands were visualized under UV-transilluminator and genotype was determined, for example in Figure 1A samples 44, 46, 47, 48, 49, 51, 52, 53, 54, 58, 59, 61, 62, and 63 showed AG polymorphism while sample no. 55 and 57 showed GG poly-morphism.

STATISTICAL ANALYSIS

Data were analyzed through statistical package of version 20 of SPSS. Data were presented as mean± SD for quantitative variables and frequency and percentage for qualitative variables. Chi-square test was app-lied to measure associations taking 95% confidence interval and P value ≤ 0.05 as significant.

RESULTS

Study population comprised of 91 subjects, out of which, 45 (49.5%) were males and 46 (50.5%) females. Mean age of the sample group was 72.4 ± 3.8 years, mean height was 5.3 ± 0.37 feet and mean weight was 65.3 ± 13.8 kg (Table-1).

Caste wise break up of participants showed that Rajputs made up 19.8% of the total subjects, followed by Mughals (16.5%), Arains (13.2%) and Syeds (8.8%). Further uncommon castes were grouped together as other castes, which comprised of 26.4% of the total sample population (Fig.2).

Study of frequency of alleles showed that major allele (M) for SNP rs2802288 in our local population is "G", while the minor allele (m) is "A". Out of a total sample population of 91 individuals, 23 (25.3 %) were homozygous for the major allele of rs2802288 (MM), while 63 (69.2%) were heterozygous (Mm) and 5 (5.5%) were homozygous for minor allele (mm). The minor allele frequency (MAF) for rs2802288 was 0.4 (Table-2).

