Hemoglobin
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597254

^{S}-Globin Gene Cluster Haplotypes in the West Bank of Palestine

Fekri Samarah [a]; Suhail Ayesh [b]; Miranda Athanasiou [c]; John Christakis [d]; Norma Vavatsi [a]
[a] Department of Biochemistry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece [b] Molecular Genetics Laboratory, Makassed Islamic Charitable Hospital, Jerusalem, Israel [c] First Paediatric Clinic, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece [d] Department of Haematology, Theagenion Cancer Centre, Thessaloniki, Greece

Online Publication Date: 01 April 2009

To cite this Article Samarah, Fekri, Ayesh, Suhail, Athanasiou, Miranda, Christakis, John and Vavatsi, Norma(2009)'^{S}-Globin Gene Cluster Haplotypes in the West Bank of Palestine',Hemoglobin,33:2,143 — 149
To link to this Article: DOI: 10.1080/03630260902861873
URL: http://dx.doi.org/10.1080/03630260902861873

Hemoglobin, 33 (2):143–149, (2009)

ISSN: 0363-0269 print/1532-432X online
DOI: 10.1080/03630260902861873

informa
healthcare

SHORT COMMUNICATION

β^S-GLOBIN GENE CLUSTER HAPLOTYPES IN THE WEST BANK OF PALESTINE

Fekri Samarah,[1] Suhail Ayesh,[2] Miranda Athanasiou,[3] John Christakis,[4] and Norma Vavatsi[1]

[1]*Department of Biochemistry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[2]*Molecular Genetics Laboratory, Makassed Islamic Charitable Hospital, Jerusalem, Israel*
[3]*First Paediatric Clinic, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[4]*Department of Haematology, Theagenion Cancer Centre, Thessaloniki, Greece*

□ *Sickle cell disease is an inherited autosomal recessive disorder of the β-globin chain. In Palestine it is accompanied by a low level of Hb F (mean 5.14%) and a severe clinical presentation. In this study, 59 Palestinian patients, homozygotes for Hb S were studied for their haplotype background. Eight polymorphic sites in the β-globin gene cluster were examined. The Benin haplotype was predominant with a frequency of 88.1%, followed by a frequency of 5.1% for the Bantu haplotype. One chromosome was found to carry the Cameroon haplotype (0.85%). Three atypical haplotypes were also found (5.95%). Heterogeneity was observed in Hb F production, ranging between 1.5 and 17.0%, whereas the $^{G}\gamma$ ratio was homogeneous among all haplotypes with a normal amount of about 41%. Our results are in agreement with previous reports of the Benin haplotype origin in the Mediterranean.*

Keywords Sickle cell anemia, β-Globin gene, Haplotype, Palestine

Hemoglobinopathies represent a major portion of all inherited diseases worldwide. Inheritance of Hb S [β6(A3)Glu→Val, G*A*G>G*T*G]is the most important of all inherited hemoglobinopathies because of its wide distribution, chronic course, and resistance to therapy (1). Sickle cell anemia is caused by homozygosity for a mutation of adenine to thymine at position

Received 8 April 2008; Accepted 19 January 2009.
Address correspondence to Professor Norma Vavatsi, Department of Biochemistry, Faculty of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; Tel: +30-231-099-9001; Fax: +30-231-099-9004; E-mail: christak@med.auth.gr

two of the sixth codon of the β-globin gene. The disease is characterized by marked-to-modest anemia, recurrent vaso-occlusive episodes, and an increased susceptibility to infection (2). Its clinical heterogeneity is due to several factors such as Hb F levels, ratio of $^{G}\gamma$ chains to $^{A}\gamma$ chains, linked mutations, β^{S} haplotypes, coexistence of α-thalassemia (α-thal), and environmental conditions (3). Disease expression can also be affected by other parameters such as age, geographic location, time of year, and availability of modern comprehensive medical care (4). With the advent of recombinant DNA technology, the study of the Hb S mutation within the β-globin gene cluster has contributed scientific knowledge to population genetics. The sickle cell gene is linked to different DNA structures (polymorphic sites) located within the β-like gene cluster and nearby flanking sequences. These sites, when considered as a set, are called haplotypes (3). The β-like gene cluster haplotypes have been used for the detection of gene flow, in prenatal diagnosis and in human evolutionary studies (5). Early investigations of genetic variation within the β-globin gene cluster demonstrated that the Hb S mutation appeared on a few different haplotype backgrounds, suggesting a multiple origin of the Hb S mutation. Five distinct haplotypes, linked to the β^{S} mutation have been found and are known as the Benin, Bantu or Central African Republic (CAR), Senegal, Cameroon, and Arabian–Indian haplotypes (6). Apart from genetic interest, there is some evidence that specific haplotypes may affect the hematological and clinical expression of sickle cell (SS) disease. In fact, the regulatory elements inherited with these haplotypes affect Hb F expression (7). Patients who inherit a Senegal or Arabian–Indian haplotype, associated with a C>T mutation at position −158 in the 5′ area of the $^{G}\gamma$ gene, tend to have higher Hb F levels than individuals with the other haplotypes, who do not have this polymorphism (8).

