Spectrum of beta-thalassemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis

We present here an analysis of 888 unrelated beta-thal chromosomes consisting of 444 transfusion dependent children from various regions of Punjab and Islamabad Pakistan. By using Multiplex ARMS- PCR, restriction endonuclease analysis, allele specific oligonucleotide (ASO) hybridization and sequencing, 17 beta-thal mutations and 3 Hb variants were detected in 99.5% (884/888) of the chromosomes analyzed. First trimester prenatal diagnosis by chorionic villus sampling (CVS) was also carried out in seven pregnancies at risk of beta-thalassemia. Our results indicate that three most common mutations accounted for 86.8% of the beta-thal alleles in this region. These findings have important implications for prevention of beta-thalassemia through genetic counseling and prenatal diagnosis in this part of Pakistan.

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Beta-thalassemia is one of the commonest inherited hemoglobin (Hb) disorders with an overall carrier frequency of more than 5% in Pakistan. Approximately 40,000 cases of transfusion dependent children with thalassemia major are presently registered and each year nearly 5,000 affected children are born in this country of more than 150 million people.[1] In the developed countries the affected children receive regular transfusions with iron chelation therapy and bone marrow transplantation in selected patients results in extended disease free survival in majority of the cases.[2,3] However, like many other developing Asian countries, beta-thalassemia pose an increasing burden for health-care services in Pakistan and it is not possible to provide blood transfusions and iron chelation therapy to all patients with limited available national resources. Bone marrow transplantation is extremely expensive and unaffordable for the Pakistani patients. In countries like Pakistan, prevention is least expensive and most effective means to deal with beta-thalassemia. It is therefore prerequisite to investigate the molecular basis and natural history of these disorders to establish the most cost effective methods for their control and management.[4] There have previously been few studies on beta- thalassemia mutations in various regions and ethnic groups of Pakistan.[5-7] The mutation detection and prenatal diagnosis service for this most common genetic disorder is only offered in three major cities (Karachi, Lahore and Rawalpindi) in this country with high prevalence of thalassemia carriers. In this study, we have extensively investigated the spectrum of mutations in various regions of Punjab; a province of Pakistan with largest population size of more than 80 million people including the capital territory; Islamabad. The aim of the present study was to screen the mutations in various regions of Punjab and capital territory including some previously unscreened areas and establish a comprehensive mutation detection and prenatal diagnosis facility at affordable cost to prevent affected births in this part of the country.

Blood samples were collected from 444 unrelated families having at least one affected child consisting of 888 beta-globin genes from various regions of Punjab province including Faisalabad, Lahore, Sargodha, Dera Ghazi (D.G) Khan, Bahawalpur, Multan, and twin cities of Rawalpindi-Islamabad. Information on prenatal diag-

Table 1. Distribution of the beta-thal mutations in various areas of Punjab and Islamabad.

Serial #	*Mutations*	*Rawalpindi Islamabad Lahore Sargodha*	*Faisalabad Bahawalpur Multan*	*D.G.Khan*	*TOTAL*
01	IVS-I-5, G→C	133 (33.8%)	144 (42.1%)	69 (45.0%)	346 (38.9%)
02	Codons 8/9, +G	137 (34.8%)	138 (40.35%)	56 (36.6%)	331 (37.3%)
03	Codons 41/42, -TTCT	59 (15.0%)	26 (7.4%)	9 (5.9%)	94 (10.6%)
04	619 bp deletion	7 (1.8%)	9 (2.5%)	1 (0.6%)	17 (1.9%)
05	IVS-I-1, G→T	7 (1.8%)	2 (0.6%)	8 (5.2%)	17 (1.9%)
06	Codon 15, G→A	7 (1.8%)	4 (1.1%)	5 (3.3%)	16 (1.8%)
07	Codon 5, -CT	6 (1.5%)	4 (1.1%)	2 (1.3%)	12 (1.3%)
08	IVS-I-1, G→A	6 (1.5%)	6 (1.7%)	-	12 (1.3%)
09	Codon 16, -C	8 (2.0%)	-	1 (0.6%)	9 (1.0%)
10	IVS-II-1, G→A	4 (1.0%)	3 (0.9%)	-	7 (0.8%)
11	Codon 30, G→C	5 (1.3%)	-	1 (0.7%)	6 (0.7%)
12	Codon 39, C→T	3 (0.8%)	-	-	3 (0.3%)
13	Codon 26, G→A	2 (0.5%)	-	-	2 (0.2%)
14	Codon 30, G→A	1 (0.2%)	-	-	1 (0.1%)
15	Initiation Codon, T?C	1 (0.2%)	-	-	1 (0.1%)
16	Cap+1, A→C	1 (0.2%)	-	-	1 (0.1%)
17	-88, C→T	1 (0.2%)	-	-	1 (0.1%)
18	IVS-II-848, C→A	-	-	-	-
19	Codons 47/48 +ATCT	-	-	-	-
20	Hb D, G→C	1(0.2%)	3(0.9%)	-	4(0.4%)
21	Hb S, A→T	2(0.5%)	-	1(0.7%)	3(0.3%)
22	Hb E Codon 26, G→A	-	1(0.6%)	-	1(0.1%)
	Uncharacterized	2(0.5%)	2(0.6%)	-	4(0.5%)
	Total	393	342	153	888

