Molecular Heterogeneity of Hb H Disease in India

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Abstract: Alpha thalassemia is an autosomal recessive disorder caused by large deletions and/or point mutations in the α-globin genes. Hemoglobin H (Hb H) disease is most frequently due to deletion of three of the four α-globin genes associated with variable clinical severity depending on the genotype. There are few reports on Hb H disease in Indians where genotyping has been done and we have reviewed the molecular and clinical heterogeneity of these cases. An electronic search for relevant articles was conducted using two journal databases, i.e., PubMed and Science Direct using the key words “Hb H Disease”, “Hemoglobin H”, “α-thalassemia”, “mutations”, “molecular heterogeneity”, “case reports” and “India”. This review was performed based on preferred reporting items for the systematic review and meta-analysis protocols (PRISMA-P) guidelines. The molecular spectrum of Hb H disease in Indians includes the most common [-α^3.7, -α^4.2, -SA, Poly A (AATAAA→AATA–)], Hb Sallanches, rare [-SEA, -MED, IVS 1nt 1 (G→A)], Hb Koya Dora, Hb Sun Prairie, very rare [Hb Iberia, Hb Seal Rock, Hb Zürich-Albisrieden] and novel [Codon 76 (+T) and –K65] α-globin gene mutations inherited largely as compound heterozygotes with considerable clinical variability. The molecular diagnosis of Hb H disease is important for genetic counseling and management.

Keywords: Hb H Disease; α-thalassemia; molecular heterogeneity; case reports; India

1. Introduction

Alpha thalassemia (α-thalassemia) is an autosomal recessive disorder caused by large deletions and point mutations in the α-globin genes, resulting in the reduction or complete absence of α-globin chain synthesis. It is characterized by a microcytic hypochromic anemia and a clinical phenotype varying from an almost asymptomatic condition to one associated with a severe hemolytic anemia [1]. Alpha thalassemia is the most common single gene disorder globally. In India, the prevalence of carriers varies from 1% to 18% in non-tribal populations to over 90% in some tribal groups. [2]. There are two copies of α-globin genes (α1 and α2) located within the α-globin gene cluster on chromosome 16. Deletion of one or both of these cis-linked genes is the most common cause of α-thalassemias [3].

Hemoglobin H disease (Hb H) is a severe form of α-thalassemia which causes chronic hemolytic anemia of variable severity in children and adults depending on the genotype [4,5]. Hb H disease is caused by the presence of deleterious mutations most frequently affecting three of the four α-globin genes [3]. There are two forms of Hb H disease, deletional and non-deletional. In the deletional form, three of the four α-globin genes are absent and denoted as (–/–α), whereas in the non-deletional form, two α-globin genes are deleted and one of the remaining two α-globin genes is abnormal (–/αTα). Occasionally, homozygosity (αT α/αT α) or compound heterozygosity (-αT/αT α) of some mutations on the α2-globin gene reduces α-globin chain synthesis, which leads to Hb H disease [6,7]. In India, the common deletional α-globin gene mutations are -α3.7, -α4.2 and -SA, while the non-deletional α-globin gene mutations are Hb Constant Spring (α 142 T→C), Hb Sallanches (α 142 G→A) and the polyadenylation (poly A) (AATAAA→AATA–) signal mutations [8,9].
Hb Sallanches was first reported in a homozygous condition in a French family and was associated with hemolytic anemia and low Hb H levels. Subsequently, this variant was reported in the homozygous condition from Pakistan and India [10]. The heterozygous state of this variant is difficult to identify since the carriers are asymptomatic. Hb Sallanches is an unstable variant where the $\alpha_1\beta_1$ contact region is disturbed by the mutation and instability with the precipitation of $\alpha$ chains which may be due to folding defects or defects in binding to the chaperone alpha hemoglobin stabilizing protein (AHSP) [10]. The poly A (AATAAA$\rightarrow$AATA–) mutation causing Hb H disease is uncommon. The first case was reported in an Indian woman heterozygous for this mutation. The poly A deletion has also been reported in association with a $\beta^0$-thalassemia mutation and in another case with the $-SEA$ deletion resulting in a milder phenotype [11]. Homozygosity for this mutation has been reported in a British family originating from Pakistan [11].

There are only a few studies reporting the profile of mutations causing Hb H disease in Indians and the associated clinical course of the disease. Here we have reviewed the molecular heterogeneity of Hb H disease and the consequent clinical manifestations among Indians.