This study was undertaken to determine what β-globin gene cluster haplotype(s) is(are) linked to the Hb S mutation in Palestinian patients with SS disease of the entire West Bank region of Palestine, and thus trace the origin of the Hb S mutation in this population. We studied 59 patients (30 males, 29 females) with sickle cell anemia who came from different Palestinian cities and were all followed at the National Centre for Blood Diseases 'HIPPOCRATES', Rammallah, Palestine. The study was approved by the ethics committee of HIPPOCRATES. A written informed consent was obtained from each patient, before entering into the study.

Hematological data were obtained by standard methods: complete blood counts and red blood cell (RBC) indices were measured on a Sysmex K × 21 counter (Kobe, Japan). We used electrophoresis on cellulose acetate gel at pH 8.6 to separate each hemoglobin (Hb) fraction and detect Hb variants. The presence of Hb S was confirmed by electrophoresis (acid pH) (9). The $^{G}\gamma$ polypeptide chains were determined by high-performance liquid chromatography (HPLC) (10). The presence of the sickle cell mutation was

confirmed by polymerase chain reaction (PCR), followed by digestion with the restriction enzyme *Dde*I (11). DNA was isolated from peripheral blood leukocytes using the QIA amp Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations and kept at –20°C until analyzed. Haplotype analysis was done as described earlier (12), using eight restriction sites: *Hind*II 5′ to the ε gene, *Xmn*I 5′ to the $^{G}\gamma$ gene, *Hind*III within IVS-II of the $^{G}\gamma$ and $^{A}\gamma$ genes, *Hind*II 3′ to $\psi\beta$ gene, *Rsa*I 5′ to the β gene, *Ava*II for IVS-II of the β gene, and *Hinf*I 3′ to the β gene. The amplified DNA was digested with the appropriate restriction enzyme (according to the recommendations of the supplier), and the digestion products were separated on a 2% agarose gel and visualized after ethidium bromide staining under ultraviolet light.

Moderate anemia (mean Hb 8.4 ± 1.09 g/dL) was observed in the results of the hematological data of SS patients. Mean corpuscular volume (MCV) and mean corpuscular Hb (MCH) values were within normal limits. Hb F levels ranged from 1.5 to 17.0%, with an average value of 5.14% in all patients. The β gene cluster haplotypes of 118 β^S chromosomes from 59 patients with SS disease were determined (Table 1). No Senegal or Arabian–Indian haplotypes were found in the population studied. The Benin haplotype (Ben) was largely predominant (88.1%), followed by the Bantu (5.1%) and Cameroon (Cam) (0.85%) haplotypes. Seven chromosomes had atypical haplotypes, four (3.4%) had [+−−−−+++], two (1.7%) had [+−−−−++ −], and one (0.85%) had [− −++ −+− +]. Forty-nine patients were homozygous for the Benin haplotype (Ben/Ben) (83.0%), followed by the Benin haplotype in combination with an atypical haplotype (8.5%). The haplotype backgrounds in the remaining patients were as follows: two (3.4%) Bantu/Bantu, one (1.7%) Ben/Bantu, one (1.7%) Bantu/atypical Bantu A2 [+−−−−+++], and one (1.7%) Cam/atypical Bantu A6a [− − −++ −+−+] haplotype.

Our findings are in agreement with similar studies in other countries of the Mediterranean region (13–19). As shown in Table 2, the Benin haplotype is predominant, ranging from 73 to 100% in these countries.

