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Original Article

Comparative analysis of hemoglobin S and normal populations based on β-Globin Like Gene Cluster Haplotype Variation in Denizli, Turkey; Historical-Geographical perspectives and mutation age estimation

Onur Ozturk[1], Sanem Arikan[2], Ayfer Atalay[2], Erol Omer Atalay[2]

[1]Inonu University School of Medicine Department of Biophysics, Malatya, Turkey
[2]Pamukkale University School of Medicine Department of Biophysics, Denizli, Turkey

Abstract

Aim: Our study aimed to understand the genetic origin of Hb S based on comparative analysis with normal population haplotype data in the Denizli province of Turkey.

Material and Methods: We performed data obtained from previously published articles. We studied DNA samples from 12 unrelated patients with heterozygous abnormal hemoglobin S (Hb S) and 59 unrelated healthy subjects from published articles. The association of population genetic parameters such as haplotypes, diversity, differentiation, HWE and demographic analysis for two populations were performed by latest version of the Arlequin software (ver. 3.5).

Results: Our results show that normal and Hb S populations have different genetic parameters based on haplotype diversity through the history. The obtained results are highly associated with frequency haplotype [+ ---+ + +] (20.8%) in the Hb S population and the Mediterranean haplotype I [+ ----+ +] (14.4%) in the Normal population. According to historical population growth and mutation age parameter of τ values for normal and Hb S populations dated approximately 42,000 to 26,000 ybp, respectively.

Conclusions: Historically, two populations exhibit different genetic parameters and unimodal growth distribution. Our results are consistent with the Hb S mutation which occured in this region about 26,000 years ago.

Keywords: β-Globin Gene; Haplotype; Hb S; Population Genetics; Historical Analysis; Mutation Age Estimate.

INTRODUCTION

Hemoglobin S or "sickle cell Hemoglobin" (HbS) – rs334, NM_000518.4(HBB):c.20A>T (p.Glu7Val), GAG GTG (dbSNP: rs334; NG_000007.3:g.70614A>T), glutamic acid valine substitutions, at codon six of the beta-globin gene (HBB) gene encoding the β-globin component of haemoglobin. HbS result from substitutions at the second position of HBB codon six. HbS is distributed widely throughout sub-Saharan Africa, as well as parts of the Middle East and is maintained at about 10% frequency in many malaria endemic regions. The sickle allele (HbS) of HBB, which in the homozygous

state gives rise to sickle cell disease (SCD), occurs commonly in populations of African ancestry as a result of its protective effect against severe malaria (1,2,3). The first abnormal hemoglobin in Turkey was Hb S reported by Aksoy (4,5). In addition to the studies conducted in Turkey, many European researchers reported their findings in the immigrant Turkish population in their countries. HbS is the most common abnormal hemoglobin in Turkey. It is prevalent in Eti-Turks living in the Çukurova Region that is an Arabic speaking consanguineous population. In various surveys, the prevalence of HbS in this population was found to be between 3-47% (6). The overall frequency in Turkey is 0.3% and that in Thrace is 2.5%. It is also observed in Manavgat, Antalya. Haplotype analysis of HbS in Turkish patients indicated that the majority of the HbS mutations originated from Western Africa [Haplotype 19 (Benin Type)] (7,8,45) (Table 1).

This haplotype is associated with severe disease, which explains the severe clinical course of sickle cell anemia in Turkey. It is generally accepted that the first modern humans migrated from Africa via mid-Asia and probably

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Corresponding Author: Onur Ozturk, Department of Biophysics, Inonu University School of Medicine, Malatya, Turkey
E-mail: onurphysics@gmail.com

settled in mainland Southeast Asia during the last Ice Age; thereafter people migrated to other region (9). According to Oppenheimer (2012), 46,000–50,000 years ago Homo sapiens entered Europe. Most Europeans today can trace their ancestry to mtDNA lines that appeared between 50,000 and 13,000 years ago. Moreover, 20,000–30,000 years ago Central Asians moved west toward Europe (10).

