SHORT COMMUNICATION

Network analysis of beta gene (HBB) haplotypes related to Hb S (β6 Glu → Val) in Denizli, Turkiye

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Abstract

Numerous haplotypes related to the haemoglobin S mutation have been diagnosed worldwide and in the Denizli region of Türkiye. These subgroup haplotypes include Benin, Arab-Indian, Senegal, Bantu, Algeria, Cameroon, Central African Republic, Saudi and various other varieties. The current study was planned to determine the formation periods and network linkage relationships of genetic variations linked to haemoglobin S in the Denizli area and other geographical regions. The haplotype data was processed using Network software to calculate possible links between haplotypes and their formation dates. The results uphold the possibility that the haemoglobin S mutation may have come to the area through migratory pathways. According to analysis, the Benin haplotype historically formed first, followed by the Senegal and Arab-Indian haplotypes. These findings indicate that the haemoglobin S mutation might have reached the Mediterranean area long before the Silk Route migrations ≈ 2,000 years ago.

Key Words: Haplotype, Hb S, β-globin gene cluster, Phylogenetic network analysis.

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Introduction

Haemoglobin S (HbS) or "sickle cell haemoglobin" (single nucleotide polymorphism database [dbSNP]: rs334; NG_000007.3: g.70614 A>T, glutamic acid [GAG] → valine [GUG]) is associated with the sixth codon of the beta-globin gene (HBB), which encodes the beta-globin proteins subunit of hb.

The products of the beta (β) globin locus (5'-ε-Gγ-Aγ-ψη-δ-β-3') constitute a group of genes with similar functions. Deoxyribonucleic acid (DNA) sequence similarities among genes in this cluster suggest a common ancestral origin. Polymorphic loci in the β-globin locus have thus garnered significant attention from researchers.[1] These genes offer valuable support for molecular evolution research due to their structural, functional and organisational characteristics. Haplotype analysis, a commonly used method in genetic origin studies related to the β-globin gene family, involves amplifying polymorphic loci using polymerase chain reaction (PCR), applying the restriction fragment length polymorphism (RFLP) method, and statistically evaluating the relationship between the obtained results and the allele linked to the mutation. Studying these polymorphisms within the β-globin genes helps trace the phylogeny of these genes, elucidates the role of balancing selection due to the protection that heterozygotes have against severe plasmodium (P.) falciparum, and provides insights into the genetic basis of related health conditions, such as sickle cell disease.

In a previous study conducted in Denizli, Türkiye, the Benin haplotype was found to have the highest frequency among haplotypes associated with HbS (Figure 1).[2] Subsequent research in the same region, utilising statistical software Arlequin, further analysed the Benin haplotype and divided it into sub-haplotype groups, resulting in a total of 10 different haplotypes identified in the Denizli region.[3] These haplotypes include Atypical, Benin, Arab-Indian and Senegal haplotypes (Table 1).[3-9] Among the locality population in Denizli, the predominantly Mediterranean haplotype I (+ − − − − − + +) has the highest frequency at 14.4% (Table 2).[3] The current study was planned to discuss the formation mechanisms of HbS-related haplotypes reported globally and in the Denizli region through a network tree analysis.

Methods and Results

The analytical, retrospective study was completed from January to March 2024 in the Denizli province of Türkiye. The network analysis was based on previously published data on HbS-related β-globin haplotypes (12 unrelated heterozygous individuals) and 59 unrelated healthy subjects from the DNA bank.[2] Historical and genetic linkages of HbS-related β-globin locus haplotype data was determined using Network version 10 (Table 3).[10] The 12 DNA samples were collected through premarital screening programmes. The analysis focussed on individuals carrying the HbS mutation; specifically, 12

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Table-1: Haplotypes of the beta gene associated with haemoglobin S (HbS) across different populations..

