

## THE HETEROGENEITY OF THE MOLECULAR BASIS OF β-THALASSAEMIA AMONG ARABS

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**Introduction:** The molecular basis of β-thalassaemias (β -thal) was investigated in Arab populations from different countries.

**Method:** A total of 272 blood samples (5-10 ml) were collected from unrelated β-thal patients attending the Ministry of Health hospitals in Riyadh, Saudi Arabia, and were grouped according to their nationality into eight groups (i.e. Saudis, Jordanians, Egyptians, Syrians, Lebanese, Yemenis, Sudanese and Palestinians). DNA was prepared from the Buffy coat and stored frozen at 700C until required for analysis. Using Amplification Refractory Mutation System (ARMS), Denaturing Gradient Gel Electrophoresis (DGGE) and dot blot analysis, the following mutations were investigated: IVS-1-1, IVS-1-6, CD 15, CD 41/42, CD 8/9, CD 39, IVS-1-5, IVS-II-1, IVS-I-110, Cap+1, IVS-1-3' end (25 bp deletion), CD 6, IVS-II-745, CD 16, IVS-II-654, IVS-I-1 (G-A), CD 17, CD 30, -88 and -28.

**Result:** The results revealed a significant heterogeneity in the molecular basis of β -thal in Arabs from different countries where each group has a major set of 6-8 mutations accounting for majority (- 80%) of the - β thal. Interestingly IVS-I-110 and IVS-II-1 were found in all Arabs, but at a significantly variable frequency, where they were more frequent in the countries around the Mediterranean and gradually decreased to the East. On the other hand, IVS-I-5, an Asian mutation occurred at a high prevalence in the UAE populations and decreased to the west. Saudi Arabia showed an overlap of both Mediterranean and Asian mutations.

**Discussion:** The β-thal gene mutations exhibit considerable heterogeneity in the different Arab populations. These

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represent either the trend of population movement or new mutations that may have occurred independently. As in the other populations of the world, 5-7 common mutations are characteristic of each country.

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The β-thalassaemias (β-thal) constitute a group of genetic disorders that have arisen from mutations in or around the β-globin gene of haemoglobin<sup>1</sup>. Over 150 mutations are known to date, where each results in either β<sup>0</sup> or β<sup>+</sup> thalassaemias<sup>2</sup>. The β<sup>0</sup>-thal mutations cause complete absence of the β-globin chains of hemoglobin, while β<sup>+</sup> thal mutations result in a variable degree of reduction in the level of the β -globin chain<sup>3-6</sup>. Consequently, the imbalance in

the  $\alpha/\beta$ - globin chain ratios results in precipitation of the excessive  $\alpha/\beta$ -globin chains forming inclusion bodies thus adversely affecting red cell membrane and causing hemolysis of the red cells.

Although, the  $\beta$ -thal exhibits a high degree of genotype and phenotype heterogeneity, various ethnic groups have a set of 6-8 common and somewhat characteristic mutations<sup>2</sup>. However, populations in neighboring regions have significantly different pattern of mutations and clinical expression of  $\beta$ -thal<sup>7-17</sup>.

The objective of this study was to investigate the molecular basis of  $\beta$ -thal among the Arab populations.

## METHOD

The study group was composed of a total of 272 unrelated patients belonging to the Arab world (Saudi Arabia 93; Jordan 39; Egypt 40; Lebanon 30; Syria 36; Yemen 15; Palestine 12 and Sudan 12) diagnosed as having thalassemia based on the clinical presentation, hematological parameters values, red cell indices, discriminant factors, electrophoresis and levels of Hb A,<sup>18</sup> and Hb F<sup>19</sup> and where ever possible  $\beta$ -globin chain ratio<sup>20</sup>. The patients were suffering from a severe form of anemia and required blood transfusion every 3-8 weeks and were therefore, classified as having  $\beta$ -thal major.

