

Original Article

Molecular epidemiology of β -thalassemia in Pakistan: far reaching implications

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Abstract: β -thalassaemia, an autosomal recessive hemoglobinopathy, is one of the commonest genetically transmitted disorders throughout the world. Collective measures including carrier identification, genetic counseling and prenatal diagnosis are required for preventing β -thalassaemia. To achieve this objective, Identification of the spectrum of genetic mutations, especially for various ethnic backgrounds in Pakistan is necessary. Therefore, we designed a cross sectional prospective study to identify the frequency of various gene mutations in different ethnic groups of Pakistan. Over a 5-year period, DNA from 648 blood samples [including specimens of chorionic villus sampling (CVS)] were analyzed for the twelve most common β -thalassaemia mutations found in the Pakistani population by a Multiplex amplification refractory mutation system (ARMS). The most common mutation identified was Intervening Sequence 1-5 (IVS 1-5 (G-C)); accounting for 40.89% mutated alleles, and was represented in all ethnic groups. 15.7 % of the β -thalassaemia alleles were found to have Frameshift 8-9 (Fr 8-9) as the second most common mutation Other common genetic defects responsible for β -thalassaemia: IVS 1-1 (G-T) was found in 8.17%, Codon-30 (Cd-30 (G-C)) 8.02%, Codon-5(Cd-5 (-CT)) contributed 2.16% and Deletion 619 base pair (Del 619bp) affected 11.11% were found in Pakistan. This large study adds to the pre-existing data in Pakistan. Knowledge of the predominant mutation in a given ethnic group will not only help in developing a short panel of (population-specific) primers of mutations thereby providing a cost-effective method for prenatal diagnosis and also help the clinicians to counsel regarding blood transfusion regimen/ pregnancy termination.

Keywords: β -thalassaemia, genetic mutations, molecular epidemiology, Pakistan

Introduction

Thalassaemias are a heterogeneous group of inherited disorders of hemoglobin synthesis resulting in life-threatening anemia and requiring regular blood transfusion for survival [1]. It's particularly associated with people of Mediterranean, Indian subcontinent and Middle East origin. The World Health Organization (WHO) has identified control of haemoglobinopathies, particularly β -thalassaemia, in developing world, as a priority [2]. An estimated 5000-9000 children with β -thalassaemia are born per year, although no documentary registry is available in Pakistan. The estimated carrier rate is 5-7%, with 9.8 million carriers in the total population [3].

With gradual control of malnutrition and com-

municable diseases, β -thalassaemia major patients who earlier died young are now surviving long enough to seek medical attention. In developing countries like Pakistan, this poses an increasing burden for health-care services as adequate blood transfusions with effective iron chelation and bone marrow transplantation is affordable for just a few; therefore, prevention has been demonstrated to be the way forward. Effectiveness of a 20-year control program in Sardinia is evidenced by reduction of the birth rate of thalassaemia major from 1:250 live births to 1:4000 [4]. In 1995, 1999 and 2004, 296, 94 and 56 β -thalassaemia homozygote, respectively, were born (2.53, 1.07 and 0.82 patients per 1000 births) - thanks to a 10-year program in southern Iran [5]. Population screening, genetic counseling, prenatal diagnosis and option

of terminating affected pregnancies remain the mainstay strategy to devise a control program and investigating the underlying molecular defects in β -thalassemia is an important prerequisite for such programs. In Pakistan most of the patients live in rural areas but those who live in cities do have access to prenatal diagnosis services which are mostly run by charity organizations.

Currently, 217 causative molecular defects have been described so far in the β -globin gene causing β -thalassemia [6]. About 20 mutations account for 90 percent of β -globin genes in the world and it is noted that each ethnic population has its own unique set of most frequent mutations. Previously, there have been few studies investigating the spectrum of β -thalassemia mutations in various regions and ethnic groups of Pakistan [7, 8]. The aim of this study is to identify the frequency of various mutations in Karachi, the largest cosmopolitan city of Pakistan with a population in excess of 10 million, having a significant representation of all the major ethnic groups and to formulate a comprehensive and affordable mutation detection strategy for control of β -thalassemia.

