Pol. J. Environ. Stud. Vol. 20, No. 6 (2011), 1525-1530

Original Research

Sickle Cell Anemia-Associated Beta-Globin Mutation in Shagia and Manasir Tribes from Sudan

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Received: 13 January 2011
Accepted: 27 May 2011

Abstract

Sickle cell anemia is common in Africa, because it is selected by the pressure related to infection with *Plasmodium* spp. However, its distribution varies locally, especially in isolated populations. The aim of this study was to analyze the frequency of the HbS mutation in two local tribes of Arab origin, the Shagia and Manasir, which are both from the region of the 4th Nile cataract. The Shagia cohort investigated represents an isolated homogenous population and was relocated recently owing to construction of a dam; the Manasir cohort had significant admixtures from other tribes. Anthropological data and buccal swabs were collected from 126 Shagia and 90 Manasir representatives from the area and were analyzed using a PCR/RFLP assay specific for HbS, with confirmation by sequencing. The S mutation in the HBB gene was not detected among the Shagia and Manasir individuals investigated. The HbS AA genotype and A allele frequency therefore showed a prevalence of 100% in both groups. Lack of HbS mutation of the HBB gene in the previously unstudied Shagia group confirms that the frequency of the sickle cell gene in Sudan is tending to decrease in a northerly direction.

Keywords: HbS mutation, HBB gene, PCR/RFLP, Sudanese Arabs

Introduction

Africa is the continent of origin of all human genetic diversity; its eastern part is the most divergent region genetically owing to common migration and the presence of the hunter-gatherer tribes [1]. Despite this fact, areas of isolation of homogenous populations still exist and are relatively common [2]. Therefore, population studies on inheritance in Africa bridge the gap in current knowledge of human genetic variability, and provide characteristics of both heterogeneous and homogenous populations. Selective pressures have been in force for the longest period of human evolution in this region; therefore. alterations in allele frequency are expected to be more pronounced. The prime example of such diversity is the hemoglobin S (HbS) mutation that causes sickle cell disease [3]. The genetic background of this condition was suspected and described as early as 1949 by Linus Pauling, prior to the discovery of DNA [4]. Phenotypically, the HbS mutation results in the production of altered hemoglobin that dimerizes in low oxygen con-

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centrations and alters the shape of the erythrocyte. Clinical symptoms of anemia are observed among homozygous individuals, with hemolytic episodes that often cause chronic anemia. Generally the course of the disease is moderate; however, various acute clinical syndromes are linked with it, including vaso-occlusive and aplastic crises, chest syndromes, cerebrovascular events, and morbidity [5]. No significant clinical defects are observed among heterozygotes. Moreover, the mutation is associated with relative protection against life-threatening malaria (an approximate 10-fold decrease in susceptibility has been reported) [6, 7].

In a range of wide-scale genetic studies carried out so far, it has been suggested that malaria pathogens had been a major factor that has modified the regional frequency of human genetic variants that decrease susceptibility to this infection. This selective pressure has been in force for at least 10,000 years, and for this reason *Plasmodium* spp. are considered to be one of the key infectious agents that modify the human genome. It has been claimed that malaria is one of the factors that determines the increased frequency of all hemoglobin β (HBB) variants – not only hemoglobin S but also hemoglobin C (HbC) and hemoglobin E (HbE). All these are a result of a single nucleotide polymorphism (SNP) with a major codon change of the β-globin gene: HbS results from replacement of glutamic acid by valine (codon 6); HbC from replacement of glutamic acid by lysine (also codon 6), while HbE results from replacement of glutamic acid by lysine at codon 26. Altered properties of hemoglobin in the red blood cells result in limitation of infection by *Plasmodium* parasites [8].