2. Materials and Methods

This review was performed based on preferred reporting items for the systematic review and meta-analysis protocols (PRISMA-P) guidelines [12]. To evaluate the mutation spectrum of Hb H disease in Indians, a systematic literature search was carried out from two journal databases, i.e., PubMed and Science Direct. The key words used were “Hb H Disease”, “Hemoglobin H”, “$\alpha$-thalassemia”, “mutations”, “molecular heterogeneity”, “case reports” and “India”.

Two researchers performed the research article search, title and abstract screening of the studies independently. Articles written only in English were included. The inclusion criteria for selecting the studies were as follows: Hb H disease cases confirmed by molecular analysis using a combination of technologies in different studies based on the available facilities (Multiplex GAP PCR, Southern Blot analysis, Sanger’s Sequencing and MLPA) along with family analysis in individuals of Indian origin. The exclusion criteria were Hb H disease cases based only on the presence of inclusion bodies, complete blood count and HPLC chromatograms, standardization and development of molecular techniques to detect mutations on already known Hb H disease cases, comparison of two different technologies to detect Hb H disease cases and such studies where no abstracts and/or full text articles were available. The data from the research articles was extracted and the details were entered into the excel spreadsheets, which were used to write this review article.

3. Results

The initial search yielded a total of 67 studies. After applying the exclusion criteria on the 67 studies, only 14 articles finally remained. The overlapping data were also removed from the studies (Figure 1). The molecular profile of these cases of Hb H disease among Indians along with their clinical presentation is shown in Table 1. Different types of deletional (one gene and two genes) and non-deletional mutations in homozygous and compound heterozygous forms were observed along with some novel and rare mutations in the $\alpha$-globin genes. A total of 109 cases were reported from 13 states (Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Orissa, Punjab, Tamil Nadu, Uttar Pradesh and West Bengal) and one Union Territory (Chandigarh) while seven cases were reported from Durban, South Africa, one from Spain and one from New Zealand in individuals of Indian origin.
The commonest genotypes causing Hb H disease in Indians were $-\alpha^{3.7}/-SA$ and $-\alpha^{4.2}/-SA$ reported from different states (Table 1). Sarkar et al. reported a novel 33.3 kb deletion ($-KOL$) in 3 cases originating from the north eastern region of India. One of them presented clinically with hypochromic microcytic anemia, having recurrent jaundice in the past, mild icterus, a palpable and enlarged liver and spleen and was diagnosed as a case of Hb H disease with the $-\alpha^{3.7}/-KOL$ genotype. In the other two cases having unexplained anemia, the mutation was reported to be $\alpha\alpha/\alpha\alpha$ by using multiplex GAP PCR analysis; however, the other mutation remained uncharacterized [13]. Nadkarni et al. [14] reported 8 Hb H disease cases referred from different regions of India. The mutations identified were $-\alpha^{3.7}/-SA$ in 3, $-\alpha^{3.7}/-SEA$ in 2, $-\alpha^{3.7}/-\alpha^{3.7+HbSallanches}$ in 1 and $-SA/-\alpha^{3.7+HbSallanches}$ in two Hb H disease cases. All these cases had hypochromic microcytic anemia with mild splenomegaly. Most of them (6/8) had pallor, and four had jaundice. None of them had any history of blood transfusions.
Table 1. Distribution of Hb H disease cases along with their mutations and clinical presentation.