Since Anatolia, which is modern Turkey, is located at the crossroads of many different migrating and interacting populations throughout history, different abnormal hemoglobins could have been introduced into her beta globin gene pool. The beta globin locus is the most intensively studied of all human loci, not least because of its association with the severe forms of inherited hemoglobin disorders like sickle cell anemia and beta thalassemia (11). Ozturk et. al. reported that the Hb S associated haplotypes with frequencies in Denizli, Turkey. Twelve heterozygous Hb S carriers were involved in the study. According to Ozturk et al, the Hb S mutation is associated with the Benin haplotype [- - - - + + +] originating from Western Africa, which is consistent with the previous reports (6,45). In our study, we performed comparative analysis of normal and hemoglobin S population data obtained from the previously published articles (12,13).

In this study as a new approach, we aimed to understand the possible genetic drift, relationships, expansion and historical origin based on haplotype frequencies of the β-globin gene cluster of normal and Hb S population in Denizli, Turkey. The comparison of all population genetic parameters was performed statistically with latest version of the Arlequin software (ver.3.5).

Table 1. β-Globin gene cluster haplotypes in four African populations linked with HbS (45)

	HincII	HindIII		Pvu II	HincII		Hinfl	HgiAI	Ava II	Hpa I	BamHI
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
Benin	-	-	-	+	-	+	-	+	+	-	+
Algeria	-	-	-	+	-	+	-	+	+	-	+
Central	-	+	-	+	-	-	-	+	+	+	+
African	-	-	-	+	-	+	-	+	+	-	+
Republic	+	-	-		-	-	-	+	+	+	+
	-	+	+		-	+	-	+	+	+	-
	-	+	-	+	+	+	+	+	+	+	+
	-	-	-	+	-	+	-	+	+	-	+
Senegal	+	-	-				-		+	+	+
	-	+	-		-	+	+	+	+	+	+

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MATERIAL and METHODS

Sample collection

In this study, statistical analysis was performed using published haplotype data (12,13). It has been reported previously published articles that during the identification of these haplotype data were used 12 unrelated patients with abnormal hemoglobin Hb S and 59 unrelated healthy subjects DNA samples.

The Determination of Haplotypes

In this study, haplotype analysis was performed by PCR-RFLP methodology for the following polymorphic sites in the beta-globin gene cluster: HincII 5′ to ε, HindIII 5′ to Gγ, HindIII in the IVS-II 5′ to Aγ, HincII in ψβ, HincII 3′ to ψβ, AvaII in β, HinfI 3′ to β as previously reported (12,13).

Statistical Analysis

β-globin gene cluster haplotypes has been commonly used to procure data concerned with human genetic diversity, genetic relationships and evolution. In this study, haplotype analysis (14,16), Hardy-Weinberg equilibrium tests (17,19), genetic diversity and population differentiation parameters such as frequency of each haplotype, haplotype diversity (h), nucleotide diversity (π), and theta (θ) values based on the number of polymorphic

sites (S) and the mean number of pairwise differences (k), respectively, F-statistic (F_{ST}, F_{IT}, F_{IS}) is the test statistic for the analysis of molecular variance (AMOVA) (20-24), Historical-demographic analysis parameters such as neutrality tests (Tajima's and Fu's tests) (25,26), mismatch distribution analysis, tau (τ) and theta initial (θ0) were estimated assuming theta final (θ1) as infinite, sum of square deviations (SSD) and the Harpending's raggedness index (Hri) and P values of SSD (27-33) were performed using Arlequin 3.05 software program as previously reported (12,13).

RESULTS

Tables 2 and 3 show that observed frequencies for seven unrelated heterozygous loci of the β-globin gene cluster haplotypes in Hb S and normal populations respectively. The obtained results are significantly associated with haplotype [+ ---+ + +] having frequency 20.8% in the Hb S population and this haplotype is not found in the normal population gene pool. In the normal population, the highly frequency association with haplotype is the Mediterranean haplotype I [+----+ +] (14.4%), similarly this haplotype is not present in the Hb S population. Additionally, the distributions of the haplotypes are also different in between Hb S and normal population. For example, haplotype [----+ + +] (16.6%) is observed in the second place in Hb S population and it is not found in the normal population haplotype diversity.

