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RESEARCH ARTICLE

Beta globin gene cluster haplotypes of abnormal hemoglobins observed in Turkey

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ABSTRACT

Since the first observation of hemoglobin S (Hb S) in Turkey by Aksoy, the number of hemoglobin variants reported was increased. Beta globin gene cluster haplotypes are being used to determine the origin of the mutations under interest. We studied the beta globin gene cluster haplotypes for the six different abnormal hemoglobins which are Hb S, Hb D-Los Angeles, Hb G-Coushatta, Hb E, Hb E-Saskatoon and Hb J-Iran observed in Turkey. In this study, we report two novel haplotypes [- + + - - - +] and [- + - + + +] in association with the Hb G-Coushatta mutation. The haplotype for the Hb J-Iran is also reported as [- + - + + + +] for the first time in world populations from Turkey.

Key Words: Abnormal hemoglobins, haplotype, Turkey

ÖZET

Türkiye'de gözlenen anormal hemoglobinlerde beta globin gen ailesi haplotipleri

Türkiye'de hemoglobin S (Hb S)'nin ilk kez Aksoy tarafından belirlenmesinden sonra gözlenen anormal hemoglobinlerin sayısında büyük artış olmuştur. Beta globin gen ailesi haplotipleri çalışılan mutasyonun genetik kökeninin belirlenmesinde kullanılmaktadır. Çalışmamızda; ülkemizde gözlenen Hb S, Hb D-Los Angeles, Hb G-Coushatta, Hb E, Hb E-Saskatoon ve Hb J-Iran olmak üzere toplam altı anormal hemoglobin mutasyonunda beta globin gen ailesi haplotipleri incelenmiştir. Bu çalışmada; Hb G-Coushatta mutasyonu ile ilintili olan, dünyada ve Türkiye'de bildirilmemiş iki haplotip (- + + - - - + ve - + - + + + +) saptanmıştır. Hb J-Iran ile ilgili olarak ise ilk kez haplotip türü (- + - + + + +) gösterilmektedir.

Anahtar Sözcükler: Anormal hemoglobinler, haplotip, Türkiye

INTRODUCTION

The first abnormal hemoglobin in Turkey was Hb S reported by Aksoy[1,2]. To date, 42 abnormal hemoglobins have been identified in the Turkish population. In addition to the studies conducted in Turkey, many European researchers reported their findings in the immigrant Turkish population in their countries[3]. 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[4]. The beta globin gene cluster haplotypes have also been largely employed in population surveys[5]. Analysis of the normal and mutant alleles is a useful tool for determination of genetic diversity and inter-population relationships[6-8]. The beta globin gene cluster is located at human chromosome 11 including five genes arranged in the order 5'ε-Gγ-Aγ-ψβ-δ-β 3'[9]. This cluster spans an approximately 60 kb DNA region separated by 9 kb recombination hot spot into 5' and 3' regions. The recombination hot spot region is located between δ and β-globin genes. The 5' region of the beta globin gene cluster contains fetal and embryonic genes exhibiting high-level linkage disequilibrium. On the other hand, the cumulative recombination rate for the 3' region of the beta globin gene cluster is high compared to the 5' region[8,10,11]. With all of these characteristic features, beta globin gene cluster haplotypes are a useful tool for determination of the genetic structure and origin of the populations, including the possible associations with mutations and hereditary diseases like thalassemias and abnormal hemoglobins of interest. In this study, we aimed to investigate the beta globin gene cluster haplotypes of the abnormal hemoglobins observed in Turkey.

MATERIALS and METHODS

This study was performed on 33 DNA samples obtained in the premarital screening programs. In case of Hb S mutation, 12 unrelated heterozygous individuals were included and the associated haplotypes were determined by Arlequin 3.0 software with unknown gametic phase[12]. For the other abnormal hemoglobins, family studies were applied in order to determine the haplotypes in association with the mutations causing the abnormal hemoglobins. The studied abnormal hemoglobins were Hb D-Los Angeles (3 samples), Hb J-Iran (4 samples), Hb E (4 samples), Hb E-Saskatoon (3 samples) and Hb G-Coushatta (4 samples). Written informed consent had already been obtained from all individuals and/or their parents and deposited in the Pamukkale University Biophysics Department DNA Bank as anonymous samples for further investigations. These heterozygous cases were diagnosed at the molecular level as previously published[13,14]. DNA sequencing results are shown in Figure 1. Abnormal hemoglobins studied in this manuscript are from different parts of Turkey (Figure 2).