		Hinc II 5' to ε	Hind III 5' to Gγ	Hind III 5' to Aγ	Hinc II Ψβ	Hinc II 3' to ψβ	Ava II B	Hinf I 3' to β			
Benin *		−	−	−	−	+	+	−			
Benin ●		−	−	−	−	+	+	−			
Bantu $\frac{4}{5}$		−	+	−	−	−	+	−			
Algeria		−	−	−	−	+	+	−	*		
Cameroon		−	+	+	−	+	+	+			
Central		−	+	−	−	−	+	−	●		
African		−	−	−	−	+	+	−	*		
Republic		+	−	−	−	−	+	−			
		−	+	+	−	+	+	−			
▲		−	+	−	+	+	+	+			
Senegal		−	−	−	−	+	+	−	*		
		+	−	−	?	?	+	−			
		−	+	−	−	+	+	+			
Arab-Indian ■		+	+	−	+	+	+	+			
Saudi		?	+	−	+	+	?	−			
		Hinc II	**Hind III**	**Hind III**	**Hinc II**	**Hinc II**	**Ava II**	**Hinf I**		**Freq.**	**SD**
Denizli, Türkiye	HAP1	+	−	−	−	+	+	+		0.208	0.08
	HAP2	−	−	−	−	+	+	+		0.166	0.07
	HAP3	−	−	−	−	−	+	+		0.125	0.06
	HAP4	−	−	−	−	+	+	−	*	0.083	0.05
	HAP5	−	−	−	−	−	+	−		0.083	0.05
	HAP6	−	−	−	−	+	−	+		0.083	0.05
	HAP7	+	+	−	+	+	+	+	■	0.083	0.05
	HAP8	−	+	−	+	+	+	+	▲	0.083	0.05
	HAP9	−	−	−	−	−	−	+		0.041	0.04
	HAP10	−	−	+	−	+	+	+		0.041	0.04

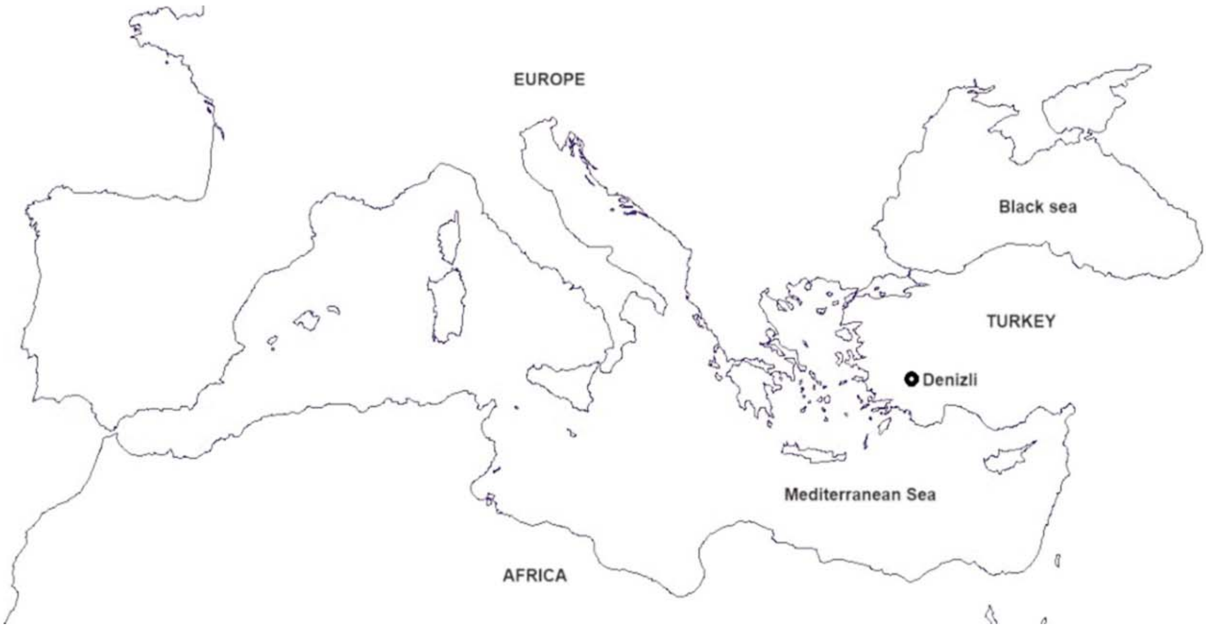

Figure-1: Locations of haemoglobin S (HbS) observed in the study.

Table-2: Haplotypes of the beta gene in Denizli, Türkiye, associated with haemoglobin A (HbA) in healthy population

No	Haplotype	Freq.	SD
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
9	−++−+++	0.033898	0.016730
10	+−−−−−−	0.033898	0.016730
11	−−−−−+−	0.033898	0.016730
12	+++++++	0.025424	0.014552
13	−++++++	0.016949	0.011934
14	++−−+++	0.016949	0.011934
15	−+−++−−	0.016949	0.011934
16	−+−−−−−	0.016949	0.011934
17	+−−−+++	0.016949	0.011934
18	++−+++−	0.016949	0.011934
19	+−−−−−+	0.016949	0.011934
20	−++−−−+	0.008475	0.008475
21	−+−−−−+	0.008475	0.008475
22	++−−−−+	0.008475	0.008475
23	+−−−++−	0.008475	0.008475
24	−+++++−	0.008475	0.008475
25	−+−−+−+	0.008475	0.008475
26	++−++−+	0.008475	0.008475
27	−+−−+−−	0.008475	0.008475
28	−+−++−+	0.008475	0.008475
29	−+−+++−	0.008475	0.008475
30	+++−+−+	0.008475	0.008475