| Sr.No. | Mutation (HGVS Nomenclature) | HbH Genotype | No. of Cases | Clinical Presentation                                                                 | Ref. |
|--------|------------------------------|---------------|--------------|--------------------------------------------------------------------------------------|------|
| 1      | α2 Poly A (AATAAA>AATA–) homozygous (HBB:c.*111_*112delAA) | αPoly Aα/αPoly Aα | 4            | Patient 1: H/O anemia and reticulocytosis and no history of receiving any blood transfusions. Patient 2: H/O weakness, easy fatigability and had jaundice twice. Patients 3: H/O severe anemia and mild splenomegaly (spleen 4.5 cms below the costal margin). Patient 4: H/O intermittent fever intermittently, splenomegaly (spleen 9 cms below the costal margin) and regular blood transfusions after 30 days of interval, had underwent splenectomy at the age of 3 years. | [11] |
| 2      | 3.7 kb (type I) deletion α-2+ 33.3 kb deletion α | -α3.7/-Kol | 1            | Hypochromic microcytic anemia, H/O recurrent jaundice in the past, mild icterus, a palpable and enlarged liver and spleen, both of which measured 3 cm below the costal margin. | [13] |
| 3      | 33.3 kb deletion α | α/-Kol trait | 2            | Mild and unexplained anemia                                                                |      |
| 4      | 3.7 kb (type I) deletion α-2+ α 23601 bp deletion (NG_000006.1:g.34247_38050del + NG_000006.1:g.19464_43064del23601) | -α3.7/-SA | 3            | H/O Microcytic hypochromic anemia with mild splenomegaly, (3 cm below the costal margin), H/O pallor and jaundice. |      |
| 5      | 3.7 kb (type I) deletion α-2+ α 19301 bp deletion (NC_000016.10:g.169818_174075del + NC_000016.10:g.169818_174075del + HBA2:c.314G>A) | -α3.7/-SEA | 2            | Microcytic hypochromic anemia with splenomegaly (3 cm below the costal margin), H/O pallor and jaundice. | [14] |
| 6      | 3.7 kb (type I) deletion alpha-2 + α2 CD 104 (G→A) (NC_000016.10:g.169818_174075del + NC_000016.10:g.169818_174075del + HBA2:c.314G>A) | -α3.7/-α3.7+HbSallanches | 1            | Microcytic hypochromic anemia with splenomegaly (3 cm below the costal margin), H/O pallor and jaundice. |      |
| 7      | α 23601 bp deletion +3.7 kb (type I) deletion α-2 + α2 CD 104 (G→A) (NG_000006.1:g.19464_43064del23601 + NC_000016.10:g.169818_174075del + HBA2:c.314G>A) | -_SA/-α3.7+HbSallanches | 2            | Microcytic hypochromic anemia with splenomegaly (3 cm below the costal margin), H/O pallor and jaundice. |      |
| Sr.No. | Mutation (HGVS Nomenclature) | HbH Genotype | No. of Cases | Clinical Presentation | Ref. |
|-------|-------------------------------|---------------|--------------|-----------------------|------|
| 8     | 3.7 kb (type I) deletion α:2+ α 23601 bp deletion (NG_000006.1:g.34274_38050del + NG_000006.1:g.19464_43064del23601) | α\^3.7/-SA | 3 | Moderate anemia with presentation in the third decade | |
| 9     | α2 CD104 (G→A) homozygous (HBA2c:314G>A) | α\^HbSallanches\^-α\^3HbSallanches\^- | 6 | Severe anemia with early presentation | |
| 10    | α2 Poly A (AATAAA>AATA–) homozygous (HBB: c.111_112delAA) | α\^Poly A\^-α\^-Poly A\^- | 3 | Moderate anemia with presentation in the second decade | |
| 11    | 3.7 kb (type I) deletion α:2 + α2 CD104 (G→A) (NC_000016.10:g.169818_174075del + HBA2c:314G>A) | α\^3.7/-α\^- | 1 | Moderate anemia. | |
| 12    | 3.7 kb (type I) deletion α:2 + α2 142 (T→G) (NC_000016.10:g.169818_174075del + HBA2c:427T>G) | α\^-3.7/α\^-Seal Rock\^- | 1 | Identified during family screening in the forth decade with mild anemia | [15] |
| 13    | 3.7 kb (type I) deletion α:2 + Novel α\^6 deletion/Large deletion involving α1 and α2 (NC_000016.10:g.169818_174075del + Novel α\^6 deletion/Large deletion involving α1 and α2) | α\^-3.7/- | 15 | Larger deletions showed early presentation with severe anemia and shorter deletions showed mild anemia | |
| 14    | α2 142 (T→G)+ Large deletion involving α1 and α2 HBA2c:427T>G + Large deletion involving α1 and α2 | α\^-Seal Rock\^-α\^- | 1 | Severe anemia with early presentation | |
| 15    | α2 CD 19 (-G) homozygous HBA2c:60delG | α2 CD 19 (-G) homozygous | 1 | Severe anemia with early presentation | |
| 16    | α CD 76 (+T) α + CD 104 (G→A) + HBA2c:314G>A | α\^76+T\^-α\^-HbSallanches\^- | 1 | Moderate anemia with presentation in the third decade | |
| 17    | α2 Poly A (AATAAA>AATA–) + α2 CD 130 (G→C) HBBc:*111_*112delAA + HBA2c:391G>C | α\^-Poly A\^-α\^-Hb-Sun Prairie\^- | 1 | Moderate anemia with early presentation | |
| 18    | α2 Poly A (AATAAA>AATA–) + α CD 76 (+T) α HBBc:*111_*112delAA | α\^-Poly A\^-α\^-76+T\^- | 1 | Severe anemia with early presentation | |
| 19    | α2 CD 104 (G→A) + CD 59 (G→C) HBA2c:314G>A + HBA2c:176G>C | α\^-α\^-HbSallanches\^-α\^-Zurich-Albisrieden\^- | 1 | H/O recurrent microcytic hypochromic anemia with reticulocytosis, intermittent blood transfusion needs and jaundice, splenomegaly (4 cm below the left costal margin). | [16] |
Table 1. Cont.

