Haptoglobin Genotypes And Longevity Among The Ghanaian Population

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Abstract: Haptoglobin (HP), an acute phase glycoprotein with antioxidant, anti-inflammatory and immune-modulatory functions may be an excellent candidate gene to investigate human longevity. The gene is polymorphic and the proteins expressed have different functional capacities due to their distinct biochemical and biophysical properties. The study aimed at determining the possible role of haptoglobin genotypes as genetic markers for longevity. One hundred and thirty three healthy elderly people above 50 years were recruited for the study. The average age of 37 (28%) males was 70.9 years and 96 (72%) females was 75.7 years. Blood samples were collected from participants for hematological analysis, HP genotyping and determination of oxidative stress. A positive correlation between superoxide dismutase activity and age ($p = 0.002$) was observed in this study population. A negative correlation was observed between age and total white blood cells ($p = 0.020$), age and neutrophil ($p = 0.028$), age and platelet counts ($p = 0.006$). HP1 and HP2 allelic frequencies were found to be 49.5% and 50.5%. Genotypic frequencies for HP2-2, HP2-1, and HP1-1 were 38%, 25% and 37% respectively, showing a departure from the Hardy-Weinberg equilibrium. The HP1 and HP2 genotypic polymorphisms did not appear to influence longevity in the Ghanaian population and none of the genotypes conferred a survival advantage.

Index Terms: Allele, Haptoglobin, Genotype, Ghanaian, Longevity, Polymorphism, Population

1 INTRODUCTION

Aging in humans is characterized by a decline of multiple physiological functions in various cells and organs leading to an increased propensity to death. It is a major risk factor in several human diseases such as cancer, diabetes, cardiovascular diseases and neurodegenerating conditions [1]. The decline of physiological functions with age is attributed to cellular changes occurring over time. A decrease in cell numbers leading to less effective homeostatic mechanisms and a decrease in the ability of cells to step up activity when challenged or stressed thus increases the vulnerability of the organism to its environment [2]. Longevity, defined as age at death is a complex phenotype and substantial evidence suggest that it is influenced by an interplay of both genetic and environmental factors [3]. A study of the genetic contribution to longevity in human done using twinregistries and population-based samples showed that twin registries range between 20% and 30% whereas those from population-based samples are slightly lower, ranging between 15% and 25% [4].

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There is limited information available in literature on candidate gene studies in associations in other populations [4],[5] necessitating the need for research into other candidate genes. Haptoglobin (HP), an acute phase protein produced mainly by the hepatocytes of the liver, is a non-enzymatic antioxidant which binds cell free hemoglobin (Hb) due to intravascular haemolysis preventing iron stimulated formation of reactive oxygen species or (ROS) free radicals [6]. Free radicals have been suggested as one of the causes of aging resulting from oxidative stress caused by ROS. The targets of these free radicals are biomolecules resulting in autoxidation of lipids, cross-linking of proteins and nucleic acids, and peptide fragmentation [7]. In humans, the HP locus is located on the long arm of chromosome 16 (16q22.1) and is polymorphic with two main alleles, HP1 and HP2, resulting in three distinct genotypes HP1-1, HP2-1 and HP2-2 [8]. The protein is made up of two types of polypeptide chains, a heavy β-chain and two light α-chains, (α1 and α2) [9]. The genetic polymorphism in humans arises from differences in the α-chains [10]. Two types of α1 chains have been identified based on their movement on polyacrylamide gel; α1F chain moves fast and α1S chain moves slow [11]. The HP1-1 phenotype consists of homodimers of two α-β units whiles the HP2-1 and HP2-2 consist of linear polymers and cyclic polymers respectively [12]. Hp1-1-Hb complexes are more rapidly cleared than the Hp2-2-Hb and Hp2-1-Hb complexes, resulting in significantly higher antioxidant function [13]. This is due to its shape and small size hence its sieving ability through the subendothelial space from serum to bind free Hb released at sites of vascular injury [14]. Hp1-1 also has higher hemoglobin binding affinity [15]. Haptoglobin has been documented to interact with other longevity genes such as APOA1 [16] and APOE [17]. Haptoglobin also has anti-inflammatory [18], angiogenic [19] and immune-modulatory [18] functions in extravascular tissues and body fluids making it an excellent candidate gene for longevity studies. Studies carried out in the central Italian population concerning HP genotypes and longevity showed that HP1-1 genotype is associated with increased probability of young subjects to attain increased lifespan. On the other hand, carriers of HP2-2 and HP2-1 alleles displayed an overall significant disadvantage in reaching old age [10]. This study therefore seeks to determine the possible association of HP genotypes