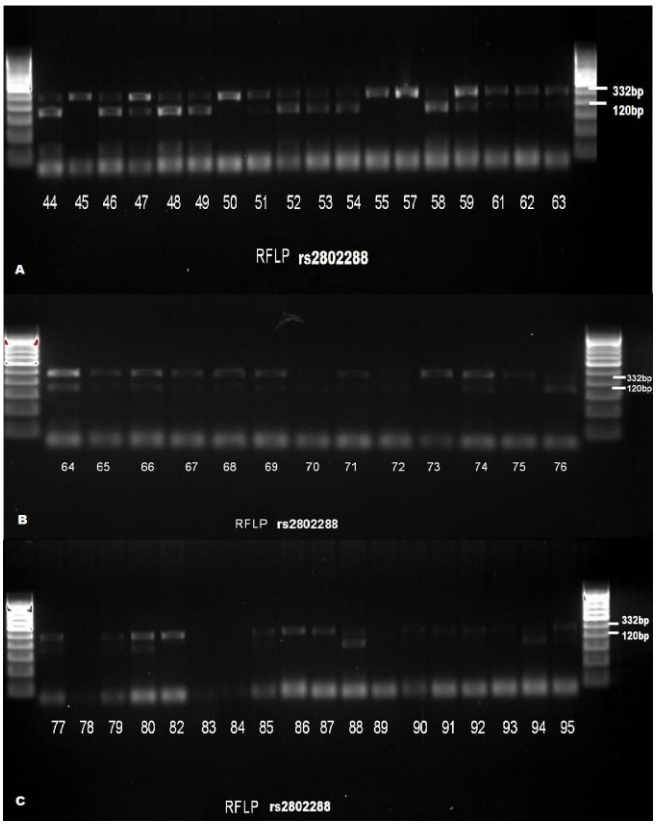

Fig. 1: *RFLP products of SNP rs2802288.*

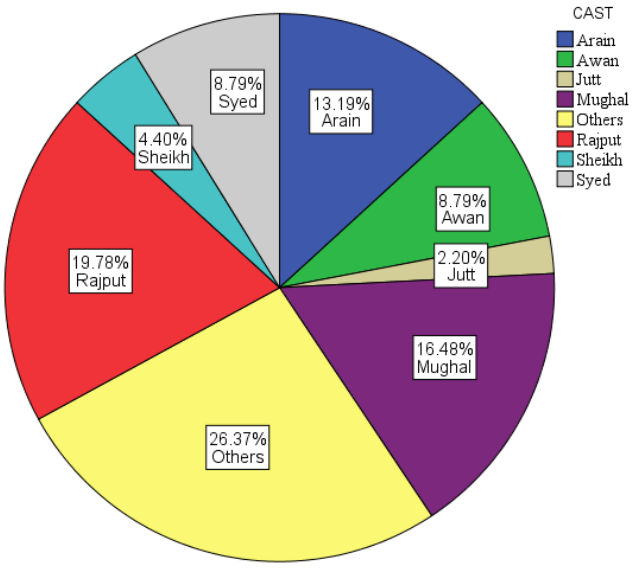

Fig. 2: *Caste-wise breakup of the study participants (N = 91).*

The effect of various alleles on age was studied as an effect modifier. The data were stratified for various age groups and showed that there was no significant

association between different age groups and the frequency of various alleles of SNP rs2802288 (*P*= 0.98) When alleles of rs2802288 were tested for a relation between prevalence of three common chronic diseases, there was no significant relation found (Table-3). Majority (67%) of the diabetics were found to be heterozygous for AG alleles, while 29.4% were found to be homozygous for G allele and 2.9% were homozygous for A allele. Among non-diabetics 70.2% were found to be heterozygous while 7% and 22.8% were homozygous for A and G alleles, respectively. As for hypertensives, 65% were found to be heterozygous while 8.3% and 26.7% were homozygous for allele A and G respectively. Majority (75%) of individuals with Ischemic Heart Disease (IHD) were heterozygous, while 20% were homozygous for G allele and 5% for A allele. The association of lifestyles with presence of co- morbidities was also studied. Most of the morbidities were also studied (Table-4).

Table-1: *Physical characteristics of study participants*

Parameter		*Frequency n (%)*	*Mean ± S.D*	*Minimum*	*Maximum*
Gender	Male	45 49.5)	-	-	-
	Female	46 (50.5)	-	-	-
Age (years)		-	72.4 ± 3.8	70	87
Height (feet)		-	5.3 ± 0.37	4.1	5.9
Weight (kg)		-	65.3 ± 13.8	40	105

Table-2: *Frequency of alleles and genotypes of the FOXO3 SNP rs2802288 and Minor Allele Frequency (MAF)*

Alleles/Genotypes		*Frequency (n)*	*Percentage (%)*	*MAF*
Alleles of rs2802288				
Major (M)	G	109	59.9%	0.4
Minor (m)	A	73	40.1%	
Genotypes of rs2802288				
Homozygous major (MM)	GG	23	25.3%	0.4
Heterozygous (Mm)	GA	63	69.2%	
Homozygous minor (mm)	AA	5	5.5%	

Table-3: *Prevalence of Diabetes, hypertension and IHD in different alleles of rs2892288*

Co-morbidity		*rs2802288*						*P-value*
		AG		*GG*		*AA*		
		N	*%*	*n*	*%*	*N*	*%*	
Diabetes	Yes	23	67.6%	10	29.4%	1	2.9%	0.597
	No	40	70.2%	13	22.8%	4	7.0%	
Hypertension	Yes	39	65.0%	16	26.7%	5	8.3%	0.205
	No	24	77.4%	7	22.6%	0	0%	
IHD	Yes	15	75.0%	4	20.0%	1	5.0%	0.812
	No	48	67.6%	19	26.8%	4	5.6%	

Table-4: *Association of various lifestyles with presence or absence of co-morbidity*

Lifestyle	*Co-morbidity*				*Total (n)*	*P-value*
	None		*Present*			
	n	*%*	*n*	*%*		
Sedentary	3	15	17	85	20	0.109
Average	12	21.8	43	78.2	55	
Athletic	7	43.7	9	56.3	16	
Total	22	24.2	69	75.8	91	

Most of the people with sedentary lifestyle (85%) suffered from a co-morbid condition. Out of 55 individuals with average lifestyle, 55 (78 %) suffered from a co-morbidity, while out of a total of 7 individuals having

athletic lifestyle, 9 (56%) had co-morbidities (P= 0.109).