Table 2. β-globin gene cluster haplotypes for the seven loci in association with the Hb S population in Denizli, Turkey

No	Haplotype	Frequency	s.d.
1	+ - - - + + +	0.20833	0.08468
2	- - - - + + +	0.16667	0.07771
3	- - - - - + -	0.12500	0.06896
4	- - - - + + -	0.08333	0.05763
5	- - - - - + -	0.08333	0.05763
6	- - - - + - +	0.08333	0.05763
7	+ + - + + + +	0.08333	0.05763
8	- + - + + + +	0.08333	0.05763
9	- - - - - - +	0.04167	0.04167
10	- - + - + + +	0.04167	0.04167

Maximum-likelihood haplotype frequencies generated by Arlequin 3.5 software
Sum of 10 listed frequencies : 1.000000 / No. of gene copies in sample: 24 / s.d.: Standard deviation

We analysed the genetic differentiation of normal and Hb S populations using the AMOVA (24) performed by Arlequin ver. 3.05.

AMOVA results showed that 8.32% significant variance was observed between the two populations. We calculated the fixation index (F_{ST}) to measure the degree of genetic differentiation in between these populations. There were high (8.32%) significant differences in the normal and Hb S populations. F_{ST} values up to 0.05 indicate negligible genetic differentiation (F_{ST}: 0.08321 (CI% 95, 0.02279 - 0.11505), p=0.00782 ±0.00242) (Table 4). The FST p < 0.05 demonstrated that significant genetic differentiation was observed between the groups. According to Loveless and Hamrick (1984) (34), outcrossing populations typically demonstrate high levels of genetic diversity within populations and low genetic differentiation among populations. Moreover, when gene flow is high, the differentiation between populations decreases (18). In our results, F_{IT} is not significant (p > 0.05) and its value is close to zero (F_{IT}: 0.06633, p-value = 0.51906). Since our F_{IT} results (F_{IT}: 0.06633, Table 4) are close to zero, genetic drift did not occur in these populations tested. It should be considered that our results are under HWE. F_{IS} values were found statistically insignificant (P>0.05) and there was no departure from the Hardy–Weinberg equilibrium in any of these populations. Similarly, F_{IS} value is close to zero (F_{IS}: -0.01841 p-value = 0.66373). F_{IS} has the value of zero in the populations, indicating that random mating becomes predominant. Finally, the exact test of population differentiation showed differences between two populations (Table 4).

Table 3. β-globin gene cluster haplotypes for the seven loci in association with Normal population in Denizli, Turkey

No	Haplotype	Frequency	s.d.
1	+ - - - - + +	0.144068	0.032465
2	+ + - + + + +	0.127119	0.030796
3	- + - + + + +	0.084746	0.025748
4	+ - - - - + -	0.076271	0.024539
5	- - - - - - -	0.067797	0.023242
6	- - - - - - +	0.059322	0.021839
7	- - - - - + +	0.050847	0.020310
8	+ + + - + + +	0.050847	0.020310

Maximum-likelihood haplotype frequencies generated by Arlequin 3.5 software
Sum of 30 listed frequencies : 1.000000 / No. of gene copies in sample: 118 / s.d.: Standard deviation

We worked out two nucleotide diversity parameters for each population: π, the average heterozygosity per site (35,36) and θ, the population mutation parameter (37). Haplotypic diversity (h) has high level and similar for two population (Normal pop.: 0.93 ±0.01, Hb S pop.: 0.91 ±0.02; Table 5). Nucleotide diversity (π) has low and different values (Normal pop: 0.43 ±0.25, Hb S pop.: 0.28 ±0.18; Table 5).

Fu's Fs statistic showed a significant negative value for the two populations, indicating similar population expansion throughout history for these populations (Table 5). Tajima's D values showed that neutral equilibrium between populations (insignificant (p > 0.05) for two populations). On the other hand, Tajima's D estimate shows that heterozygotes have a selective advantage in these populations.

We analyzed the statistical demographic parameters for two populations. The distribution of pairwise differences is revealed to be statistically nonsignificant. The mismatch

distribution results for these populations proved to be distribution has shape of unimodal graphic (Fig. 1). This shape of distribution result was also corrected by the levels of Harpending's raggedness index and P values of SSD (Table 6) (27). The SSD and rg test statistics accepting the null hypothesis of population expansion (Table 6) and the shape of distributions look like to be unimodal (28,31).

In terms of the time estimations parameter values of τ and historical population parameters θ (θ_0 and θ_1) also show historical growth period for populations (Table 5). The mean population age for normal and Hb S populations in Denizli depend on results of estimations parameter values of τ, dated approximately 42,000 ybp (95% CI; 14,000-52,000) to 26,000 ybp (95% CI; 11,000-36,000), respectively (Table 5).