with longevity among the Ghanaian population.

2 MATERIALS AND METHODS

2.1 Population, Study design and Methods

This was a cross-sectional study involving one hundred and thirty three healthy individuals above 50 years among the aged who attended the Help-Age Ghana Centre at La Accra, Ghana. The centre serves as counselling, recreational and screening site for the aged. The study was approved by the Ethical Research and Protocol Review Committee of the University of Ghana Medical School. Written informed consent was obtained from all subjects prior to their inclusion in the study. Demographic data was obtained from participants by a questionnaire. Whole blood sample (5ml) was collected by venepuncture into anticoagulant EDTA tubes. Hematological parameters were measured immediately with an autoanalyzer. The blood sample was then centrifuged at 4000rpm for 10mins. Aliquots of the serum, buffy coat and the red cells were stored at 4°C for further analysis.

2.2 Superoxide dismutase (SOD) activity

The SOD activity levels were determined by a colorimetric method using SOD Assay kit (Cayman Chemicals, Michigan-USA). Two hundred and fifty microliters (250µl) of erythrocytes were lysed with deionized ice-cold distilled water (4oC) and centrifuged at 6,000rpm for 20 minutes. An aliquot (1 mL) of the erythrocyte lysate was collected and placed on ice. The erythrocyte lysate was diluted 1 in 100 with sample buffer, and 10µl of the diluted solution used to determine Copper/Zinc Superoxide dismutase activities as described by the manufacturer.

2.3 DNA Extraction

Genomic DNA was extracted from the buffy coat using QIAGEN DNA mini kit (QIAGEN Co., Germany) according to the manufacturers' protocol and stored at -20°C.

2.4 Genotyping

HP1S, HP1F, and HP2 alleles were determined by allele specific polymerase chain reaction (PCR) previously described by Yano et al [20]. HP1S allele was amplified by forward primers C51(5'-GCAATGATGTCACGGATATC-3') and reverse primer S2(5'-TTATCCACTGCTTCTCATTG-3'), HP2 allele was amplified by F3(5'-CAGGAGTATACACCTTAAATG-3') and C42(5'-TTACACTGGTAGCGAACCGA-3') primer pair, HP1F allele by F3(5'-CAGGAGTATACACCTTAAATG-3') and C72(5'-AATTTAAAATTGGCATTTCGCC-3') primer pair. Briefly DNA amplifications were performed in a 25µl reaction mix containing 5µl of genomic DNA, 0.125µl Taq polymerase (5U/µl), 1.0µl of each primers at 10µM, 0.5µl each of dNTPs at 10mM and 3µl of 10x PCR buffer. PCR amplification conditions were an initial denaturation at 94°C for 15 minutes, followed by thirty five cycles of denaturation at 94°C for 40sec, annealing at 52°C for 1min for C51-S2 and F3-C72 primers and at 58°C for 1min for F3-C42 primers, followed by final extension at 72°C for 2min. Ten microliters (10µl) of the PCR product was resolved in 2% agarose gel containing ethidium Bromide. PCR product sizes of 1,400bp, 1,200bp and 935bp were obtained for the HP1F, HP1S, and HP2 alleles respectively.

2.5 Statistical analysis

The data was analyzed with PASW Statistics Student Version 18. Differences between categorical variables were assessed using chi-square test for proportions. Comparison of measures of centrality was done by analysis of variance (ANOVA), unpaired t-test or Kruskal-Wallis test as appropriate. Pearson's correlation test was used to assess the correlation between continuous variables. A p-value of less than 0.05 was considered significant.