HbC and HbE variants are detected mainly in West Africa and Southeastern Asia, while HbS is common in Sub-Saharan Africa, the Middle East, and the Mediterranean region. The HbS mutation is also observed in Northern Africa, as well as in Iraq, Iran, Afghanistan, India, Georgia, Azerbaijan, Turkey, Tajikistan, and among Caucasians. In Israel the HbS variant is found exclusively among Israeli Arabs, while in Africa three areas can be distinguished where the mutation is the most common, namely Lower Nigeria (including the Genua Basin in Central West Africa), the area around Democratic Republic of the Congo, and the area around Senegal (Western Africa) [9]. So far, five major haplotypes of the HbS mutation have been observed and named after geographical regions. In Western Africa the Senegal, Benin, and Cameroon haplotypes are found, while Bantu predominates in Eastern and Central Africa, with Arab-Indian being a dominant haplotype in the Middle East and Asia [10]. The Benin haplotype spread most probably from Central West Africa to Northern Africa, and is also found among the majority of patients with sickle cell anemia in the Western part of Saudi Arabia. Moreover, the Bantu haplotype is found in the entire Northern Africa region and in the area of the Mediterranean Sea, and is probably of Central West African ancestry [9].

According to World Health Organization estimates, as many as 5% of the world's population carries sickle cell disease or thalassemia. As expected, the prevalence of sickle cell disease is highest in Africa, where 150,000 to 300,000 homozygous individuals are born every year [11], with as many as 33,000 in the area around Nigeria alone [12]. Systematic screening of more than 30,000 newborn babies in the Republic of Congo demonstrated that 1.4% of individuals are homozygous for the hemoglobin S mutation [13].

In this study the frequency of HbS mutation was analyzed among representatives of the isolated Shagia and Manasir tribes who reside in Sudan, in the region of the 4th Nile Cataract.

Experimental Procedures

Geopolitical Conditions and Description of the Study Group

Shagia Tribe

The region of the 4th Nile Cataract is inhabited by the largely isolated tribe of Shagia people. These are Africans of Semitic origin who live on both banks of the Nile from Korti to the 3rd Cataract, and in portions of the Bayuda Desert. The Shagia are partly nomadic and partly an agricultural people [14]. The ethnic identity of the Shagia cannot be determined easily to be Arab or African. This is related both to their nomadic lifestyle and the historical assimilation of other populations. Given the geographical location, the group examined was partially isolated.

The Merowe High Dam is being erected near Umm Duwemi village. The current plans for industrial development necessitate the relocation of three Shagia villages, namely Abu Haras, Shibabit and El Higiena, on the right bank of the Nile River between the towns of Karima and Abu Hamad, which administratively is a part of the Northern Province of Sudan. Genetic material was collected from 126 Shagia inhabitants of the three villages (71 (56.4%) female, and 55 (43.6%) male; mean age 23.9±17.3 years, median 20 years). A full family and medical history was recorded for all individuals enrolled in the study.

Manasir Tribe

The region of the 4th Nile Cataract is also inhabited by the Manasir tribe, who live approximately 80 km from the three Shagia villages described above and represents a population with admixture of other populations (Beniamer, Ababda, Foraui, and Kesinger tribes).

The Manasir people are of Arab origin (they migrated originally from the Arab Peninsula) and a considerable part of this population lives as Bedouins in the Bayudah Desert, while others inhabit communities closer to the Nile.

Samples from 91 representatives of this tribe were collected to serve as a reference group representing the 4th Nile Cataract area. In this group 46 individuals (51.1%) were female and 45 (48.9%) were male (mean age 42±19 years, median 41.3 years).

Due to high rate of illiteracy in the analyzed cohort – oral consent for participation in the study was obtained in the presence of local authorities representative.

DNA Extraction

Buccal swabs were collected from all individuals who consented to participate in the study. The collected swabs were dried carefully in separate areas in order to avoid cross-contamination. For DNA extraction, a BuccalAmp DNA Extraction Kit (Epicentre, Madison, USA) was used. Extractions were performed according to the manufacturer's protocol. The DNA was re-suspended in TRIS-EDTA buffer (QIAgen, Hilden, Germany) and stored at 4ºC for further analysis.