TABLE 1 Frequencies of β-Globin Gene Cluster Haplotypes in SS Patients from Palestine

Haplotypes	5′ε *Hind* II	5′$^{G}\gamma$ *Xmn* I	$^{G}\gamma$ *Hind* III	$^{A}\gamma$ *Hind* III	3′$\psi\beta$ *Hind* II	5′β *Rsa* I	5′β *Ava* I	3′β *Hinf* I	Number of chromosomes (*N*)	%
Benin	−	−	−	−	+	−	+	+	104	88.1
Bantu	−	−	+	−	−	+	+	+	6	5.1
Cameroon	−	−	+	+	+	−	+	−	1	0.85
Atypicals	+	−	−	−	−	+	+	+	4	3.4
	+	−	−	−	−	+	+	−	2	1.7
	−	−	+	+	−	+	−	+	1	0.85
Total									118	100%

TABLE 2 The Distribution of Chromosomes Bearing the Benin Haplotype in the Mediterranean Basin

Country	Number of chromosomes	Benin haplotype (%)	Other haplotypes (%)	References
Palestine	118	88.1	11.9	This study
Greece	82	96.4	36	(13)
Egypt	28	100.0	0.0	(14)
Jordan	20	80.0	20.0	(14)
Lebanon	100	73.0	27.0	(15)
Sicily	38	100.0	0.0	(16)
Tunisia	66	94.0	6.0	(17)
Algeria	20	100.0	0.0	(18)
Turkey	138	91.0	9.0	(19)

Haplotype analysis is a powerful tool for the study of the origin and evolution of structural gene mutations, such as that affecting the β-globin chain (20). The association of specific haplotypes with a geographically distinct population has provided information on the history and spread of the sickle cell mutation (21). There are five major β^S-globin gene cluster haplotypes in the world: four in Africa (Senegal, Benin, Bantu or CAR, and Cameroon) and one in Asia (Arabian–Indian). The Benin haplotype has been reported to be associated with β^S genes in Algeria, Tunisia, Egypt, Jordan, Lebanon, Sicily, Greece, and Turkey (Table 2) (13–19). Historic data from these countries indicate that the Benin-associated β^S gene has traveled from Central West Africa to North Africa and various Mediterranean countries. Except for a brief report on six chromosomes from the West Bank of Palestine (22), no comprehensive information about the haplotype background of the sickle cell mutation in Palestine is available. In this study, Benin was found to be the most frequent haplotype in patients with SS disease in the West Bank of Palestine. The homozygous form of the Benin haplotype accounts for 83% of all haplotypes, followed by the double heterozygous form of Benin/atypical as the second most frequent haplotype (8.5%). The very high frequency of the Benin haplotype in our study suggests that the β^S mutation present in Palestine may have originated from the Benin region and was brought to Palestine along the slave trade routes. The Arab slave trade brought slaves into the Middle East from Africa as house slaves. The Arab slave trade, based on Islamic law, followed the same tradition of slavery within Africa. The sons and daughters of slaves were emancipated and blended rapidly into the local society (23). These data are consistent with the views of Kulozik et al. (24) and Nagel (25), who suggested that people from West Africa carrying the Benin β^S haplotype had migrated to North Africa, the Mediterranean, and the Southwestern part of the Arabian Peninsula.

Seven chromosomes carried atypical haplotypes: four (3.4%) had [+−−−−+++], two (1.7%) had [+−−−−++ −], and one (0.85%) had the [− −++ −+− +]

haplotype pattern. The occurrence of the atypical haplotypes in heterozygous combination with typical African haplotypes could be explained by a recombination event or point mutation or gene conversion at the restriction fragment length polymorphism (26). The atypical haplotypes reported in our study were identical at the 3′ region, whereas the differences were in the 5′ region. The [+−−−−+++] atypical haplotype was reported previously by Srinivas et al. (27) as Bantu A2 based on the similarities of restriction sites on the 5′ region of the β gene. The [+−−−−++−] atypical haplotype differs from Bantu A2 in that it lacks the 3′β *Hinf*I restriction site, and thus reported here as Bantu A2c. The third atypical haplotype was similar to the Bantu A6 atypical haplotype, but as the *Ava*II site for this particular atypical haplotype was absent, it was designated as Bantu A6a (28). We can postulate that the atypical haplotypes encountered in our study could have resulted from a crossover close to the "hot spot" between the 5′ and 3′ portion from a typical haplotype in the 5′ region. The relatively high frequency of the Bantu A2 and Bantu A2c atypical haplotypes reported in our SS patients, and their presence also in β^A and β-thal chromosomes in the Palestinian population (unpublished data), suggest that they represent a common haplotype in our country. It is possible that they arose from a normal wild-type chromosome that serves as the site (background) of two independent mutations leading to two different chromosomes; one carrying the sickle gene and the other the β-thal mutations.

