International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: 10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Cross Sectional Analysis indicates that alpha thalassemia deletion mutation is prevalent in a South Indian Population

C. Maheswari, A. Sheebanancy, S. Himalini and V. J. Kavitha*

Department of Biotechnology, Mother Teresa Women's University, Kodaikanal– 624101 India

***Correspondence Info:**
Dr. V.J. Kavitha
Assistant Professor
Department of Biotechnology
Mother Teresa Women's University
Kodaikanal– 624101 India.
E-mail: kavithahla@gmail.com

Abstract

Thalassemia is a group of genetic disorders characterized by quantitative defects in globin chain synthesis. Deletions of either one (α -thalassemia 2) or both (α -thalassaemia 1) α-globin genes on chromosome 16 account for over 95% of alpha thalassemia cases. The subsequent absence or decrease of hemoglobin production results in microcytosis with varying degrees of anemia. The present study was undertaken to investigate the prevalence of α thalassemia in a South Indian population, Nadars, inhabiting Tamil Nadu and to compare them with Asian Populations. 31 random samples were collected and genotyped using Gap PCR. Statistical analysis was done using Excel, ARLEQUIN, MEGA and GRAPH PAD. The study population Nadars has a frequency 32% of αα/-α3.7. The two-tailed p value for Fisher's exact equals 0.0033, highly statistically significant. The dendrogram shows that Nadars clubbed with Malay population that showed a double mutation for -4.2. This means that the mutation rate for α thalassemia in both populations is significant and Nadars need to be studied in depth for the -4.2 mutation. The principal component analysis pointed out Nadars is perhaps unique because of αα/-α3.7 genotype and the need of the hour is to screen all south Indian populations for thalassemia mutations in general and alpha thalassemia in particular.

Keywords: Alpha thalassemia, Gap PCR, Mutation, Genotyping

1. Introduction

The thalassemia are among the most common genetic disorders worldwide, occurring more frequently in Mediterranean region, the Indian subcontinent, Southern Asia, and West Africa [1-3]. Thalassemia is a hemoglobin disorder characterized by the absence or reduced synthesis of globin chains, α, β, γ, δ and ζ of human Hb. The two main types of thalassemia are α- and β-thalassemia [4,5]. Hemoglobin (Hb) is the oxygen-carrying protein packaged within circulating erythrocytes. It has been extensively studied in terms of its structure function relationship, genetics and hereditary disorders [6].

Alpha-thalassemia is the most common hemoglobin disorder in the world. Deletions of either one (α -thalassemia 2) or both. (α -thalassaemia1) α-globin genes on chromosome 16 account for over 95% of alpha thalassemia cases [7-9]. Genomic deletions involving the α-globin gene cluster are the most common molecular defects causing α-thalassemia (α-thal), a recessively inherited disorder characterized by a quantitative reduction of α-globin chain production leading to a mild microcytic and hypochromic anemia, HbH (β4) disease or Hb Bart's (γ4) hydrops fetales[10].

Alpha thalassemia is divided into deletion and non-deletion types. There are at least 40 different deletions in this locus. The size of the deletion is important and affects the clinical phenotype of hydrops fetalis. The globin gene cluster occurs on the short arm of chromosome16 and includes the α globin genes as well as the embryonic genes. Common α-thalassemia deletions that spare the embryonic gene allow for the production of functional embryonic hemoglobin early in gestation. In contrast, the large deletions lack the benefit of embryonic hemoglobin. These large deletions are particularly severe. Non-deletion mutations may have a more severe phenotype than most of the deletion mutations. The most common non-deletion α-thalassemia mutation is Hemoglobin Constant Spring; this mutation of the stop codon results in 31 amino acids being added to the α

chain. Mutations of a terminal codon to a coding sequence often lead to an elongated α chain that is unstable and produced at a very limited rate [11-13].