Genotyping for the point mutation at codon 6 (A20T) of the HBB gene (HbS mutation) was carried out using the method published by Ayatollahi [14]. The PCR was performed using the following primers: forward 5'-ACACAACTGTGTTCACTAGC-3' [EMBOSS:001] and reverse 5'-CAACTTCATCCACGTTCACC-3' [EMBOSS:001]. The reaction was carried out in a total volume of 20 µl that contained 40 ng of template DNA, 4 pM of each primer, 1 x PCR buffer [10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P40] (MBI Fermentas), 1.5 mM MgCl2 dNTP (MBI Fermentas), and 0.5 U of Taq polymerase (MBI Fermentas). The amplification was performed with initial denaturation at 94ºC for 5 min, and then 37 cycles of denaturation at 94ºC for 20 s, annealing at 53ºC for 40 s, and extension at 72ºC for 40 s. The final incubation at 72ºC was extended by 8 min. The amplified fragments (110 bp) were digested with the restriction endonuclease Eco81I, which has a recognition site at codon 6 in the HBB gene. For the RFLP assays, an aliquot of 16 µl of PCR product was incubated at 37ºC for 18 h with 10 U ofe Eco81I. The sizes of the restriction fragments were 56 bp and 54 bp for the normal allele (A20), while the mutant allele (T20) remained uncleaved and gave a band of 110 bp. The fragments were separated by electrophoresis on 4% agarose gel stained with ethidium bromide. The results were recorded by photographing the gels under UV light [15].

Some of the results of the PCR-RFLP method were further confirmed by capillary sequencing using an Applied Biosystems 3130 Genetic Analyzer under standard sequencing conditions and the set of primers described above (Department of Genetics and Pathology).

The authors state that their research conforms to the Helsinki Declaration and to local legislation. Patients' informed consent was a prerequisite. Institutional ethical clearance from the Ethics Committee of Pomeranian Medical University was obtained.

Results

In the Shagia group (n=126) the S mutation in the HBB gene was not found (Table 1), it was also not observed among representatives of the Manasir tribe. Therefore, the frequency of the AA genotype and the A allele was 100% in both groups (Fig. 1). In order to confirm the results of PCR-RFLP, sequencing was performed. The examples showed 100% homogeneity with the expected sequences (Fig. 2). Within the analyzed sequence an additional SNP was identified (rs713040), which had a frequency consistent with that observed previously in African populations. This confirms the validity of the study and the accuracy of the result obtained for the HbS mutation.

Table 1. Frequency of the mutation causing sickle cell anemia in different parts of Sudan with reference to the results obtained in the Shagia and Manasir tribes [10].

Area	Percentage of sample	Percentage of controls	Percentage of total population (29 million)
West	73 (n=189)	38 (n=118)	30
South	3.1 (n=189)	18 (n=118)	16
Centre and North	23.8 (n=189)	42 (n=118)	42
North Sudan	0 (n=216)	-	4.5

Discussion

In this study no individuals with the sickle cell gene were identified. The frequency of the HbS mutation in the region of the 4th Nile Cataract in Sudan has not been studied previously, although data are available for more northern regions and Southern Sudan. Studies of genetic variants of hemoglobin S have been performed also in the eastern part of Sudan among the Negroid Fulani and Masaleit tribes, among whom the sickle cell gene was found in 15.7% and 21% of individuals, respectively. In contrast, the frequency of this mutation among Arab populations of North Africa is up to 3% [16].

The results of genotyping for the sickle cell gene can be compared with the data reported by Abdelrahim et al. [12], who analyzed a study group that consisted of 189 patients with sickle cell anemia and a control group composed of 118 patients chosen randomly from individuals referred to a hospital in Khartoum. In that study it was demonstrated that the frequency of the HbS variant was highest in the Messeryia tribe. It was reported that the highest frequency of the sickle cell gene is noted in the area of Southern Sudan, and that the prevalence is lower in the central part of the country. Data from the north remain incomplete; the

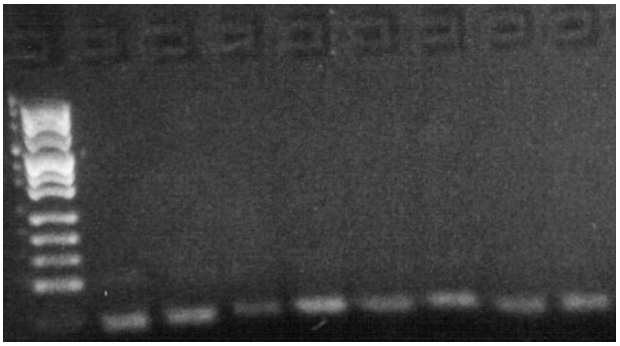

Fig. 1. Record of the PCR-RFLP products for detection of hemoglobin sickle cell mutation.