Table 4. (AMOVA) F-statistics calculated for seven loci differentiation among populations of between Normal and Hb S

		Distance method: Pairwise difference		
Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	1	6.587	0.12991 Va	8.32
Among individuals within populations	69	96.948	-0.02635 Vb	-1.69
Within population	71	103.500	1.45775 Vc	93.37
Total	197	286.056	1.48449	

Fixation Indices: Significance tests (1023 permutations)

F_{IS} : -0.01841 p-value = 0.66373 ± 0.01228
F_{ST} : 0.08321 p-value = 0.00782 ± 0.00242
F_{IT} : 0.06633 p-value = 0.51906 ± 0.01389

Exact Test of Sample Differentiation Based on Haplotype Frequencies
Global test of differentiation among populations, Insignificant P > 0.05, significant P ≤ 0.05
d.f.: degrees of freedom / F_{IS}: Inbreeding Subpopulation / F_{ST}: Subpopulation Total / F_{IT}: Inbreeding Total

Table 5. Summary of molecular diversity for two populations

Populations	n	No. of haplo	k (95%CI)	θS	h	π	Tajima's D		Fu's FS		Mismatch distribution		
							D	P	F_S	P	τ (95%CI)	θ_0	θ_1
Normal	59	30	12.65 (8.13-19.34)	1.30 ±0.56	0.93 ±0.01 0.91±0.02	0.43 ±0.25	3.00	0.99	-16.88	0.00	3.46 (4.15-1.16)	0.01	25.82
Hb S	12	10	5.91 (2.65-12.89)	1.87 ±0.90	0.91±0.02	0.28 ±0.18	0.21	0.59	-3.86	0.01	2.12 (2.93-0.94)	0.00	99999

Table 6. Values of the mismatch distribution test statistics SSD and rg against a null hypothesis of population expansion

	Goodness-of-fit tests			
Populations	SSD	SSD-P value	rg	rg P value
Normal	0.00352	0.330	0.02279	0.590
Hb S	0.00792	0.330	0.07538	0.280

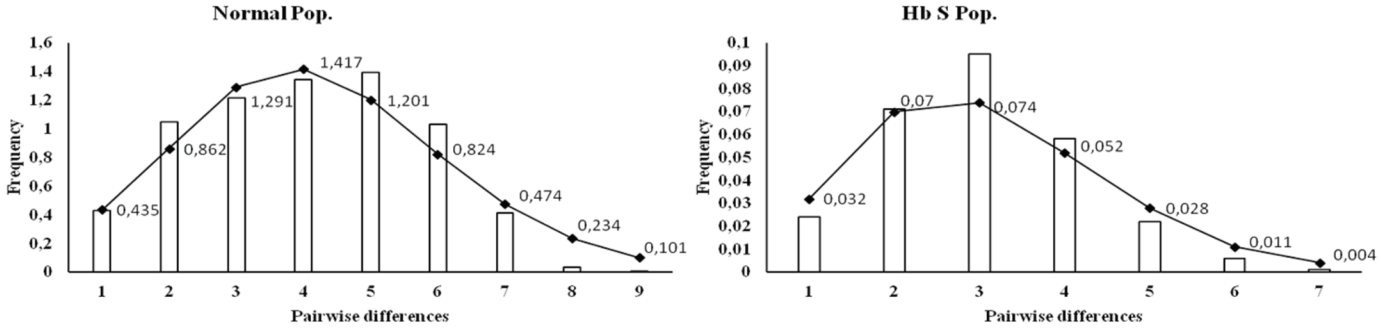

Figure 1. The observed pairwise difference (bars) and the expected mismatch distributions (solid line) under the sudden expansion model of Normal and Hb S populations.

Hardy–Weinberg Equilibrium (HWE) means that there will be no change in allelic or genotypic frequencies from one generation to the next. If the populations are under HWE (P > 0.05), no experiencing alterations were found in gene frequencies and genotypes as a result of genetic drift, selection, or deviation of random mating. According to our results, both populations (normal and Hb S) were in HWE (P > 0.05) for each of the seven polymorphic loci examined by Arlequin 3.5 software (Table 7).