For the study of the molecular basis of  $\beta$ -thal in these patients, blood (10 ml) was drawn in EDTA tubes and used to obtain buffy coat by centrifugation. The Buffy coat was carefully removed from the top of the red cell layer and used for DNA extraction by using a modification of the procedure of Kunkel and co-workers<sup>21</sup>. The DNA was used to detect 20  $\beta$ -thalassemia mutations (Table 1) commonly encountered in Asians and Mediterranean populations by using the Amplification Refractory Mutation System (ARMS)<sup>22</sup>. Oligonucleotide primers used were prepared in our laboratory<sup>22</sup>. The patients DNA and set of controls were used during the Polymerase Chain Reaction (PCR) using a Perkin-Elmer Thermocycler to conduct the amplification under the conditions described previously<sup>22,23</sup>. The PCR product was visualized after electrophoresis on a 1.5 Nusieve and 1.5% agarose gel followed by ethidium bromide staining and ultraviolet light illumination. Denaturing gradient gel electrophoresis and dot blot analysis was used for some of the mutations.

Table 1.  $\beta$ -thalassemia mutations tested in Arabs

Location	Mutation	Effect	$\beta$ -thal type
IVS-I-110	G A	Internal IVS change*	+
IVS-I-5	G C	Consensus change*	+
CD 39	C T	Nonsense mutant**	0
IVS-I-3' end	-25	Splice junction changes*	0
IVS-II-1	G A	Splice junction change*	0
CD 6	- A	Frameshift mutant**	0
CD 8/9	+ 1	Frameshift mutant**	0
Cap + 1	A C	Cap site mutant	+
IVS-I-1	G T	Splice junction change*	0
IVS-I-1	G A	Splice junction change*	0
IVS-II-654	C T	Internal IVS change*	0
CD 41-42	(-CTTT)	Frameshift mutant**	0
CD 16	- 1	Frameshift mutant**	0
IVS-I-6	T C	Consensus change*	+
CD 15	TGG-TAG	Nonsense mutant	0
-88	C T	Transcriptional mutant*	+

IVS-II-745	C	G	Internal IVS changes	+
CD 30	G	C	Consensus changes*	+
CD 17	A	T	Nonsense mutant**	+
-28	A	G	Transcriptional mutant	+

\* RNA processing mutants

\*\* Nonfunctional RNA

## RESULT

The mutations identified included: IVS-I-110, IVS-II-1, CD 39, IVS-I-1, CD 8/9, IVS-I-6, IVS-II-745, IVS-I-5, CD 6, and IVS-L-3' end and Cap+1. Of these IVS-L-5, CD 6 and IVS-L-3' end and Cap<sup>+1</sup>, were identified only in Saudis and were not found in any of the other Arabs (Table 2). The CD 41/42, CD 15 and CD 16 were not identified in any of the Arab populations.