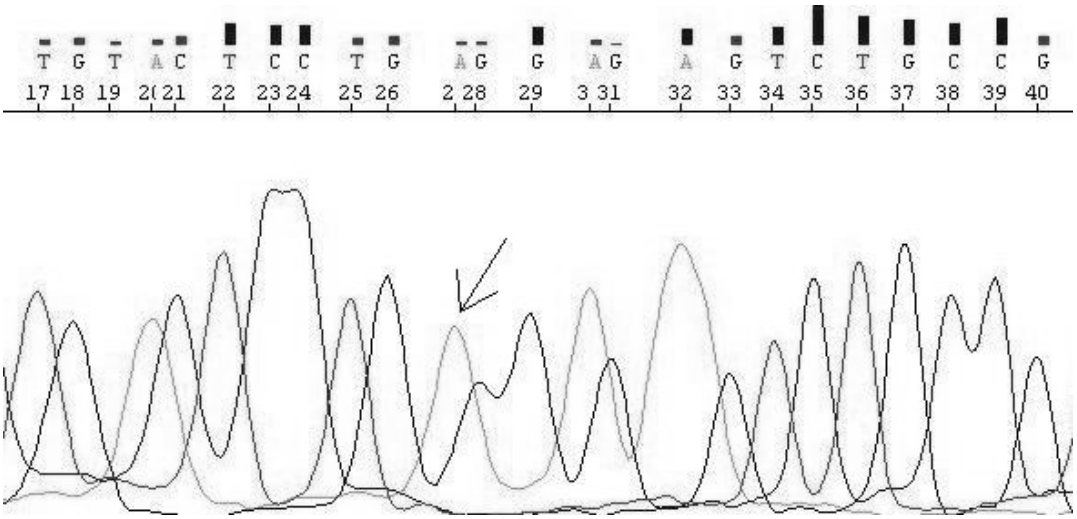

Fig. 2. Capillary electrophoresis results confirming the PCR-RFLP results. Rs344 SNP is marked with an arrow.

results of the current study provide additional data for this region [12] and are presented in Fig. 3. Results obtained in this study remain in accordance with the published reports on the frequency of the HbS mutation in Africa. Around three main areas with high mutation prevalence (Bantu, Benin, Senegal), this frequency is lower. In general the mutation prevalence is decreasing in the northern and northeastern direction [9].

With regard to ethnicity, Sudan may be divided into two parts. The northern part of the country is mostly of Arab ancestry, while the southern part is inhabited by negroids. The population of the northern provinces of Sudan is 1,293,279. The aim of the current study was to fill the gap in knowledge of the distribution of African HbS variants among individuals of Arab origin and to provide unique genetic information on the local tribes. It must be noted that this genetic information for the Shagia tribe may soon be lost owing to the forthcoming relocation of the tribe and the construction of a new dam in this area. Three housing estates have been built in the desert for the residents of the threatened area, and access has been provided to schools, mosques, hospitals, electricity, and a primitive water supply system. It may be presumed that, in the near future, sharing of the same community by thousands of people who belong to different tribes will result in new social arrangements, customs, and culture. The research described herein provided an opportunity to record anthropological, genetic, and ethnographic data on this place and its population. From the scientific point of view, the population of the Shagia tribe that resides in the area examined is attractive for its homogeneity, which is a result of geographical, genetic, and cultural isolation.