| Sr.No. | Mutation (HGVS Nomenclature)                                                                 | HbH Genotype                         | No. of Cases | Clinical Presentation                                                                 | Ref.   |
|--------|--------------------------------------------------------------------------------------------|--------------------------------------|--------------|---------------------------------------------------------------------------------------|--------|
| 20     | α2 Poly A (AATAAA>AATA–) homozygous or large deletion + α2 Poly A (AATAAA>AATA–) heterozygous (HBB:c.*111_*112delAA) | αPoly A/αPoly A_{\alpha}or αPoly A_{\alpha}→1 | 1 **         | Hypochromic microcytic anemia $                                                           | [17]   |
| 21     | 3.7 kb (type I) deletion α-2+ α 23601 bp deletion (NG_000006.1:g.34247_38050del + NG_000006.1:g.19464_43064del23601) | -α^{3.7}/-SA                          | 7            | Hypochromic microcytic anemia $                                                           |        |
|        | 4.2 kb deletion + α 23601 bp deletion (NG_000016.10:g.169818_174075del + NG_000006.1:g.19464_43064del23601) | -α^{4.2}/-SA                          | 3            | Hypochromic microcytic anemia $                                                           | [18]   |
| 22     | 3.7 kb (type I) deletion α-2+ α 16401 bp deletion (NC_000016.10:g.169818_174075del + NG_000006.1:g.24664_41064del16401) | -α^{3.7}/_-MED                        | 1            | Hypochromic microcytic anemia $                                                           |        |
| 23     | α2 Poly A (AATAAA>AATA–) homozygous (HBB:c.*111_*112delAA)                                      | αPoly A/αPoly A_{\alpha}              | 2            | Hypochromic microcytic anemia $                                                           |        |
| 24     | α IVS 1-1 (G→A) + α 23601 bp deletion HBA2:c.95+1G>A + NG_000006.1:g.19464_43064del23601     | αIVS 1-1 (G→A)_{\alpha}/-SA           | 3            | Hypochromic microcytic anemia $                                                           | [19]   |
| 25     | α2 CD104 (G→A) homozygous (HBA2c:314G>A)                                                     | αHb Sallanches/αHb Sallanches_{\alpha} | 1            | H/O recurrent jaundice and hemolytic anemia with reticulocytosis, hepatosplenomegaly (liver 2 cm and spleen 2 cm below the costal margin, respectively). | [19]   |
| 26     | 3.7 kb (type I) deletion α-2+ α 23601 bp deletion (NG_000006.1:g.34247_38050del + NG_000006.1:g.19464_43064del23601) | -α^{3.7}/-SA                          | 1            | Not mentioned                                                                           |        |
| 27     | 3.7 kb (type I) deletion α-2+ α 16401 bp deletion (NC_000016.10:g.169818_174075del + NG_000006.1:g.24664_41064del16401) | -α^{3.7}/_-MED                        | 2            | Not mentioned                                                                           | [20]   |
| 28     | 3.7 kb (type I) deletion α-2+ α 19301 bp deletion (NC_000016.10:g.169818_174075del + NG_000006.1:g.26264_45564del19301) | -α^{3.7}/_-SEA                        | 7            | Not mentioned                                                                           |        |
| Sr.No. | Mutation (HGVS Nomenclature) | HbH Genotype | No. of Cases | Clinical Presentation |
|--------|-----------------------------|--------------|-------------|----------------------|