Materials and methods

The studied population included members of the five major ethnic groups in Karachi: Punjabi, Pathan, Sindhi, Baluchi, Immigrants (from India after the 1947 partition of Subcontinent) and others like Saraikees, Kashmiri, Memon and Hazara as well. Over a period of five years, collected venous blood samples (in EDTA) from 466 individuals having at least one affected family member known to have β -thalassemia major/ HbE- β -thalassemia/ HbE homozygotes/ β -thalassemia trait. Chorionic villus sampling (CVS) at 11 to 15 weeks gestational age for 143 couples referred by thalassemia clinics (for pregnancies at risk of having affected child) was also used to obtain allele information. In all, 648 mutated alleles were identified. The diagnosis of β -thalassemia trait, β -thalassemia major and Hb E thalassemia were established from clinical data, hematological indices and hemoglobin electrophoresis (cellulose acetate hemoglobin electrophoresis was done by HELENA SAS-MX manual gel tank system), DNA was extracted from whole blood by using Genomic DNA Purification Kit (Gentra system USA). For detection of mutations, a PCR based method Multiplex ARMS was used which previously has been

used as a mutation-screening tool as mentioned in a number of publications [9, 10]. Primers were designed for simultaneous detection of the following previously described mutations [7, 8, 10] in a single reaction: IVS 1-5(G>C), Fr 8-9 (+G), IVS 1-1(G>T), Cd-30(G>C), Cd-5(-CT), Del 619bp, Cd-15(G>A), Fr 41-42(-TTCT), Fr 16(-C) and Cap +1(A>C) along with two Hb variants: HbS and HbE.

Results

By using the methods mentioned above, mutations were characterized in 640 (98.75%) of the alleles studied; however, there were eight instances where the allele remained uncharacterized. The population study included 53% males and 47% females. Also included were 143 CVS specimens, which showed homozygosity in 45 CV samples whereas 89 of the CV biopsies showed mutations in one allele and were diagnosed as carriers; 9 were normal. The overall distribution of the β -thalassemia mutations in different ethnic groups of Pakistan settled in Karachi is summarized in **Table 1**. The genetic heterogeneity in Karachi is reflected by the identification of all the common β -thalassemia alleles and two Hb variants but following eight mutations were more common: IVS 1-5(G>C), Fr 8-9(+G), Del 619 bp, IVS 1-1(G>T), Fr 41-42(-TTCT), Cd-30(G>C), Cd-5(-CT) and Cd-15(G>A), accounting for 93.9% of the β -thalassemia alleles. However, the distribution was uneven as depicted in **Figure 1**.

Although IVS 1-5(G>C) was the most common mutation (40.89% of the sample), its frequency varied from 20% in the immigrant (from India) population to 76.9% in the Balochis. It should be noted that southwest Iran which shares border with Balochistan also has a high prevalence of IVS 1-5(G>C) [11]. The second most frequent mutation was Fr 8-9(+G), constituting 15.7% of the allele pool. Indeed, Fr 8-9(+G) was the most common mutation in the Pathans (31.3%) as it was in people of Saraikee origin (47%). Higher prevalence of Fr 8-9(+G) in the Pathans has been reported previously also [7, 8] but other studies [15] have commented that IVS 1-5(G>C) is the most common mutation in southern Punjab- the Saraikee belt. This can be attributed to the small count of Saraikee individuals in our study cohort. Deletion 619 bp was found to be the third most common, 11.11% and interestingly, it was identified most commonly (44.6%) in members of the Memon community. The

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Table 1. Distribution of β -thalassemia mutations in Pakistan's various ethnic groups