The heterogeneity of Hb F expression in each patient (ranging from 1.5 to 17.0%) could not be explained solely by age difference and presumably is under the control of other factors yet to be discovered. The high Hb F level (17%), which was in a patient homozygous for the Benin haplotype, is most probably due to his age (2 years). On the contrary, the $^G\gamma$-globin gene expression is remarkably homogeneous with a normal amount approaching (41%). It has been suggested previously that a C>T mutation at position −158 5′ to the $^G\gamma$ gene is associated with a high $^G\gamma$-globin chain ratio (8), as observed in Saudi Arabia (76.5%) (29) and Indian populations (71.78%) (30). In our SS patients, characterized by the common β^S Benin haplotype with low Hb F production and a normal $^G\gamma$-globin gene expression (41%), the *Xmn*I site is absent. These data are similar to those reported by Labie et al. (31) for the patients from Benin.

The coinheritance of α-thal has been proposed by several investigators as a modifier of sickle cell disease (32). We did not study the role of α-thal and its prevalence in our group of patients, because the background data from our hospitals and laboratories indicated that it was a very rare disease in Palestine.

In conclusion, the Benin haplotype is the most common haplotype in SS patients in Palestine. It is associated with fairly low Hb F and $^G\gamma$ levels. Its relation to the clinical expression of the disease remains to be evaluated.

ACKNOWLEDGMENTS

We thank Mrs. Evangelia Allawnah, Chairman of the National Centre for Blood Diseases "HIPPOCRATES," Rammallah, Palestine, for her help in sample and data collection. F. Samarah was supported by a grant from the State Scholarships Foundation (IKY), Greece.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. In: Higgs DR, Weatherall DJ, eds. *The Haemoglobinopathies.* Vol 6. London: W.B. Saunders Company. *Bailliere's Clin Haematol.* 1993:215–262.
2. Powars DR. β^S-Gene cluster haplotypes in sickle cell anemia. Clinical and hematologic features. *Hematol/Oncol Clin N Am.* 1991;5(3):475–493.
3. Serjeant GR. Geography and the clinical picture of sickle cell disease. An overview. *Ann NY Acad Sci.* 1989;565(1):109–119.
4. Powars DR. β^S-Gene-cluster haplotypes in sickle cell anemia. Clinical implications. *Am J Ped Hematol/Oncol.* 1990;12(3):367–374.
5. Sutton M, Bouhassira EE, Nagel RL. Polymerase chain reaction amplification applied to the determination of β-like globin gene cluster haplotypes. *Am J Hematol.* 1989;32(1):66–69.
6. Pangier J, Mears JG, Dunda-Belkhodja O, Beldjord C, Nagel RL, Labie D. Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa. *Proc Natl Acad Sci USA.* 1984;81(6):1771–1773.
7. Nagel RL, Labie D. DNA haplotypes and the β^S globin gene. *Prog Clin Biol Res.* 1989;316B:371–393.
8. Labie D, Dunda-Belkhodja O, Rouabhi F, Pagnier J, Ragusa A, Nagel R. The −158 site 5′ to the $^G\gamma$ gene and $^G\gamma$ expression. *Blood.* 1985;66(6):1463–1465.
9. Betki K, Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. *Nature.* 1959;184(Suppl. 24):1887–1888.
10. Huisman THJ. High-performance liquid chromatography as a method to identify haemoglobin abnormalities. *Acta Haematol.* 1987;78(2–3):123–126.
11. Kulozik AE, Lyons J, Kohne E, Bartram CR, Kleihauer E. Rapid and non radioactive prenatal diagnosis of β-thalassemia and sickle cell disease: Application of the polymerase chain reaction (PCR). *Br J Haematol.* 1988;70(1);455–458.
12. Murhekar KM, Murhekar MV, Mukherjee MB, Gorakshakar AC, Surve R, Wadia M. Red cell genetic abnormalities, β-globin gene haplotype and APOB polymorphism in the Great Andamanese, a primitive Negrito tribe of Andaman and Nicobar Islands, India. *Hum Biol.* 2001;73(5):739–744.
13. Boussiou M, Loukopoulos D, Christakis J, Fessas Ph. The origin of the sickle mutation in Greece: Evidence from β^S globin gene cluster polymorphism. *Hemoglobin.* 1991;15(6):459–467.
14. El-Hazmi MAF, Warsy AS, Bashir N, Beshlawi A, Hussain IR, Temtamy S, et al. Haplotypes of the β-globin gene as prognostic factors in sickle-cell disease. *East Med Health J.* 1999;5(6):1154–1158.
15. Inati A, Taher A, Bou Alawi W, Koussa S, Kaspar H, Shbaklo H, et al. β-Globin gene cluster haplotypes and Hb F are not the only modulators of sickle cell disease in Lebanon. *Eur J Haematol.* 2003;70(2):79–83.
16. Schilirò G, Samperi P, Consalvo C, Gangarossa S, Testa R, Miraglia V, et al. Clinical, hematological, and molecular features in Sicilians with sickle cell disease. *Hemoglobin.* 1992;16(6):469–480.
17. Abbes S, Fattoum S, Vidaud M, Goossens M, Rosa R. Sickle cell anemia in the Tunisian population: Haplotypes and Hb F expression. *Hemoglobin.* 1991;15(1&2):1–9.
18. Mears JG, Beldjord C, Benabadji M, Belghiti YA, Baddou MA, Labie D. The sickle gene polymorphism in North Africa. *Blood.* 1981;58(3):599–601.
19. Aluoch JR, Killinc Y, Aksoy M, Yüregir G T, Bakioglu I, Kutlar A, et al. Sickle cell anemia among Eti-Turks: Haematological, clinical and genetic observations. *Br J Haematol.* 1986;64(1):45–55.