Haplotype analysis was done by polymerase chain reaction (PCR)-based restriction enzyme digestion for the beta globin gene cluster of the following polymorphic restriction sites: *Hinc*II 5' to ε, *Hind*III 5' to $^{G}\gamma$, *Hind*III in the IVS-II 5' to Aγ, *Hinc*II in ψβ, *Hinc*II 3' to ψβ, AvaII in β, and *Hinf*I 3' to β as previously published[15,16]. The restriction sites for the haplotype analysis are shown in Figure 3. Fok I enzyme digestion approach was also used for the differentation of Hb E and Hb E-Saskatoon[17].

RESULTS

Abnormal hemoglobins included in this study were Hb S, Hb D-Los Angeles, Hb G-Coushatta, Hb J-Iran, Hb E and Hb E-Saskatoon from Denizli, Burdur, Kocaeli and Samandag-Hatay, as shown in Figure 2. Two different approaches were followed. The first approach was the generation of associated haplotypes by Arlequin 3.0 software with unk-

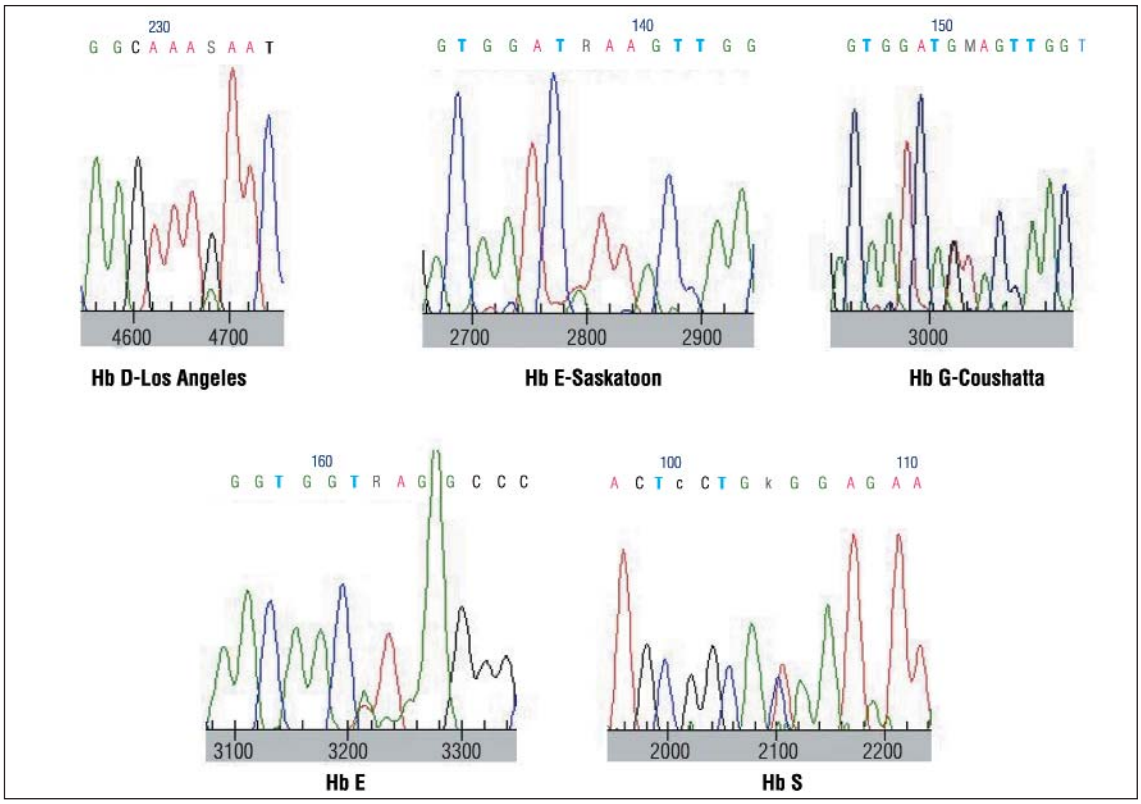

Figure 1. DNA sequencing data of the abnormal hemoglobins observed.