This work was a part of a large archaeological research project that has been carried out by the Archaeological Museum in Gdańsk since 1996, in agreement with the National Corporation for Antiquities and Museums in Khartoum. The project included surface and excavation works in the 4th Nile cataract region as well as the collection of anthropological and genetic data.

In our study the mutated hemoglobin S variant was not found among isolated Shagia people or Mansir representatives. This may be explained partially by a detailed review of family relationships in the Abu Haras village, inhabited by the Shagia tribe, which disclosed a high degree of consanguinity among the inhabitants. Cultural conditioning predisposes to marriage within one tribe (in the case of Abu Haras, one village), with virtually no admixture of external genetic material. The genetic similarities observed in this population might be explained by geographical isolation.

Fig. 3. Frequency of the sickle cell S mutation in Sudan [10] in comparison with the results obtained in the Shagia and Manasir tribes.
(Figure legend: light gray 25-50%; gray 1-5%; white <1%).

The gene polymorphism frequency is reflected in the family structure here, with the exception of El Hygiena village, which was too small for precise characterization. Analysis of the family situation in Shibabit village, where farmers predominate, shows that females and children are sedentary while men follow agricultural work, which suggests that genetic mixtures become more likely. Within these three villages both isolated and mixed populations were investigated, as were individuals from the Manasir tribe, who represent a population with higher genetic diversity that lives in the same area, and were analyzed for comparison. Given that in this study both an isolated tribe (Shagia) and a migratory tribe (Manasir) were investigated, it is possible that the results that indicate a lack of HbS mutation in the area of the 4th Nile cataract also reflect a low frequency of this mutation in Northern Sudan. It must be noted that the lack of HbS mutation in both groups, regardless of lifestyle, marriage pattern, or extent of isolation might be a result of the fact that populations of Arab ancestry tend not to mix with Negroids. Therefore, the observed frequency of HbS may be related not only to the isolation status but also to the general race affiliation.

With regard to the origin of the HbS mutation, two hypotheses exist. The first claims that it is a novel mutation, which as a result of climate change and migration from the Arabian Peninsula was transmitted to India, Eastern Saudi Arabia, and Equatorial Africa. The second theory, the so-called multiple mutation hypothesis, emerged with the introduction of new molecular markers to genetic research. It has been suggested that several independent mutational events occurred in the S gene, which resulted in the evolution of several divergent haplotypes [12]. Mears suggests that the HBB S mutation could have appeared independently in Atlantic Africa (Senegal), Western Africa, and Bantu-speaking Africa [17, 18]. The results of research published recently seem to prove that the S mutation has appeared only recently in the gene pool of eastern Africa [19], and has developed separately in different locations [20]. The mutation has spread either from the Middle East or from Western Africa [16]. The gene mutation for sickle cell anemia is found also in several regions of Saudi Arabia [21]. It is difficult to assess whether its presence is the result of migratory transfer from Africa, or whether it occurred in the Saudi population independently. The high frequency of the mutation in the western provinces of Saudi Arabia may be associated with immigration from Africa. It should be emphasized, however, that malaria is present in some regions of Saudi Arabia, so the mutational events in the HBB gene might have occurred independently.

Conclusions

Lack of the HbS mutation of the HBB gene in the previously unstudied Shagia group confirms that the frequency of the sickle cell gene in Sudan tends to decrease in a northerly direction. This study provides additional knowledge on the distribution of HbS in Africa, in both mixed and isolated populations. A limitation of the study is related to the relatively small sample size. But the authors aimed to perform the study in small, local populations. Moreover, it was performed as a part of rescue anthropological research in the area from which the population would be transferred before its unique features were lost.

Competing Interests

The authors declare that they have no competing interests. All individuals were inhabitants of the 4th Nile cataract area. Due to the high rate of illiteracy in the analyzed cohort, oral consent for participation in the study was obtained in the presence of local authorities.

Acknowledgements

The authors wish to thank Mahmoud el Tayeb M.Sc. Ph.D. and the entire Shagia tribe for the support and kindness that allowed them to complete this research.

This study was supported by a grant from the Ministry of Science and Higher Education, Republic of Poland (No. N N404 140837).

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