20. Nagel RL, Fleming A. Genetic epidemiology of the β^S gene. *Baillierre`s Clin Haematol.* 1992;5(2):331–365.
21. Nagel RL. The origin of the Hemoglobin S gene: Clinical, genetic and anthropological consequences. *Einstein Q J Biol Med.* 1984;2:53–62.
22. Abd El-Latif M, Filon D, Rund D, Oppenheim A, Kanaan M. The β-IVS-1-6 (T→C) mutation accounts for half of the thalassemia chromosomes in the Palestinian population of the mountain regions. *Hemoglobin.* 2002;26(1):33–40.
23. Segal R. *Islam Black Slaves.* New York: Farrar, Straus and Giroux. 2001.
24. Kulozik AE, Wainscoat JS, Serjeant GR, Kar BC, Al-Awamy B, Essan GJF, et al. Geographical survey of the β^S-globin gene haplotypes: Evidence for an independent Asian origin of the sickle cell mutation. *Am J Hum Genet.* 1986;39(2):239–244.
25. Nagel RL. Severity, pathobiology, epistatic effect, and genetic markers in sickle cell anemia. *Semin Hematol.* 1991;28(3):180–201.
26. Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH, et al. Nonuniform recombination within the human β-globin gene cluster. *Am J Hum Genet.* 1984;36(6):1239–1258.
27. Srinivas R, Dunda O, Krishnamoorthy R, Fabry ME, Georges A, Labie D, et al. Atypical haplotypes linked to the β^S gene in Africa are likely to be the product of recombination. *Am J Hematol.* 1988;29(1):60–62.
28. Rahimi Z, Merat A, Gerard N, Krishnamoorthy R, Nagel RL. Implications of the genetic epidemiology of globin haplotypes linked to the sickle cell gene in Southern Iran. *Hum Biol.* 2006;78(6):719–731.
29. Geever RF, Wilson LB, Nallaseth FS, Milner PF, Bittner M, Wilson JT, et al. Direct identification of sickle cell anemia by blot hybridization. *Proc Natl Acad Sci USA.* 1981;78(8):5081–5085.
30. Rahimi Z, Karimi M, Haghshenass M, Merat A. β-Globin gene cluster haplotypes in sickle cell patients from southwest Iran. *Am J Hematol.* 2003;74(3):156–160.
31. Labie D, Pagnier J, Lapoumeroulie C, Rouabhi F, Dunda-Belkhodja O, Chardin P, et al. Common haplotype dependency of high $^G\gamma$-globin gene expression and high Hb F levels in β-thalassemia and sickle cell anemia patients. *Proc Natl Acad Sci USA.* 1985;82(7):2111–2114.
32. Embury SH, Clark MR, Monroy G, Mohandas N. Concurrent sickle cell anemia and α-thalassemia. Effect on pathological properties of sickle erythrocytes. *J Clin Invest.* 1984;73(1):116–123.