**Different IgVH gene or allele, clilnically relevant change in the prognostic subgroup;° different IgVH gene or allele, no clinically relevant change in the prognostic subgroup.*

nosis and genetic counseling was provided to the families at the time of blood collection in the transfusion centers. For detection of mutations various PCR based methods including Multiplex ARMS-PCR, restrictions endonuclease analysis, radioactive and non radioactive dot blot hybridization and direct genomic sequencing were used. Multiplex ARMS-PCR was used for simultaneous detection of three most common mutations in the Pakistani population i.e. IVS-I-5 (G→C), codons 8/9 (+G) and codons 41/42 (-TTCT) using specific ARMS primers in a single reaction.

The DNA samples were screened for 19 previously reported beta-thal mutations and 3 Hb variants; Hb S, Hb D and Hb E in this population. By using the techniques mentioned above mutations were characterized in 884 (99.5%) of the alleles studied. In four sick children one allele remained uncharacterized even after sequencing. The distribution and frequencies of these mutations in various regions of Punjab and the capital territory are shown in the Table. The distribution of mutations in Punjab and the capital territory is different from the overall pattern in Pakistan as reported previously.[6,7] The three most common mutations; IVS-I-5 (G→C), codons 8/9, (+G) and codons 41/42 (-TTCT) constitute 86.8% of the alleles characterized while IVS-I-5 (G→C) was the most common mutation (39.0%) in all regions with highest in South Punjab (45.0%) i.e. D.G. Khan, Bahawalpur and Multan region whereas codons 8/9 (+G) the second most frequent mutation (37.3%) was number one in the patients from Rawalpindi-Islamabad (34.9%). The deletion of TTCT between codons 41 and 42 of the beta-globin gene was the third most common mutation (10.6%) in the overall samples analyzed in this study. The rest of 14 beta-thal mutations and 3 Hb variants (Hb S, Hb E and Hb D) were less common or rare and altogether accounted for only13.9 % of the alleles.

In this study the third most frequent mutation was codons 41/42(-TTCT) instead of deletion of 619 bp (1.8%) unlike previous reports. Punjab predominantly comprised the local Punjabi ethnic group and immigrants from Indian Punjab who settled in Pakistan after the 1947 partition of subcontinent. Although population of Rawalpindi-Islamabad consists of majority Punjabis but people from all regions of the country have migrated and settled in these twin cities due to capital of Pakistan. The genetic heterogeneity in the capital territory is reflected by the identification of 17 beta-thal alleles and two Hb variants in the patients from this area.

The South Punjab area (D.G. Khan, Bahawalpur and Multan) has not been studied exclusively in the previous reports. We analyzed 153 beta-thal chromosomes from this region and characterized mutations in 100% of the alleles by using only 9 sets of ARMS-PCR primers and ASO probes for the known mutations and HbS by restriction endonuclease analysis. The frequency of IVS I-5 (G→C) was 45.0 % which is the highest in the areas studied.

There are only a few centers located in big cities offering prenatal diagnosis of beta-thalassemia to a small fraction of Pakistani population whereas for Faisalabad the third biggest city of Pakistan with associated large rural population and surrounding areas no such facility exists. We established the first trimester prenatal diagnosis by chorionic villus sampling (CVS) under the framework of this study. Based on this mutation data, retrospective first trimester prenatal diagnosis through chorionic villus sampling (CVS) was carried out in 7 pregnancies at risk of having affected child.[8] The CVS was taken at 11-15 weeks of gestation and mutations were characterized by ARMS-PCR. Out of seven, two pregnancies were homozygotes for beta-thalassemia and the couples opted for termination of the pregnancy, four were carriers and one fetus was negative for any beta-thal mutation. The awareness among the local population is increasing through our genetic counseling and thalassemia education program as an increasing number of couples with affected children are contacting us for genetic counseling and prenatal diagnosis.[10] On the basis of this analysis, it is suggested to identify the carriers of beta-thalassemia in relatives of each patient, educate them and communicate the consequences of marrying another carrier.

Lack of a national level screening program for beta-thalassemia and tradition of consanguineous marriages in Pakistan has resulted in a large number of carrier couples who have 25% risk of having an affected child in each pregnancy. Majority of these couples are from the low income rural population groups with very little or no access to the medical facilities. Screening of all pregnancies and prenatal testing of at-risk fetuses are strongly advocated as the cost of screening is much less than the postnatal service cost.[9] Establishment of screening facilities for all pregnancies at the major public and private sector hospitals in Pakistan will help to control the birth of affected children.

This data strongly supports and provides the basis for the establishment of mutation detection and prenatal diagnostic service at NIBGE for the whole country in general and for Faisalabad and surrounding areas in particular for prevention of affected births in Pakistan. Although a complete prevention of thalassemia in Pakistan seems not possible due to several factors including large population size, limited available medical facilities, a low literacy rate and poor economic condition of general public but by looking at the present developing awareness, increasing literacy rate and positive response of the families to genetic counseling and prenatal diagnosis it is expected that rate of annual affected births will come down gradually.

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