Genes that regulate both synthesis and structure of different globins are organized into 2 separate clusters. The α-globin genes are encoded on chromosome 16 and the γ, δ, and β- globin genes are encoded on chromosome 11. Each individual normally carries a linked pair of α-globin genes, 2 from the paternal chromosome, and 2 from the maternal chromosome. Therefore, each diploid human cell has four copies of the α- globin gene. The four α-thalassemia syndromes thus reflect the disease state produced by deletion or no-function of one, two, three, or all four of the α-globin genes [14](Table 1).

Table 1: Characteristic of the Thalassemia Syndromes [15]

Syndrome	Molecular basis	Laboratory values	Clinical Feature
α Thalassemia			
α Thalassemia silent carrier	One α - gene deletion (α-/αα) Heterozygous α-thalassemia	No anemia or RBC morphology abnormalities; Asymptomatic may have l-2 % Hb Bart's at birth	Asymptomatic
α Thalassemia trait (minor)	Two α-gene deletion (--/αα) Heterozygous α-thalassemia-1 Two α-gene deletion (-α/-α) Homozygous α-Thalassemia 2	Mild anemia, microcytosis, and hypochromia; 4-6% Hb Bart's at birth	Asymptomatic
Hb H disease (Hb variants related to mutation in α-globin chain)	Three α-gene deletion (--/-α) α-thalassemia-1 α- thalassemia-2 Hb Constant Spring α-thalassemia-1/Hb Constant Spring	Moderate anemia, microcytosis, hypochromia, RBC fragments; Hb Bart's prominent at birth α- chain has extra 31 amino acids	Jaundice, gallstones, splenomegaly, occasionally need transfusion; antioxidant drugs can precipitate hemolysis
Hb Bart's Hydropsfetalis	Four α-gene deletion (--/--) Homozygous ao thalassemia	Severe anemia, nucleated RRCs; only Hb H, Bart's, and Portland present	Death in utero or shortly after birth

α-globin genes and its two pseudo genes are embedded within two highly homologous 4 kb duplication units. Single gene deletions are either the leftward type involving the X homology blocks and deletion of 4.2 kb DNA, or the rightward type, involving the Z homology blocks and deletion of 3.7 Kb of DNA. Both types of single alpha gene deletions occur due to mispairing of homologous sequences within Z or X homology blocks. Unequal crossing over results in the deletion of one alpha gene on one chromosome (-α) and triplicated alpha genes on the other (ααα)[14]. Alpha thalassemia has been documented in different areas of India [16] (Figure 1).

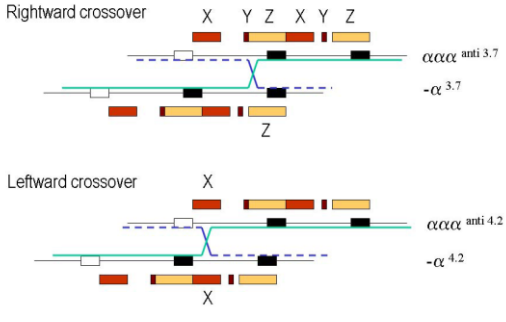

Figure 1: Displaced, but homologous, crossing-overs which produce the –a3.7 (Z boxes) and the –a4.2 (X boxes)[20]

The large Indian population is multiethnic and divided into subgroups, which practise caste endogamy and clan exogamy. Various evolutionary forces such as natural selection, mutation and recombination, migration and genetic drift play an important role to regulate the frequency of the mutation. Two of the α-globin gene deletion mutants studied (-3.7 and -4.2) appeared to be present in tribal as well as non tribal population of Eastern India along with β- globin gene defects[17,18].

Dravidians of South India is more a culture and a unique social institution, with aunique linguistic family subdivided into many gene pools, differing in their origin, migration and settlement [19]. Nadars a Tamil speaking Dravidian caste are concentrated in Chennai, Madurai, Theni, Virudhunagar, Thirunelveli, Kanyakumari and Nagercoil districts of Tamil Nadu. In terms of occupation they were regarded initially as "toddy-tappers" while latter adapted into, merchants, professionals and agriculturists. They are considered as one of the earliest inhabitants of South India [20]. The present study aims to provide data on the incidence of the various forms of *α* globin in Nadars, a large south Indian in the context of their laboratory presentations, and to examine the data to elucidate the possibility of an ethnicity based alpha thalassemia screening programme.