Figure 2. Locations of the abnormal hemoglobins observed in this study.

[HbS: Denizli (B), Hb G-Coushatta: Kocaeli and Denizli (A,B), Hb J-Iran: Burdur (B), Hb E: Samandağ-Antakya (C), Hb E-Saskatoon: Denizli (B)].

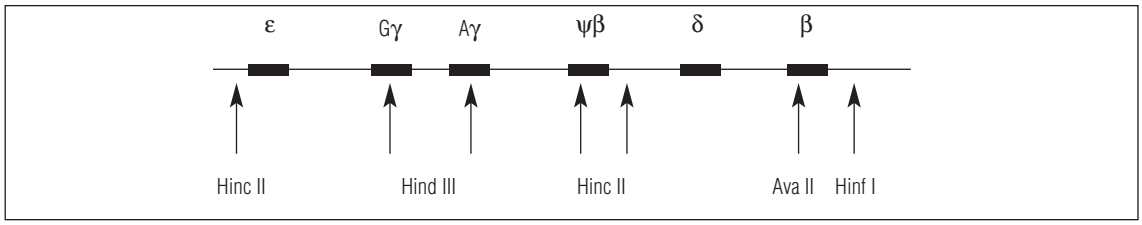

Figure 3. Beta globin gene cluster haplotype analysis for the seven loci.

nown gametic phase, used for the 12 heterozygous Hb S carriers. The second approach was to determine the associated haplotypes using family studies. The beta globin gene cluster haplotype analysis was done according to Falchi et al.[15]. In total, seven loci were studied as shown in Figure 3. The beta globin gene cluster haplotypes are shown in Table 1 from each of the heterozygous Hb S carriers. Arlequin-generated haplotypes are also shown in Table 1. According to the results obtained, DS01 [- - - - + + +] and DS02 [+ - - - - + +] are the associated haplotypes with frequencies of 50% and 17%, respectively, for the Hb S mutation. The results of the family members of the other abnormal hemoglobins are included in Table 2 and Table 3. According to our results, haplotype [- + + - + - +] is associated with Hb E-Saskatoon. Associated haplotypes for the other abnormal hemoglobins are as follows: haplotype [- + - + + + +] for Hb J-Iran, haplotype [- + + - + + +] for Hb D-Los Angeles, haplotype [+ - - - - + -] for Hb E, and haplotype [- + - + + + +] and [- + + - - - +] for the Hb G-Coushatta mutations studied (Table 4).

DISCUSSION

Since the first observation of Hb S in Turkey, many publications have reported the other abnormal hemoglobins under the framework of developing up-to-date molecular techniques and many university laboratories working in this field have been established in Turkey. The tremendous effort and the present status of the abnormal hemoglobins have been reviewed in detail by Altay with respect to the reported abnormal hemoglobins in Turkey[3]. The premarital screening program applied by the Turkish Ministry of Health has an important role with regard to information about the abnormal hemoglobin status of Turkey, especially for the abnormal hemoglobins that do not present a clinical picture. In this collaborative study, we aimed to identify the beta globin gene cluster haplotypes of the abnormal hemoglobins observed in different parts of Turkey.

Hb S is the first hemoglobin to be discussed. All samples are from the Denizli province of Turkey and they are heterozygous carriers. We have 20 unrelated families carrying the Hb S mutation registered in the Hemoglobinopathy Control Program in our database. Twelve heterozygous carriers were involved in the study. According to our results, the Hb S mutation is associated with the Benin haplotype [- - - - + + +] originating from Western Africa, which is consistent with the previous reports[3].