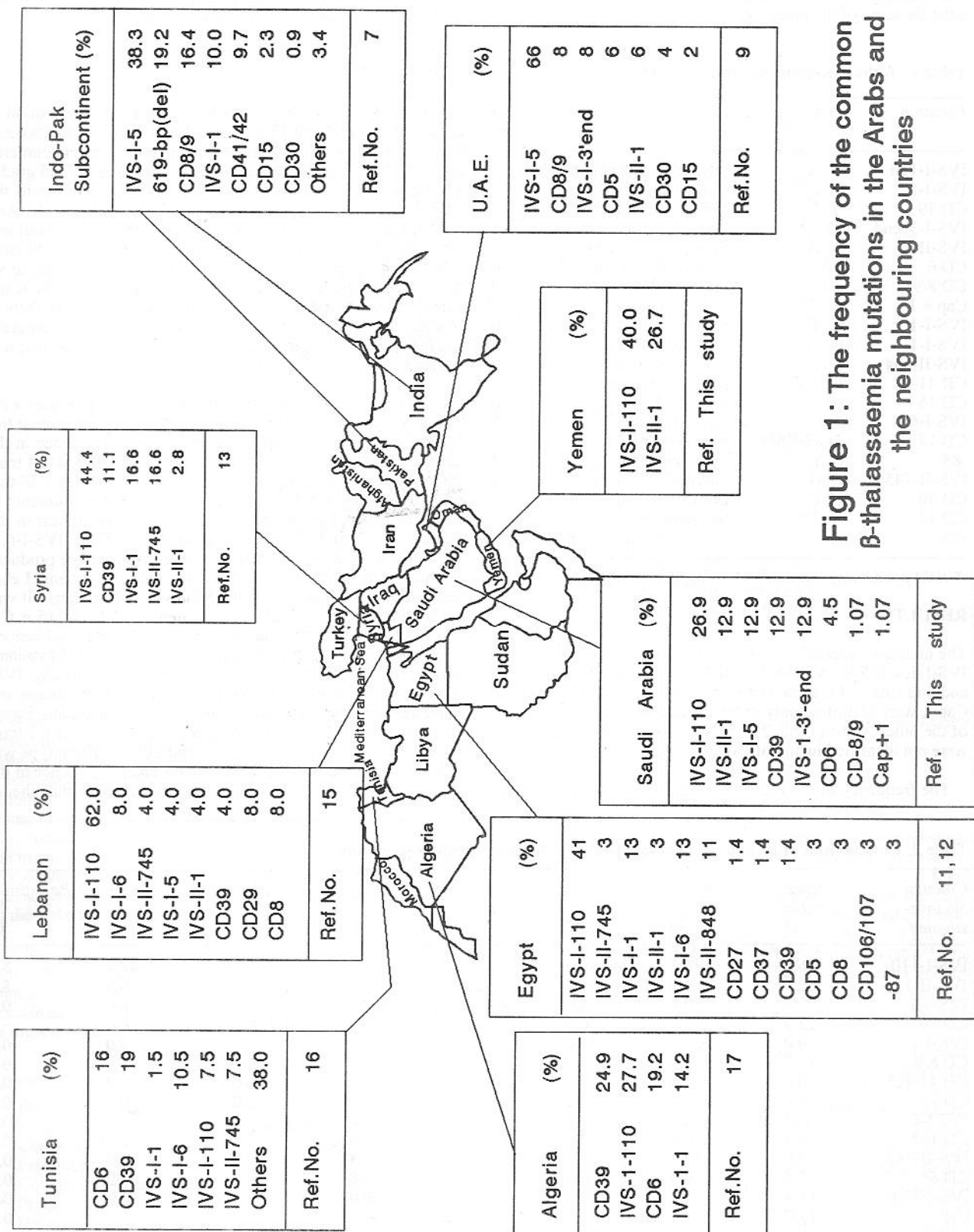
Figure 1. The frequency of the common  $\beta$ -thalassemia mutations in the Arabs and the neighboring countries

Table 2. Comparison of  $\beta$ -thalassemia mutations in different Arab populations

Country	Saudi Arabia	Jordan	Egypt	Syria	Lebanon	Yemen	Sudan	Pales- tinine
No. inve- stigated	93	39	40	36	30	15	7	12
IVS-I-110	26.9	38.5	40.0	44.4	66.7	40.0	42.9	8.3
IVS-II-1	12.9	7.7	7.5	2.8	3.3	26.7	0.0	8.3
IVS-I-5	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CD 39	12.9	0.0	0.0	11.1	3.3	0.0	0.0	0.0
IVS-I-1	0.0	5.13	15.0	16.6	-	-	0.0	0.0
CD 8/9	1.07	7.7	-	-	-	-	0.0	0.0
CD 41/42*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CD 15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IVS-I-6	0.0	5.13	12.5	-	6.6	-	14.3	0.0
CD 16*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IVS-II-745	0.0	5.13	2.5	16.6	3.3	0.0	0.0	0.0
CD 6*	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IVS-I-3'end	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cap + 1	1.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total (%)	84.94	69.2	77.5	91.66	83.3	66.7	57.2	16.6

\* Frameshift

The frequency of the identified  $\beta$ -thal mutations in the Arabs were compared and contrasted with those reported in literature for the populations in the neighboring countries. A similarity in the  $\beta$ -thal mutation frequency pattern was revealed between the Arabs and the neighboring populations (Fig 1).