2. Methods

It was ensured the volunteers participating in this study were all informed of the purpose and outcome of the

study. For genotyping purpose random samples one per household were selected from Nadar population. All the volunteers were above 18 years of age. Mentally disabled and sick people were excluded from this study. A total of 31 samples were collected from Kanyakumari District, Kodaikanal, Palani, Kerala, Theni district, Virudhunagar District, Nellai, Vilupuram. The investigation was done in accordance with the ethical principles outlined by the Indian Council of Medical Research (ICMR) guidelines for medical research involving human subjects and informed consent was obtained from Institutional Ethical clearance of Mother Teresa Womens University. DNA was extracted from mouth wash using a modified method of Ausubel *et al*[21].

Alpha thalassemia Gap PCR Insertion/Deletion polymorphism was done using specific primers to amplify the fragment from the isolated DNA as per [22].

a2/3.7-F CCCCTCGCCAAGTCCACCC
3.7/20.5-R AAAGCACTCTAGGGTCCAGCG
a2/3.7-F CCCCTCGCCAAGTCCACCC
a2-R AGACCAGGAAGGGCCGGTG
4.2-F GGTTTACCCATGTGGTGCCTC
4.2-R CCCGTTGGATCTTCTCATTTCCC

The final volume of the reaction mixture was 25 µl which contained, 8.7µl of Double distilled water, PCR Buffer 3µl, Mgcl2 2.4µl, dNTP 2.4µl, primer 1.5µl of a2/3.7 forward, 1.5µl of a2/3.7 reverse, 1.5µl of a2 R, 3µl of a2/4.2 forward 3µl of a2/4.2 reverse, 0.6µl of Taq DNA polymerase, 100ng DNA.

The thermal cycling was carried out in Cyberlab. The PCR cycling conditions were initial denaturation was at 96^0C for 2 minutes.30 cycles of denaturation at 96^0C for 1 minutes which followed by primer annealing at 55^0C for 15 minutes and by extension at 72^0C for 3 minutes. Final extension of 72^0C for 10 minutes was given. Modifications were made to the annealing and extension temperature and the concentration of DNA to achieve successful amplification.

This polymorphism is defined by the presence of aa3.7 jxn fragment (2022/2029 bp), a2 gene (1800 bp),-a4.2 jxn fragment (1628 bp).

Data was also collected from the published literature for the allele and genotype frequencies of Alpha thalassemia and compared with the study population. Frequency tables were created using Excel (2007). The frequency data was converted into genetic distances in Arlequin (v3.1). Dendrograms were constructed using Molecular and Evolutionary Genetics Analysis (MEGA v3.1). The genetic distances were also used for Principal Component Analysis (PCA) in Genetic analysis in Excel (GenAlEx v6.2). To see the prevalence of the predisposing genotype or allele for the Alpha thalassemia polymorphisms, the populations were named according to the ethnic group.

Statistical Analysis:

Genotype absolute

Analyze a 2x2 contingency table

Indian (Reference Standard)	33	19	52
Nadar	29	2	31
Total	62	21	83

Fisher's exact test:

The two-tailed P value equals 0.0033. The association between rows (groups) and columns (outcomes) is considered to be very statistically significant. The total Nadar population as per the census of India 2011, in Tamilnadu is 6,24,05,679. 1,99,69,817 will be silent carriers for alpha thalassemia deletion mutation – a3.7. 49, 92, 454 will be double mutants for -a3.7 deletion mutation if the silent carriers marry among themselves. If at least 50% of the cases have the mutation in cis orientation and they marry among themselves then the genetic load for a Barts hydropsis in the next generation is 6, 24,057.

3. Result and Discussion

Three Genotypes were identified aa/aa and aa/-a3.7 as shown in Figure 2.