Mutation	Sindhi	Baloch	Pathan	Punjabi	Immigrant (India)	Saraikkee	Memon	Hazara	Total
IVS1-5(G-C)	134	60	19	22	13	3	14	-	265
Fr 8-9	38	7	21	10	12	8	6	-	102
Del 619	34	-	1	-	7	1	29	-	72
IVS1-1(G-T)	30	4	-	4	5	-	10	-	53
Cd-30(G-A)	30	-	11	4	1	2	4	-	52
Fr 41-42	9	-	1	8	12	2	-	3	35
Cd-5(-CT)	4	-	8	1	1	-	-	-	14
Cd-15(G-A)	8	2	-	4	2	-	-	-	16
Cap +1(A-C)	2	-	1	2	2	-	1	-	8
Fr 16(-C)	-	3	3	-	-	-	0	-	6
Hb S	-	3	-	-	1	-	-	-	4
Hb E	5	-	-	4	3	-	1	-	13
Uncharacter-ized	-	-	2	1	4	1	-	-	8
Total	294	78	67	60	63	17	65	3	648

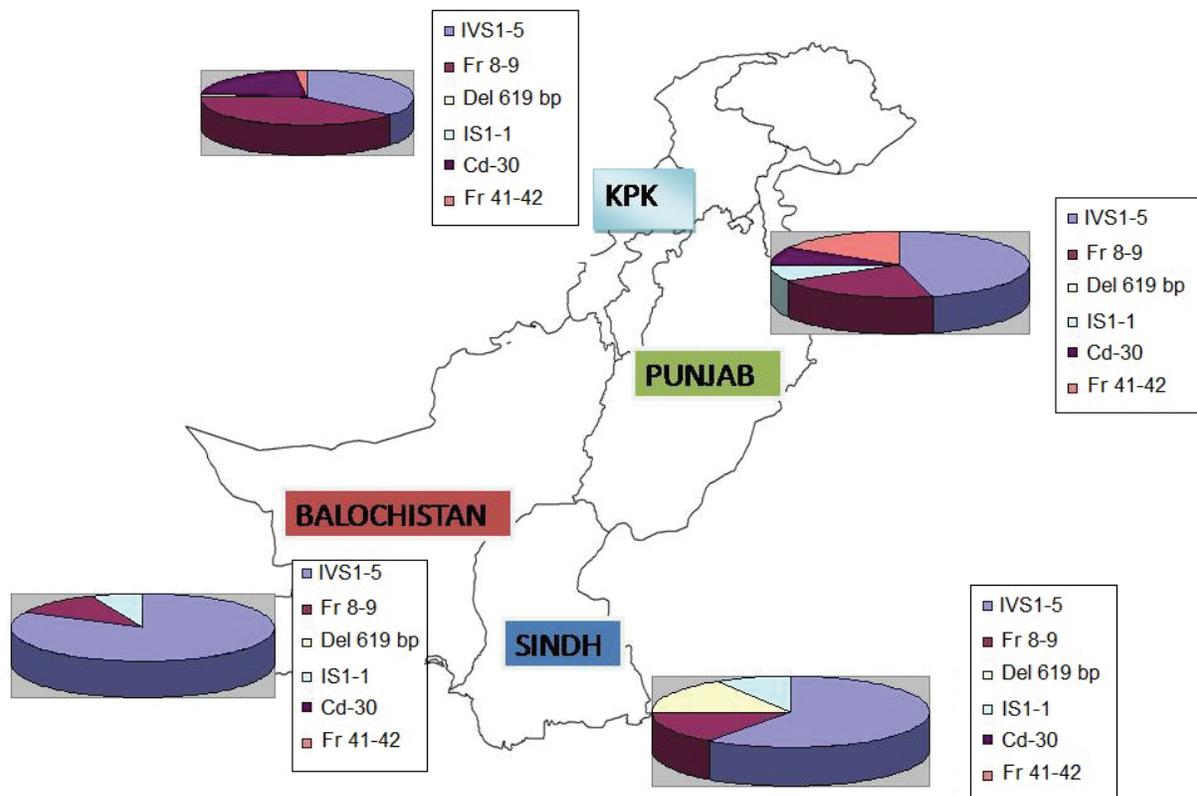


Figure 1. Distribution of different genetic mutations in different provinces.

same has been reported by Colah R et al [12] in Gujrat, India as well for the Lohana community (historically, after accepting Islam the Lohanas were titled as Memons).