unrelated heterozygotes were included. Haplotype determination for these samples was carried out using Arlequin 3.0 software, with the gametic phase being unknown. Written informed consent had been previously obtained from all the subjects or their guardians.[2] All the samples were sourced from Denizli, and each individual was identified as a heterozygous carrier. The haplotype data presented was derived through the use of PCR-RFLP methodology, which was applied to 7 polymorphic loci (HincII 5′ to ε, HindIII 5′ to Gγ, HindIII in IVS-II 5′ to Aγ, HincII in ψβ, HincII 3′ to ψβ, AvaII β, and HinfI 3′ to β).3 RFLP data analysis was conducted using Arlequin version 3.5 to determine the genetic variations (haplotypes) linked to HbS in the Denizli area.[11] Possible links were calculated between haplotypes and their formation dates using the obtained haplotype data (Table 3).

The rho (ρ) value in the Network software quantified the age of an ancestral node in terms of mutational units. This mutation age was then converted into calendar years by multiplying it by the mutation rate, which was 1 mutation per 20,180 years for human mitochondrial DNA in the segment spanning np 16,090-16,365. To determine the age of a specific "root" haplotype, the mutation rate was applied to all available descendant sequences, and the arithmetic mean of the distances to the root haplotype was calculated (Figure 2). This approach was referred to as "rho" estimation.[3]

The haplotypes associated with HbS and their frequencies in both the global context and the Denizli region were noted (Table 1). Using the Network software, the

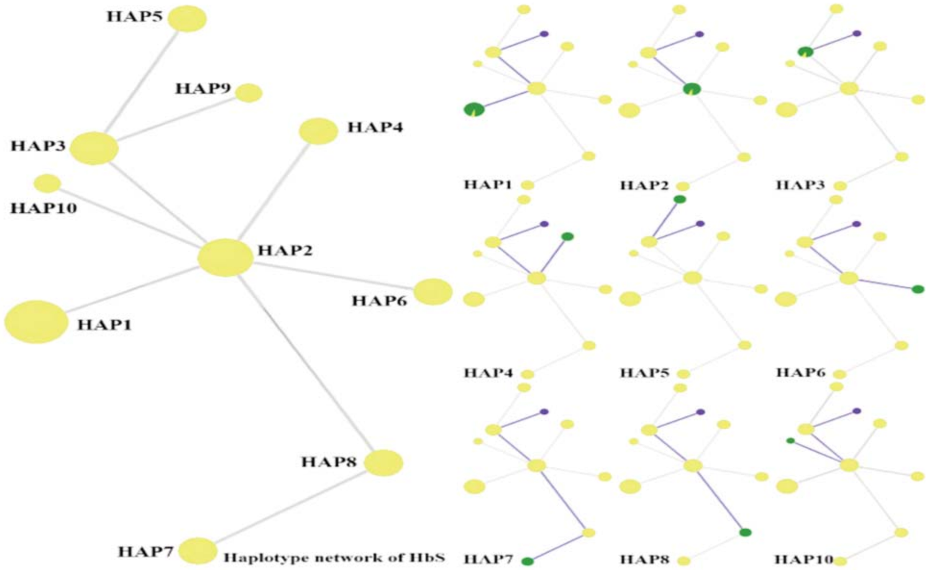

Figure-2: Phylogenetic network analysis on beta gene haplotypes linked to haemoglobin S (HbS). The diameters of the circles represent the prevalence of each beta gene haplotype within the population. Blue paths indicate the network connectivity of haplotypes, while green circles that are most likely to occur after the ancestral haplotype (HAP 9).

Table-3: Estimation of the age of beta gene haplotypes associated with haemoglobin S (HbS) in Denizli, Türkiye.