3 RESULTS

The study comprised 37 (28 %) and 96 (72 %) healthy aged males and females with mean ages of 70.9 years and 75.7 years respectively. The minimum age was 62 years for males and 53 years for females while the maximum ages were 88 years and 91 years respectively. The modal ages were 68 years and 75 years for the males and females respectively. The entire study population had a minimum age of 53 years and a maximum of 91 years. The median age was 73 years, the mean age was 71.8 years and the modal age was 75 years. The allelic frequencies for HP1 and HP2 were found to be 49.5 % and 50.5 % respectively with a 95% confidence interval (Table 1).The difference between the observed and expected haptoglobin genotypic frequency in the aged population was found to be statistically significant representing a departure from the Hardy-Weinberg equilibrium (Table 2). A positive significant linear relationship (p-value of 0.002) was recorded between age and SOD activity among the study population. Total white blood cells, neutrophil and platelet counts also showed a significant reciprocal relationship (r = -0.206, -0.194, and -0.241 respectively) with age. No relationship was observed between age and lymphocyte counts (*p*= 0.122)and Hb levels (*p*= 0.575).

Table 1: Haptoglobin allelic frequencies for the study population

Haptoglobin Allele	Population studied	N*	Allelic frequency % (95 % C.I)
HP1	Aged (53-90 years)	100	49.5 (39.7 – 59.3)
HP2			50.5 (40.7 – 60.3)

**Sample size*

Table 2: Observed and expected HP genotypic frequencies in the study Population

HP genotype	Observed number	Expectednumber	p-value (χ^2)
HP 1-1	37	24.5	
HP 2-2	38	25.5	< 0.000001
HP 2-1	25	50.5	

p-value < 0.05 is significant

Table 3: Correlations between Age and SOD Activity, WBC, LYM, NEUT, PLT and HGB among the study population

	AGE	
	PEARSON CORRELATION	*P-VALUE*
SOD ACTIVITY	0.345*	0.002
WBC	-0.206*	0.020
LYM	-0.137	0.122
NEUT	-0.194*	0.028
PLT	-0.241**	0.006
HGB	-0.500	0.575

**. Correlation is significant at the 0.05 level (2-tailed).*
***.Correlation is significant at the 0.01 level (2-tailed).*

Table 4: Comparison of SOD activity and Gender

	Gender	**N**	**Mean**	***p*-value**
SOD Activity	Male	38	224.49 ±139.1	0.525
	Female	95	207.62 ±133.3	

N is sample number

Comparison of SOD activity between genders showed males had a higher mean value than females (Table 4). The difference was however not statistically significant (p-value is 0.525). Analysis of the distribution of haptoglobin genotypes among the age categories of the study population showed no age category had a significant association with any of the haptoglobin genotypes (Table 5). Levels of SOD activity measured among the genotypes showed no significant association between the various haptoglobin genotypes and SOD activity (p-value of 0.488) be it low, normal or increased (Table 6).

Table 5: Distribution of haptoglobin genotypes among the age categories in the study population

Age groups (Years)	**HP Genotypes**			**Total**
	1-1	2-1	2-2	
50-59	3	1	4	8
60-69	15	9	10	34
70-79	14	11	18	33
80-89	5	3	6	14
90-99	0	1	0	1
Total	37	25	38	100

Pearson Chi-square (χ2) 5.527, p-value = 0.700

Table 6: Distribution of SOD activity among the haptoglobin Genotypes

Haptoglobin genotypes	**SOD Activity**			
	Low	**Normal**	**Increased**	**Total**
HP 1-1	1	14	22	37
HP 2-1	2	8	15	25
HP 2-2	0	14	24	38
Total	3	36	61	100

Assuming reference limits of 30.49 U/ml – 129.57 U/ml (Mean ± 2 S.D, mean =80.03, S.D = 24.77) being SOD activity measured in young people, SOD activity in the study subjects was categorized as low (<30.49 U/ml), normal (30.49 – 129.57) and high (> 129.57 U/ml). Pearson Chi-square (χ2) 3.485, p value is 0.488.