Discussion

Pakistan is a country with a remarkable racial mix from a long history of invasions and com-

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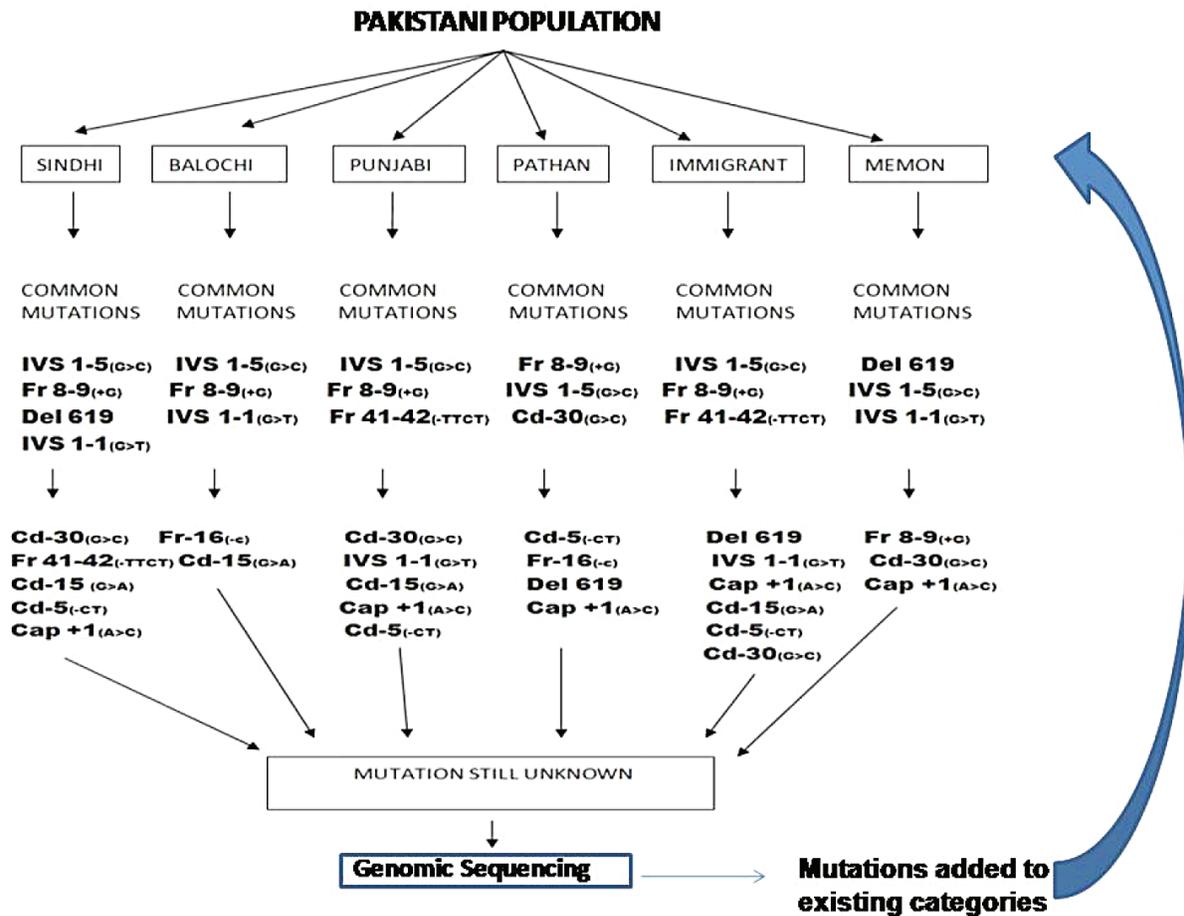