									Age estimation based on the beta gene ancestral haplotype			
		ε	G_{γ}	A_{γ}	ψη	3'ψη	β	3'β	Years	Years *SD*	Rho statistic	Sigma *SD*
Haplotype	HAP1	+	−	−	−	+	+	+	50,853	16,951	2.52	0.84
	HAP2	−	−	−	−	+	+	+	32,288	16,144	1.6	0.3
	HAP3	−	−	−	−	−	+	+	15,135	15,135	0.75	0.75
	HAP4	−	−	−	−	+	+	−	40,360	13,453	2	0.66
	HAP5	−	−	−	−	−	+	−	26,906	13,453	1.3	0.66
	HAP6	−	−	−	−	+	−	+	40,360	13,453	2	0.66
	HAP7	+	+	−	+	+	+	+	67,266	13,453	3.33	0.66
	HAP8	−	+	−	+	+	+	+	53,813	19,025	2.66	0.94
	HAP9	−	−	−	−	−	−	+	**Ancestral haplotype**			
	HAP10	−	−	+	−	+	+	+	30,270	10,090	1.5	0.5

Note: Years refer to the duration elapsed since the emergence of beta gene an ancestral haplotype.

estimated historical occurrence times of haplotypes were calculated based on the ancestral haplotype (Table 3). This calculation relied on the Haplotype 9 (HAP 9) (− − − − − − +), which was statistically the most similar to the ancestral haplotype (− − − − − − −).[3] The standard deviations (SD) presented in Table 3 are computed for both chronological years and sigma mutation units. In this calculation using Network software, rho estimation is independent of demographic parameters and instead depends on the relationships between haplotypes.

The phylogenetic network analyses of HbS-related haplotypes were conducted based on their historical occurrence times and intra-population frequencies, showing the phylogenetic analysis of each haplotype associated with HbS in the Denizli region.

Discussion

The network analysis of HbS-related β-globin gene family haplotypes provides insights into possible interactions, gene exchange, environmental influences, migration and the mechanism of mutation formation. The current study examined the worldwide haplotypes of the HbS mutation to construct a possible haplotype tree for HbS in Denizli, Türkiye. Previous data suggests that the HbA (healthy population) and HbS populations in Denizli dated back to around 42,000 to 26,000 years before present (Ybp), respectively.3 It is estimated that anatomically, modern humans, "Homo sapiens sapiens", settled down in Europe and western Asia approximately 45,000 years ago.[12] Data on European mitochondrial DNA diversity corroborate this, suggesting a Paleolithic demographic expansion from a limited population approximately 40,000 years ago.[12] The homo sapiens entered Europe between 50,000 and 46,000 years ago, and the Central Asians migrated west towards Europe 20,000 to 30,000 years ago.[13]

The estimated ages of haplotypes (Table 3) in the current study align with the homo sapien population movement data. For instance, the occurrence of HAP 4 (Benin) 8%, HAP 7 (Arab-Indian) 8%, and HAP 8 (Senegal) 8% in the Denizli region was calculated as 40,360, 67,266, and 53,813 years subsequent to the emergence of an ancestral haplotype, respectively. The age of formation of the HbA (healthy population) in Denizli as 42,000 Ybp suggests that HAP 4, HAP 7 and HAP 8 haplotypes were introduced to the regional population through migration. The atypical haplotypes ranking highly (Table 3) are subgroups of the Benin (HAP 4) haplotype based on phylogenetic network analysis (Figure 2).

Another supporting finding for HbS mutation coming to the region through migration pathways is that the Benin haplotype (HAP 4) is absent among normal population haplotypes (Table 2), while the Arab-Indian (HAP 7) and Senegal (HAP 8) haplotypes rank second and third, respectively. The formation of the Arab-Indian (HAP 7) and Senegal (HAP 8) haplotypes approximately 27,000 and 13,000 years after the Benin (HAP 4) haplotype (Figure 2) suggests that more efficient migration may have led to the development of HbS over normal population haplotypes. These results indicate that historically, the Benin haplotype formed first, followed by the Senegal and Arab-Indian haplotypes. This supports the notion that the HbS mutation arrived in the Mediterranean region long before migrations related to the Silk Route. Historically, the Silk Route migrations date back to approximately 2000 Ybp.

Studies utilising Network analysis based on genetic data can be valuable for anthropological, paleoclimatic, archaeological and phylogeographical research.

Conclusion

The age of formation of the HbA (healthy population) as 42,000 Ybp suggests that HAP 4, HAP 7 and HAP 8 haplotypes were introduced to the regional population through migration. Another supporting finding is that the Benin haplotype (HAP 4) is absent among normal population haplotypes, while the Arab-Indian (HAP 7) and Senegal (HAP 8) haplotypes rank second and third, respectively. The formation of the Arab-Indian (HAP 7) and Senegal (HAP 8) haplotypes approximately 27,000 and 13,000 years after the Benin (HAP 4) haplotype suggests that more efficient migration may have led to the development of HbS over normal population haplotypes.

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OO: Concept, design, data collection, analysis, interpretation of results and preparation.