Hb E and Hb E-Saskatoon are observed in Turkey and reported by different researchers[3]. Both of these abnormal hemoglobins behave like Hb A_2 in alkaline pH hemoglobin electrophoresis. In acidic pH, these hemoglobins move like human adult hemoglobin Hb A. Due to their similar electrophoretic properties, these hemoglobins should be identified by molecular techniques. The reason for the molecular identification of Hb E is due to its clinical importance, being thalassemic hemoglobin. Fok I enzyme digestion is a valuable approach for the differentiation of these hemoglobins[17]. DNA sequencing can also be used for the verification of the results. Hb E-Saskatoon was reported in three unrelated families from Antalya, Aksaray and Kayseri[3]. Our case is the fourth unrelated family from Denizli pro-

Table 1. Beta globin gene cluster haplotypes of Hb S in Denizli province

Beta globin gene cluster				Case	5'-ε	Gγ	Aγ	5'-ψβ	3'-ψβ	5'-β	3'-β
No.	**Haplotype (*)**	**Frequency**	**S.D. (+/-)**	**No.**	**Hinc II**	**Hind III**	**Hind III**	**Hinc II**	**Hinc II**	**Ava II**	**Hinf I**
DS01	- - - - + + +	0.500000	0.100862	01	-/-	-/-	-/-	-/-	+/+	+/+	+/-
DS02	+ - - - - + +	0.166667	0.082860	02	+/-	-/-	-/-	-/-	+/-	+/+	+/-
DS03	- + - + + + -	0.062500	0.052007	03	-/-	+/-	-/-	+/-	+/+	+/-	+/+
DS04	- - - - + + -	0.062500	0.049400	04	+/-	-/-	-/-	-/-	+/-	+/+	+/+
DS05	+ + - + + - +	0.041667	0.042201	05	-/-	-/-	-/-	-/-	+/+	+/+	+/+
DS06	- + - + + - +	0.041667	0.040569	06	+/-	+/-	-/-	+/-	+/-	+/+	+/-
DS07	+ - - - - - +	0.041667	0.039646	07	+/-	-/-	-/-	-/-	+/-	+/+	+/+
DS08	- - + - + + +	0.041667	0.034471	08	+/-	-/-	-/-	-/-	+/-	+/+	+/+
DS09	+ + - + - + -	0.020833	0.034019	09	+/-	-/-	-/-	-/-	+/-	+/-	+/+
DS10	+ - - - - + -	0.020833	0.030982	10	+/-	+/-	-/-	+/-	+/+	+/-	+/+
-Sum of 10 listed frequencies > 1.000000				11	-/-	+/-	-/-	+/-	+/+	+/+	+/-
(*) Generated by Arlequin 3.0 Software				12	-/-	-/-	+/-	-/-	+/+	+/+	+/+

Table 2. Beta globin gene cluster haplotypes of the Hb E-Saskatoon, Hb J-Iran and Hb E families

	Hemoglobin	**5'-ε Hinc II**	**Gγ Hind III**	**Aγ Hind III**	**5'-ψβ Hinc II**	**3'-ψβ Hinc II**	**5'-β Ava II**	**3'-β Hinf I**
				Hb E-Saskatoon Family				
Mother	Hb A/A	+/-	+/-	-/-	+/-	+/-	+/+	+/-
Father	Hb A/E-Saskatoon	+/-	+/-	+/-	-/-	+/-	+/-	+/+
Son	Hb A/E-Saskatoon	-/-	+/+	+/-	+/-	+/+	+/-	+/-
Associated Haplotype		**-**	**+**	**+**	**-**	**+**	**-**	**+**
				Hb J-Iran Family				
Mother	Hb A/A	+/-	+/+	+/-	+/-	+/+	+/+	+/-
Father	Hb A/J-Iran	-/-	+/+	-/-	+/+	+/+	+/+	+/+
Daughter	Hb A/J-Iran	-/-	+/+	+/-	+/-	+/+	+/+	+/-
Son	Hb A/J-Iran	-/-	+/+	+/-	+/-	+/+	+/+	+/-
Associated Haplotype		**-**	**+**	**-**	**+**	**+**	**+**	**+**
				Hb E Family				
Mother	Hb A/E	+/+	-/-	-/-	-/-	-/-	+/+	+/-
Father	Hb A/A	+/+	-/-	-/-	-/-	-/-	+/+	+/+
Daughter -1	Hb A/E	+/+	-/-	-/-	-/-	-/-	+/+	+/-
Daughter- 2	Hb A/E	+/+	-/-	-/-	-/-	-/-	+/+	+/-
Associated Haplotype		**+**	**-**	**-**	**-**	**-**	**+**	**-**

Table 3. Beta globin gene cluster haplotypes of the Hb D-Los Angeles and Hb G-Coushatta families