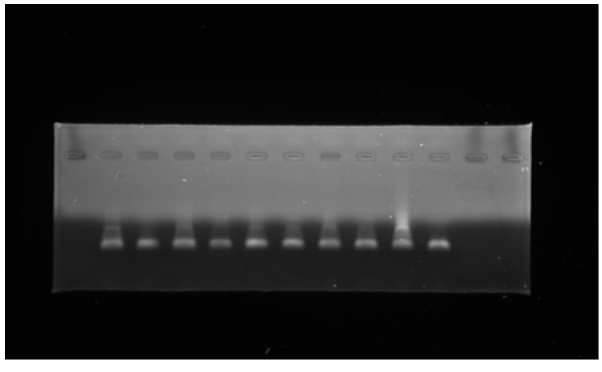

Figure 2: Gel photo of Alpha Thalassemia Deletion Mutation in Nadar population

The graphical representation showed that the Normal (αα) genotype is more in the study population and also in compiled populations. Sarawak, Orang Asli, Sahara, Kuda and South East Asian have equal frequency of double homozygote –α3.7/-α3.7 about 19%. The Heterozygote –α4.2/αα genotype was comparatively low in the compiled study population. The Nadar population has a frequency of 68% for αα/αα and 32% of αα/-α3.7 genotype. The Malay was the only population that showed a double mutation for -4.2.