Table 7: Comparison of age and SOD activity amonghaptoglobin genotypes

HP genotypes	**Age (Mean± SD)**	***p*-value**	**SOD Activity Median (Range)**	***p*-value**
HP 1-1	70.43 ± 7.79	0.790	213.20 (23.8 – 428.33)	0.877
HP 2-1	71.68 ± 8.09		199.50 (20.78 – 485.10)	
HP 2-2	71.53 ± 8.54		210.55 (31.44 – 499.38)	

The distribution of SOD activity and age in terms of the mean and median values was found not to be significantly different among the haptoglobin genotypes (p-values of 0.877 and 0.790 respectively) [Table 7].

4 DISCUSSION

Results indicate that HP1/HP2 polymorphism does not affect the probability of an increased life span in the Ghanaian population. The 49.5% HP1 allelic frequency obtained is close to the 52% previously reported in the general Ghanaian population [21] which falls within the 95% CI of this study. Analysis of the distribution of HP genotypes among age categories showed no significant association with any age class (*p*-value of 0.700). This is inconsistent with findings in the Italian and Polish populations [10], [22]. In the Italian populations, the HP1-1 genotype and the HP1 allele were significantly associated with increased probability of young subjects to attain increased lifespan whiles carriers of HP2 allele displayed an overall significant disadvantage in reaching old age [10]. In the Polish study, a considerable increase of HP1-1 genotype was observed in subjects aged 81–91 years [22]. The conflicting results obtained from this study among Ghanaian population may be partly explained by the large difference between the size of populations sampled in these separate studies. Ahaptoglobinemia and/or hypohaptoglobinemia which may be present among the

Ghanaian population sampled, may also be responsible for the conflicting results obtained. Teye et al. [23] in 2003 reported a 13.8% prevalence of these phenotypes in the Ghanaian population. SOD activity measured was found not to be significantly associated with any genotype (*p*-value = 0.877). There was also no association between the distribution of the levels of SOD activity and genotype (*p*-value = 0.488). Comparing the mean ages of the population sampled and their genotypes showed no significantly association (p-value = 0.790) indicating no relationship between age and genotype. These results further indicate that the Hp genotype did not influence the level of free radicals and thus aging according to the free radical theory [7]. There was however a significantly high positive correlation between SOD activity and age (p-value of 0.002). This increase in enzyme activity seems to be a compensatory and adaptive effect to the increasing levels of ROS with aged. This agrees with other studies in the aged [24], [25] and consistent with the free radical theory of aging [7]. Studies on other anti-oxidative enzymes activities such as glutathione reductase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in erythrocytes were also reported to increase with age which may be due to positive feedback mechanism in response to rising lipid peroxidation [26]. Other studies however discovered decreased SOD activity with age [27], [28] attributed probably to high degree of oxidative damage to biomolecules with age due to increasing oxidative stress resulting in decreased synthesis of the enzyme, or mutations that lead to reduction in its activity. The increased or decreased SOD activity with age may thus reflect the strength of the individuals' anti-oxidative system and DNA repair system to oxidative damage. Comparison of SOD activity between genders showed women had a lower mean value (207.62U/ml) than men (224.49U/ml) suggesting higher ROS levels in men than women. This is consistent with studies that reported higher mitochondrial oxidative stress in men than women due to higher levels of oestrogen in women [29]. Correlation results between age and the hematological parameters also indicated significant negative relationship between total white blood cells, neutrophil and platelet counts consistent with studies that showed blood cells decreased with age [30], [31], [32].

5 Conclusion

These results suggest that the HP1 and HP2 genotypic polymorphisms did not influence longevity in the Ghanaian population and no genotype conferred a survival advantage. Further investigations in a larger population to determine protein expression and their level of expression may better elucidate its role in longevity in the Ghanaian population.

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