	Hemoglobin	5'-ε Hinc II	Gγ Hind III	Aγ Hind III	5'-ψβ Hinc II	3'-ψβ Hinc II	5'-β Ava II	3'-β Hinf I
				Hb D-Los Angeles Family				
Mother	Hb A/Hb D-Los Angeles	+/-	+/-	+/-	-/-	+/-	+/+	+/+
Father	Hb A/A	+/+	-/-	-/-	-/-	-/-	+/+	+/-
Daughter	Hb A/Hb D-Los Angeles	+/-	+/-	+/-	-/-	+/-	+/+	+/+
Associated Haplotype		-	+	+	-	+	+	+
				Hb G-Coushatta Family (Denizli)				
Son	Hb A/G-Coushatta	-/-	+/+	-/-	+/+	+/+	+/-	+/+
Mother	Hb A/G-Coushatta	-/-	+/+	-/-	+/+	+/+	+/+	+/+
Father	Hb AA	+/-	+/-	-/-	+/-	+/-	-/-	+/+
Associated Haplotype		-	+	-	+	+	+	+
				Hb G-Coushatta Family (Kocaeli)				
Mother	Hb A/G-Coushatta	+/-	+/-	+/-	-/-	-/-	+/-	+/+
Father	Hb A/G-Coushatta	+/-	+/-	+/-	-/-	-/-	+/-	+/+
Daughter	Hb G-Coushatta (homozygous)	-/-	+/+	+/+	-/-	-/-	-/-	+/+
Son	Hb A/G-Coushatta	+/-	+/-	+/-	-/-	-/-	+/-	+/+
Associated Haplotype		-	+	+	-	-	-	+

Table 4. Beta globin gene cluster haplotypes associated with Hb D-Los Angeles and Hb G-Coushatta as observed in world populations

Mutation	5'-ε Hinc II	Gγ Hind III	Aγ Hind III	5'-ψβ Hinc II	3'-ψβ Hinc II	5'-β Ava II	3'-β Hinf I	Ref
Hb D-Los Angeles								
- Italy	+	-	-	-	-	+	+	(28)
- Thailand	-	+	+	-	+	+	+	(29)
- Mexico	+	-	-	-	-	+	+	(30)
- Iran	+	-	-	-	-	+	+	(31)
- Turkey	+	-	-	-	-	+	+	(16)
- Turkey	-	+	-	-	+	+	+	(16)
- Turkey	-	+	+	-	+	+	+	This study
Hb G-Coushatta								
- American Indian	-	+	-	-	+	-	nr	(27)
- Chinese	-	+	+	-	+	+	nr	(27)
- Turkey	-	+	-	+	+	+	+	This study
- Turkey	-	+	+	-	-	-	+	This study

vince of Turkey. On the other hand, our Hb E family is from Samandağ-Hatay, which is in the eastern Mediterranean part of Turkey (Figure 2). Hb E is associated with Mediterranean haplotype V [+ - - - - + -] and Hb E-Saskatoon is associated with haplotype II like haplotype [- + + - + - +] according to Falchi et al., based on Orkin's classification[15,18]. Although they are from unrelated families, our Hb E-Saskatoon mutation has the same origin as reported before from Turkey[17]. This mutation was firstly reported from Orkney Islands[19]. Since the beta globin gene cluster haplotype is not known at the moment, the relationships between Turkish and Scottish mutations are still unclear.

Hb J-Iran was firstly reported by Rahbar et al. from Iran[20]. There are four reported cases from Turkey in the four different cities of Ankara, Antalya, Muğla and Denizli[14,21-23]. The beta globin gene cluster haplotype structure of Hb J-Iran is not known. The Denizli case was the fourth reported case in Turkey[14], and we studied the family members of this case to determine the beta globin gene cluster haplotype in association with the Hb J-Iran mutation. Our results show that the Hb J-Iran is associated with the haplotype IX [- + - + + + +]. The beta globin gene cluster haplotypes of the other cases reported from different parts of Turkey will help to elucidate whether or not these cases have the same genetic origin.