Table 7. Hardy-Weinberg equilibrium (HWE) test for all Loci in Normal and Hb S populations

	Locus	#Genot	Obs.Het.	Exp.Het.	P-value	s.d.	Steps done
Normal Pop	1	59	0.44068	0.49718	0.43146	0.00049	1001000
	2	59	0.44068	0.50413	0.43292	0.00052	1001000
	3	59	0.23729	0.26076	0.60571	0.00048	1001000
	4	59	0.37288	0.44039	0.24596	0.00046	1001000
	5	59	0.57627	0.50297	0.29906	0.00045	1001000
	6	59	0.49153	0.40635	0.19071	0.00042	1001000
	7	59	0.45763	0.42083	0.54751	0.00050	1001000
	Locus	#Genot	Obs.Het.	Exp.Het.	P-value	s.d.	Steps done
Hb S Pop	1	12	0.58333	0.43116	0.48771	0.00050	1001000
	2	12	0.33333	0.28986	1.00000	0.00001	1001000
	3	12	0.08333	0.08333	1.00000	0.00001	1001000
	4	12	0.33333	0.28986	1.00000	0.00001	1001000
	5	12	0.50000	0.39130	0.52885	0.00050	1001000
	6	12	0.25000	0.22826	1.00000	0.00001	1001000
	7	12	0.33333	0.28986	1.00000	0.00001	1001000

Tests for HWE for each locus within each population used an HWE test analogous to Fisher's exact test. P values were obtained using arlequin 3.5 software.
#Genot: Genotypes / Obs.Het.: Observed heterozygosity / Exp.Het.: Expected heterozygosity / s.d.: Standard deviation

DISCUSSION

Our results support that the Hb S population in Denizli province later joined the local population gene pool. This participation may have occurred through Asiatic or African migrations. According to the published data, Homo sapiens neanderthalensis (HN) constitute a group of hominids whose particular morphology developed in Europe during the last 350,000 years under the effect of selection and genetic drift, reaching its final form approximately 130,000 ybp (38). This subgroup of hominids populated Europe and western Asia until the arrival of the first modern humans, Homo sapiens sapiens (HS), approximately 45,000 ybp (39,40). Available data on European mtDNA diversity indeed support this view, since most European populations do present a signal of Paleolithic demographic expansion from a small population, which could be dated to about 40,000 ybp (41). From 50,000 to 46,000 ybp Homo sapiens entered Europe. Most Europeans today can trace their ancestry to mtDNA lines that appeared between 50,000 and 13,000 ybp. Moreover, 20,000–30,000 years ago Central Asians moved west towards Europe (9,42). Our dating results are compatible with these dating results support the Hb S populations introduced into the Anatolian population by early migration from Asia or Africa. Since known Asiatic tribal migrations were recent events (about 2,000 ybp) we had to observe genetic drifts in our data but we do not observe genetic drifts during the time course of about 40,000 ybp up to the present time. On the other hand, we observed a unimodal distribution between normal 42,000 ybp (95% CI; 14,000-52,000) and Hb S 26,000 ybp (95% CI; 11,000-36,000) populations. These results indicate that the Hb S population was originated in the genetic pool of the normal population in Denizli, Turkey about 26,000 years ago. Most strikingly the variant of the beta-globin gene that causes sickle cell disease, HbS, protects against clinical malaria caused by infection with the most virulent species, Plasmodium falciparum (43). Microepidemiological studies on the distribution of alpha-thalassaemia support the hypothesis that this condition, like the βs-mutation, has been selected because it confers protection against malaria. Population-specific DNA polymorphisms at these and other loci promise to be of considerable contribution to genetic anthropology (44).

Consequently, our results indicate that the origin of the Hb S population may have been in the Mediterranean area approximately 26,000 ybp which was not in connection with the Silk Road migrations (about 2,000 ybp). On the other hand; probably the Hb S population is developed under the effect of the environmental factors in different genetically distinct geographical locations, genetic exchange in between with HN and HD or lethal infections

agents like Plasmodium Falciparum independently from migrations on the Silk Road. If Hb S is originating from Africa, Middle East or Asia location and distributed worldwide, we should see similar haplotypes with this region population. In addition, there is a difference between the HbS population haplotype distribution obtained using published data and the published haplotype distribution. A more detailed haplotype analysis in the latest version of the arlequin software used and a list of haplotypes with a low frequency can explain why this difference exists (44).

The evaluation of such data may generate valuable information to the anthropological, paleoclimatic, archaeological and phylogeographical approaches to human biology throughout the historical period of time.

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Declaration of interests

The authors declare that they have no competing interests.

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