**Figure 1: The frequency of the common  $\beta$ -thalassaemia mutations in the Arabs and the neighbouring countries**

## DISCUSSION

This study has revealed an interesting aspect of distribution of the  $\beta$ -thalassemia mutation in the "Arab World". Mutations of frequent occurrence in the Mediterranean region are encountered in the Arabs living around the Mediterranean region and gradually decreased eastwards and are almost non-existence in the Emirates, East of Arabian peninsula. A similar but, reverse Arabian pattern is shown for the mutations reported in the Gulf and Emirates that is closer to the pattern reported for Indo-Pakistan subcontinent population, where these mutations decrease to almost non-existence in Mediterranean Arab populations. The Kingdom of Saudi Arabia occupies a central position where there is considerable overlap between mutations both of Mediterranean and Indo-Pakistan origin, with IVS-I-110 being the most frequent mutation.

IVS-I-110, and GA transition, mutation which produces a  $\beta^+$ -thal phenotype due to defective RNA processing is the most frequent mutation in all Arabs, with the highest prevalence in the Lebanese and the lowest in the Palestinians. IVS-II-1, a GA transition mutation, affecting a splice junction, produces a  $\beta^0$ -thal phenotype due to RNA processing defect is also encountered in all Arabs except the Sudanese. Its frequency is highest in the Yemenis (26.7%), followed by the Saudis (12.9%). IVS-I-6, a TC transition mutation leading to a consensus change produces  $\beta^+$  thal and is encountered in the Jordanians, Egyptians, Lebanese and Sudanese but not in the Saudis or Palestinians. It was not studied in the Syrians and Yemenis. IVS-II-745 a C G Tran version mutation producing internal IVS change and hence a  $\beta^+$ -thal phenotype is encountered in the Jordanians, Egyptians, Syrians and Lebanese, but not in the other Arabs. Similarly, IVS-I-1 a GT, transversion producing a splice junction change and hence a  $\beta$ -thal phenotype is encountered in the Jordanians, Egyptians and Syrians, but not in the other Saudis. CD 39, a CT transition resulting in a nonsense mutant and  $\beta$ -thal phenotype was encountered in the Saudis, Syrians and Lebanese, but not in the other Arabs. IVS-I-5, a G C consensus leading to  $\beta^+$ -thal phenotype, CD 8/9 which occurs in the Jordanians. These mutations may have either occurred specifically in the Saudis or reached Saudi Arabia due to genetic drift. Among these mutations, IVS-I-5 is the most frequent mutation reported in Indo-Pakistan subcontinent and some of the Gulf countries<sup>7,9</sup>. In three of the population groups studied (Sudanese, Yemenis and Palestinians), only 2 of the mutations investigated during this study were encountered. The study group from these countries was small and the pattern of common mutations may be different in these countries.

This pattern of  $\beta$ -thal mutations points to some extent, to the pattern of population movement. Over generations due to religious reasons, there has been significant population movement from the neighboring Arab and Asian countries to Saudi Arabia and this is revealed in the overlap between Asian and Mediterranean mutations encountered in this population. However, independent mutations, particularly those occurring at a higher frequency, may have occurred in different regions.

This study highlights the local variation in the  $\beta$ -thal mutation pattern in the Arab populations. The diagnostic relevance of the findings is obvious, and hence geographical origin and other relevant consideration are required and should be considered in adopting approaches to carrier detection, pre- and neonatal diagnosis using molecular methods.

## CONCLUSION

The study has provided an overview of the nature of  $\beta$ -thal mutations in Arabs. It has also shown that there are significant variations in the pattern of  $\beta$ -thal mutations amongst Arabs from different countries. Yet due to small number of study group from some of the countries and analysis of only 20 mutations has not

given an entirely complete picture of the  $\beta$ -thal mutations in Arabs. In order to clarify the entire picture of  $\beta$ -thal mutations, there is an urgent need to conduct investigations on a larger sample size of  $\beta$ -thal cases from each of the Arab countries.

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