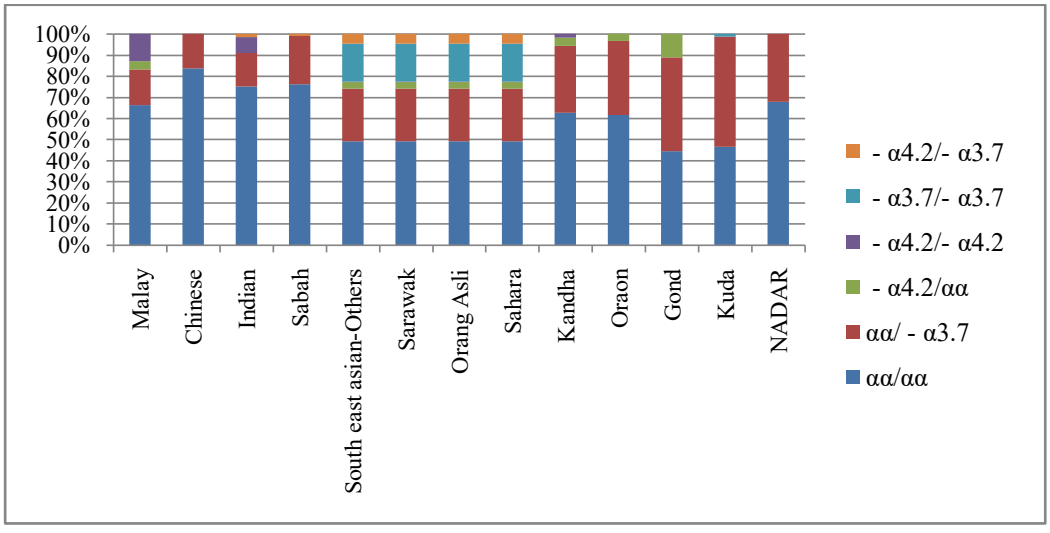

Figure 3: Graphical Representation of Asian Populations with Nadar

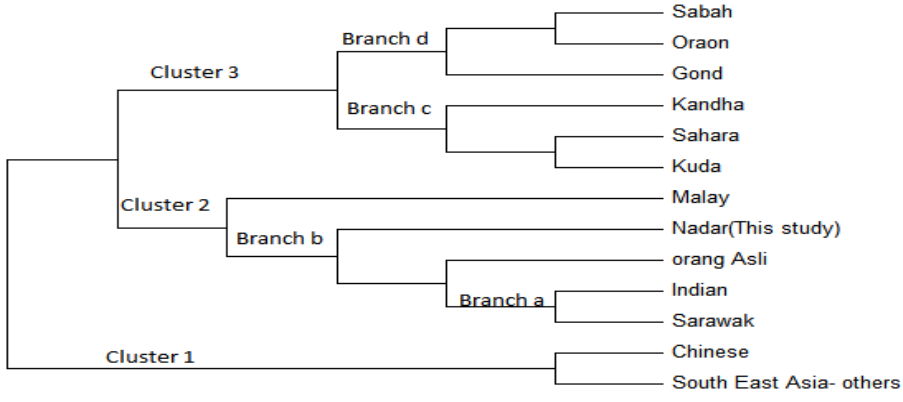

Figure 4: UPGMA dendrogram for Compiled Populations and Nadar population

The cluster analysis showed three major clusters. In cluster 1 Chinese clubbed with South East Asia. In cluster 2 Indian clubbed with Sarawak in 'a' branch and another separate branch was found for Orang Asli. In branch 'b' Nadar population clubbed with Malay. In the cluster 3 Sahara clubbed with Kuda in branch 'c' and Kandha was a separate lineage. Sabah clubbed with Oraon in branch'd' and Gond fell in the separate lineage. Nadar population clubbed with Indian Population.

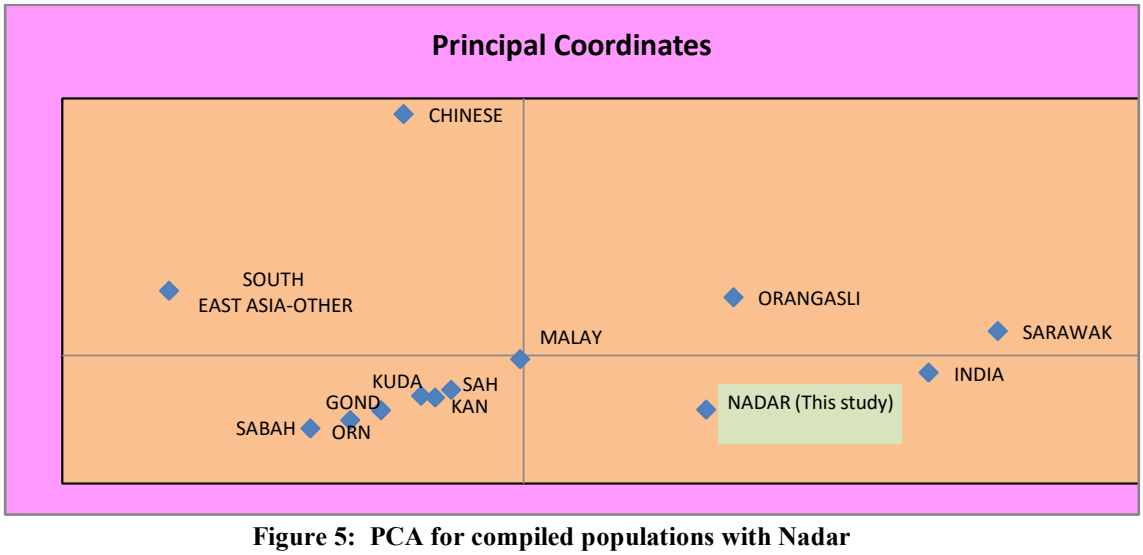

Figure 5: PCA for compiled populations with Nadar

The principle component analysis (PCA) for compiled population with Nadar population showed that Chinese and South East Asia-Others fell in the upper left quadrants. Kuda, Sahara, Kandha, Gond, Oraon, Sabah fell in the lower left quardrant. Malay was a center mid-point in the PCA. Orangasli and Sarawak fell in the upper right quadrants. India fell in the lower right quadrants. The Nadar population also fell in the lower right quadrant. The PCA for ethnic populations complied along with this Nadar population indicated that Nadar were distinct form Indian populations studied.

4. Conclusion

This study identified the genotype frequency for –a3.7 to be 32%. This frequency is the highest in the complied populations. The significance is that this high frequency is for heterozygote for silent carrier. So it is imperative to consider the genetic mutational load for future generations in regard to alpha thalassemia mutations. Because given the large size of the population the chances of double homozygotes are increased considerably. The need of the hour is to screen large populations for a thal and create awareness for preventing marriages between heterozygotes or silent carriers.