Hb G-Coushatta was reported by Schneider et al. in an American Indian family[24]. This hemoglobin variant was reported from different regions-Japan, Korea, Taiwan, China, Algeria and Turkey[13]. In Turkey, Hb G-Coushatta has also been reported by different research groups[13,23,25,26]. Hb G-Coushatta cases are from Kocaeli and Denizli. To the best of our knowledge, the Kocaeli case is the first reported homozygous case in Turkey. There are two different suggested genetic origins of Hb G-Coushatta mutations-American Indian and Chinese-based on beta globin gene cluster haplotypes and related frameworks[27]. Our families reported in this study are unrelated and live in different parts of Turkey. They have different beta globin gene cluster haplotypes that also differ from the other published cases (Table 4).

Hb D-Los Angeles is the second most common abnormal hemoglobin observed in both world and Turkish populations. There are three different beta globin gene cluster haplotypes reported in world populations (Table 4) [15,28-31]. These haplotypes are Mediterranean haplotype I [+ - - - - + +], haplotype II [- + + - + + +] and [- + - - + + +]. Most of the Hb D-Los Angeles cases observed are associated with the Mediterranean haplotype I. This haplotype was reported for the Hb D-Los Angeles from Italy, Mexico, Iran and Turkey[16,28,30,31]. Hb D-Los Angeles mutation is associated with haplotype II [- + + - + + +] in Thailand[29]. On the other hand, one family was reported as a novel haplotype as [- + - - + + +] from Turkey[16]. In this study, we report a family carrying Hb D-Los Angeles mutation in association with the Thai haplotype of [- + + - + + +]. Two different hypotheses have been proposed for the origin of the Hb D-Los Angeles mutation. It may have arisen in the Mediterranean area, independently from other populations, and/or the mutation may have arisen only once, most likely in Asia, based on the prevalence in Punjab (India) and Northwest China[28]. The Hb D-Los Angeles frequency in Denizli Province is similar to the frequency observed in Xinjiang Province, People's Republic of China, where it accounts for 55.6% of total hemoglobin variants observed[13,32]. The beta globin gene cluster haplotypes of the Chinese and Indian cases are not known at present. As far as Asian cases are concerned, only Thailand cases are known. Since most cases reported from different populations are related with the Mediterranean haplotype I except Thai cases, the hypothesis regarding the Asian origin of this mutation is under discussion. To prove the hypothesis of the Asian origin, Chinese and Indian cases should be identified.

In conclusion, we identified the beta globin gene cluster haplotypes of six different abnormal hemoglobins - Hb S, Hb D-Los Angeles, Hb G-Coushatta, Hb E, Hb E-Saskatoon and Hb J-Iran. Table 5 summarizes the beta globin gene cluster haplotypes observed in Turkey. For the Hb J-Iran, haplotype [- + - + + + +] is the first published beta globin gene cluster haplotype in world populations. Haplotypes [- + - + + + +] and [- + + - - - +] are also novel haplotypes in association with the Hb G-Coushatta mutation reported from Turkey, being the third and fourth different origins in the world populations. Since the prenatal diagnosis is applied at gene level, haplotype identification of the mutation and associations with the mutations of interest contribute to understanding the genetic structure of the fetus, helping in the diagnosis, and molecular mechanisms. In this study, we also emphasize the importance of premarital screening programs and the collaborative research between research groups and the Ministry of Health Centers in the different cities of Turkey. These abnormal hemoglobins were observed primarily in the Hemoglobinopathy Laboratories of the Turkish Ministry of Health local authorities. A national database and registry system based on the close collaboration between all research facilities will elucidate and improve the research on the hemoglobin molecule, its genetic structure and expression at the molecular level in Turkey.

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Table 5. Beta globin gene cluster haplotypes associated with abnormal hemoglobins observed in Turkish samples

Mutation	5'-ε Hinc II	Gγ Hind III	Aγ Hind III	5'-ψβ Hinc II	3'-ψβ Hinc II	5'-β Ava II	3'-β Hinf I
Hb S	-	-	-	-	+	+	+
Hb D-Los Angeles	+	-	-	-	-	+	+
	-	+	-	-	+	+	+
	-	+	+	-	+	+	+
Hb G-Coushatta	-	+	-	+	+	+	+
	-	+	+	-	-	-	+
Hb E-Saskatoon	-	+	+	-	+	-	+
Hb J-Iran	-	+	-	+	+	+	+
Hb E	+	-	-	-	-	+	-

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