Figure 2. Individual with β -thalassaemia trait based on haematological indices.

mercial interactions leading to considerable genetic diversity. This supplemented by a strong cultural preference for consanguineous marriage has been responsible for a relatively high prevalence of recessively inherited disorders like β -thalassemia. To sustain children affected with β -thalassemia, monthly blood transfusions accompanied by iron-chelation therapy is needed and requirements for treating one annual birth cohort for one year are 90,000 units of blood plus 22 million dollars worth of deferoxamine [3]. Genetic risk factor information such as identification of β -globin gene mutations should be integrated routinely into epidemiological studies [13], followed by genetic counseling and prenatal diagnosis to reduce birth rate of affected infants [2, 4] to undo the financial burden caused by this disease. We elucidate with this study, the frequencies of different β -thalassemia mutations in the residents of Kara-

chi of various origins. This city is the financial hub of the country and therefore, people from all regions of Pakistan have migrated over here. Also are included the (post Indo-Pak partition) immigrants from India. The resultant genetic heterogeneity can be seen by the identification of all common β -thalassemia mutations. Results are coherent with statistics provided by Ahmed S, et al [7] in 1996 by characterizing 1216 alleles and also with those given by Khan SN and Riazuddin S [8] who had analyzed 602 β -thalassemia mutations. These investigators reported a spectrum of 19 mutations and found IVS 1-5(G>C) to be the commonest mutation with greater prevalence in Southern Pakistan whereas Fr 8-9(+G), the second most common mutation was found more in Northern Pakistan. We also noted that eight rather than five (as previously reported) mutations comprised 93% of the spectrum. Another facet pointed by this

analysis is the heterogeneity of genetic spectrum in the immigrant (from India) population residing in Sindh. These people migrated from all parts of the present India at the time of partition of Sub-continent and thus represent the same genetic versatility as has been reported by investigators from India [12] which itself has an ethnically diverse population. Because of the practice of endogamy, gene variants are trapped within extended family groupings and biradris. Lack of a national level screening program and the limited chances of success for changing the social structure on a large scale to avoid consanguineous marriages (which actually does not have a sound ethical footing as well [16]) emphasize the need for a policy to provide both family studies and premarital or antenatal screening for the relatives of affected children. Carrier diagnostics at the hematological and HPLC level are the key elements of routine epidemiological study and identification of the mutation and genetic counseling is the procedure reserved to the couples at risk. Knowing the specific mutation panels relevant for the various ethnicities will improve the feasibility of DNA diagnostics in developing countries like ours. Indeed, mutation micro mapping on a caste and biradri basis might serve the purpose even better as differences have been noted in this sub-classification as well [14]. Efforts should be made to increase awareness about the available diagnostic facilities for the prenatal diagnosis in Pakistan.

Based on the outcome of our study, we have formulated a cost effective proposal for detection of β -thalassemia mutations as shown in **Figure 2**. The strategy is based on first inquiring the ethnic background of the individual with β -thalassemia trait so that DNA can be 'triaged' to the 'common mutation panel' found in the particular ethnic group. If the mutation is not found in the common mutation panel, the DNA is further tested using the uncommon mutation panel. If mutation is not identified in primary and secondary panels as suggested in **Figure 2** then sample should be send to reference lab for DNA sequencing. Lastly, it is also worth mentioning that genetic epidemiological studies like ours have more than local relevance- the information can be used in other countries like the USA, Canada, Europe and Australia in which there is a major influx of Pakistani migrants who present as ethnic minorities for diagnosis, counseling and management.

Take home message: IVSI-5(40.89%), Fr8-9 (15.7%), & IVSI-I(8.17%), were the most common genetic mutations identified in Pakistan. Knowledge of the predominant mutation in a given ethnic group will not only help in developing a short panel of (population-specific) primers of mutations thereby providing a cost-effective method for prenatal diagnosis and also help the clinicians for genetic counseling and pregnancy termination.

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