References

[1] Momin Abdulrahaman A, Mangesh P Bankar, Gouri M Bhoite The prevalence of α Thalassemia in South Western Maharashtra. *Biomedical Research* 2012; 23 (1): 152-154.

[2] Mohanty, D. Genetic disorders in haematological practice in India. *Community Genet*. 2002; 5(3):197-200.

[3] Molecular Basis of Thalassemia Michela Grosso, Biochimicae Biotecnologie Mediche, University of Naples Federico IIItaly 2012.

[4] Chong,Y.Met al., 2006 Screening Of Concurrent A-Thalassaemia Thalassaemia Carriers.

[5] Chen, FE, Ooi C, Ha SY, et al. Genetic and clinical features of haemoglobin H disease in Chinese patients. *N Engl J Med* 2000; 343: 544-50.

[6] Waye John S.PhD David H.K. Chui, MD clinically important genes The α-globin gene cluster: genetics and disorders. *Clin Invest Med* 2001; 24(2):103-9.

[7] George E. Beta-thalassemia major in Malaysia, an Ongoing Public Health Problem. *Med J Malaysia* 2001; 60(1): 397-400.

[8] George E. Thalassaemia carrier diagnosis in Malaysia. Thalassaemia Diagnostic Communityin Change. Berkerley: University of California Press.

[9] Fucharoen, S, and Winichagoon, P. Haemoglobinopathies in Southeast Asia: molecular biology and clinical medicine. *Hemoglobin*. 1997 Jul; 21(4):299-319.

[10] Higgs D.R., Weatherall D.J., The Alpha Thalassemias, *Cell .Mol. Life Sci.* 66 (2009) 1154–1162.

[11] Vichinsky EP. Alpha thalassemia major--new mutations, intrauterine management, and outcomes. *Hematology Am Soc Hematol Educ Program*. 2009:35–41.

[12] Singer ST, Vichinsky EP. Changing outcome of homozygous alpha-thalassemia: cautious optimism. *J Pediatr Hematol Oncol.*2000; 22:539-542.

[13] Lorey F, Vichinsky EP, et al. Universal newborn screening for Hb H disease in California. *Genet Test*. 2001; 5:93-100.

[14] Higgs, D. R., et al. A review of the molecular genetics of the human alpha-globingene cluster. *Blood* 1989; 73: 1081-1104.

[15] Tangvarasittichai Surapon. Thalassemia Syndrome, Advances in the Study of Genetic Disorders, Dr. Kenji Ikehara (Ed.), 2011 ISBN: 978-953-307-305-7, InTech, Available from:http://www.intechopen.com/books/advances-in-the-study-of-genetic-disorders/thalassemia-syndrome.

[16] Brittenham, G., Alpha globin gene number: population and restriction endonuclease studies. *Blood* 1980; 55:706-708.

[17] Gajra, B., Chakrabarty, S., Sengupta, B., Bose, S., Dasgupta,I., De. M. and Talukder, G. Molecular heterogeneity of alpha thalassaemia and its relationship with other haemoglobinopathies in a tribal population from Sian village, Birbhum, West Bengal. *The Nucleus*, 2003; 46 (1&2):29-33.

[18] Sen, R. and Talukder, G.: Alpha thalassaemia- A Review. *The Nucleus*, 2005; 48(3):111-134.

[19] Pitchappan RM. Castes, migration, immunogenetics, infectious diseases and South India. *Community Genetics*, 2002; 5: 157-161.

[20] Harteveld CL, Losekoot M, Haak H, Heister GA, Giordano PC, Bernini LF: Anovel polyadenylation signal mutation in the alpha 2-globin gene causing alpha thalassaemia. *Br J Haematol* 1994; 87:139-143.

[21] Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K. Current Protocols in Molecular Biology (Wiley, New York) 2001.

[22] Tan Arnold S.-C, Thuan C. Quah, Poh S. Low and Samuel S. Chong A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for a – thalassemia. *Blood* 2001; 98: 250-25.1 doi:10.1182/blood.V98.1.250.