DISCUSSION

The genetic makeup of any individual not only determines that individual's susceptibility to different diseases but also has great impact on the life expectancy and aging in that individual.[4,9] One such gene is *FOXO3*, which has been subject of vigorous research in recent years. In present study, for the first time, we deter-mined the frequency of a polymorphism of *FOXO3A* gene, rs2802288 in local population. The results of this study show that the minor allele frequency (MAF) for rs2802288 was 0.4. In a similar study carried out on the southern Chinese population of the Red River Basin, the minor allele for rs2802288 was also A with minor allele frequency (MAF) 0.38 which is very close to the value obtained by our study on Pakistani (Lahore) population.[10] Anselmi *et al.* studied rs2802288 in Southern Italians and reported A as the minor allele for this SNP with an MAF of 0.49.[11]This indicates that MAFs may vary among various ethnic groups. The ex-tent of these variations cannot solely be explained by demographic differences, instead, it brings to light the ethnic bias in genetic studies.[12,13]

Several studies have pointed towards the association of SNPs of *FOXO3* with longer lifespan. Present study showed weak association between allelic variations and age (P= 0.98). Study conducted on the southern Chinese population of the Red River Basin showed that the A allele for rs2802288 was associated with advanced age (P=0.005).[10] Donlon *et al.* identified 13 variants of FOXO3 forming a longevity haplo-type on chromosome 6.[14] This haplotype modifies the binding of transcription factors. It was found to be more frequent in Asian population, lesser in white and was almost non-existent in the African population. These variants delay aging by energy homeostasis, modifying glucose metabolism, protecting against environmental stress, regulating immune response, control cell cycle and apoptosis.[15] Various genetic factors are involved in pathogenesis of age related diseases like diabetes mellitus, hypertension and ischemic heart disease.[2,16,17] Minor alleles of *FOXO3* SNPs were shown to be protective against coronary artery disease related mortality in, white and black individuals of America and also in Japanese.[18] Our genetic study failed to identify any association between variations in *FOXO3* SNP rs2802288with age related co-morbid conditions. These differences in results may be attributed to small sample sizes, different selection criteria, different evaluation methods for evaluation of phenotypic variables and specially differences between individuals belonging to different ethnic groups. Current study reported a very high prevalence of co-morbidities in the sedentary group against a considerable low prevalence in the athletic group (*P*=0.109). Similar studies in other populations showed that physical activity reduces risk of ischemic heart disease,[19,20] hypertension,[21] diabetes[22] and other age-related diseases. Hamer *et al.* established the association of healthy ageing with physical activity in an eight-year follow-up study. The participants who remained active during the 8-year period of the study showed healthy ageing as opposed to those participants who remained inactive. Finally, it is evident that the genetic variations in *FOXO* gene SNPs can occur in different populations that may determine their role in ageing process of that population. Although, significant association of this SNP with longevity was observed in previous studies, the current study showed a very weak association. The factors responsible for these variations must further be explored to better understand the link between genetics and human process of aging.

CONCLUSION

It is concluded that the minor allele of FOXO3 SNP rs2802288 and its frequency (i.e. MAF) varies in different populations. This pioneer study on local population shows minor allele as "A" with an MAF of 0.40. Present study points towards a weak association bet-ween genetic variations in *FOXO3A* gene and susceptibility to co-morbidities and longer life span in our local population. Nevertheless, evidence from literature suggests that a significant association may exist with rs-2802288 or similar SNPs which warrants further exploration. This pioneer study on local population may serve a gateway for further studies to find a possible association between *FOXO3* gene and the process of aging and related co-morbidities in our local population that may help in better understanding of the complex mechanisms involved in human aging.

LIMITATIONS OF STUDY

The difference in findings of present study and above-mentioned studies may be attributed to the comparatively smaller sample size of present study. Also, the co-morbidities were assessed on history and medical record. These chronic diseases often go undiagnosed, therefore, in addition to history, a more objective method of assessment may provide more reliable results. Further studies with larger sample size and objective methods of assessment may be able to provide more statistically significant results.

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AUTHOR'S CONTRIBUTION

AF, KPL: Substantial contributions to conception and design, acquisition of data and drafting the article.
AK, MJS, AA, RS: Acquisition of data and analysis and interpretation of data.

CONFLICT OF INTEREST

None to declare.

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