| 29     | \(\alpha\text{2 Poly A (AATAAA}\rightarrow\alpha\text{A–)}\) homozygous (HBB:c.*111_*112delAA) | \(\alpha^{\text{Poly A}}\alpha/\alpha^{\text{Poly A}}\alpha\) | 4 | Not mentioned |
| 30     | 3.7 kb (type I) deletion \(\alpha\text{-2} + \alpha\text{2 CD 104 (G} \rightarrow \text{A)}\) (NC_000016.10:g.169818_174075del + HBA2.c.314G>A) | \(-\alpha^{3.7}/\alpha\text{Hb Sallanches }\alpha\) | 4 | Not mentioned |
| 31     | \(\alpha\text{23601 bp deletion} + 3.7 \text{kb (type I) deletion }\alpha\text{-2} + \alpha\text{2 CD 104 (G} \rightarrow \text{A)}\) (NG_000006.1:g.19464_43064del23601 + NC_000016.10:g.169818_174075del + HBA2.c.314G>A) | \(-\alpha^{SA}/-\alpha^{3.7+}\text{Hb Sallanches}\) | 1 | Not mentioned |
| 32     | \(\alpha\text{23601 bp deletion} + \alpha\text{2 CD 104 (G} \rightarrow \text{A)}\) (NG_000006.1:g.19464_43064del23601 + HBA2.c.314G>A) | \(-\alpha^{SA}/\alpha\text{Hb Sallanches}\alpha\) | 1 | Not mentioned |
| 33     | \(\alpha\text{19301 bp deletion} + \alpha\text{2 CD 104 (G} \rightarrow \text{A)}\) (NG_000006.1:g.26264_45564del19301 + HBA2.c.314G>A) | \(-\alpha^{SEA}/\alpha\text{Hb Sallanches}\alpha\) | 1 | Not mentioned |
| 34     | \(\alpha\text{2 CD104 (G} \rightarrow \text{A)}\) homozygous (HBA2.c.314G>A) | \(\alpha^{\text{Hb Sallanches}}\alpha/\alpha^{\text{Hb Sallanches}}\alpha\) | 1 | Hypochromic microcytic anemia with reticulocytosis, H/O pallor, jaundice, thalassemic bone changes, an enlarged liver (2.5 cm below the costal margin) and spleen (5 cm below the costal margin). [21] |
| 35     | \(\alpha\text{2 Poly A (AATAAA} \rightarrow \alpha\text{A–)}\) homozygous (HBB:c.*111_*112delAA) | \(\alpha^{\text{Poly A}}\alpha/\alpha^{\text{Poly A}}\alpha\) | 14 | Hypochromic microcytic anemia with splenomegaly and indirect hyperbilirubinemia and elevated Serum LDH |
| 36     | \(\alpha\text{2 Poly A (AATAAA} \rightarrow \alpha\text{A–)}\) + \(\alpha\text{2 CD 130 (G} \rightarrow \text{C)}\) (HBB:c.*111_*112delAA + HBA2.c.391G>C) | \(\alpha^{\text{Poly A}}\alpha/\alpha^{\text{Hb San Prairie}}\alpha\) | 1 \(c\) | Severe anemia with intermittent blood transfusion [22] |
| 37     | \(\alpha\text{2 Poly A (AATAAA} \rightarrow \alpha\text{A–)}\) + \(\alpha\text{2 CD 142 (A} \rightarrow \text{C)}\) (HBB:c.*111_*112delAA + HBA2.c.428A>C) | \(\alpha^{\text{Poly A}}\alpha/\alpha^{\text{Koya Dora}}\alpha\) | 1 | Hypochromic microcytic anemia with splenomegaly with indirect hyperbilirubinemia and elevated Serum LDH |
| 38     | \(\alpha\text{2 Poly A (AATAAA} \rightarrow \alpha\text{A–)}\) + \(\alpha\text{2 CD 22 (C} \rightarrow \text{T)}\) (HBB:c.*111_*112delAA + HBA2.c.69C>T) | \(\alpha^{\text{Poly A}}\alpha/\alpha^{\text{CD 22 (C–T)}}\alpha\) | 2 | Hypochromic microcytic anemia with splenomegaly with indirect hyperbilirubinemia and elevated Serum LDH |
| Sr.No. | Mutation (HGVS Nomenclature) | HbH Genotype | No. of Cases | Clinical Presentation | Ref. |
|-------|-----------------------------|--------------|--------------|-----------------------|-----|
| 39    | α2 Poly A (AATAAA>AATA–) + α2CD 104 (T→C) HBB:c.*111_*112delAA + HBA2:c.313T>C | αPoly A / α/αHb Iberia α | 1 | Hypochromic microcytic anemia with splenomegaly with indirect hyperbilirubinemia and elevated Serum LDH | |
| 40    | α2 CD104 (G→A) homozygous (HBA2:c.314G>A) | αHb Sallanches / α/αHb Sallanches α | 1 | Hypochromic microcytic anemia and intermittent blood transfusion | [23] |
| 41    | 3.7 kb (type I) deletion α-2+α23601 bp deletion (NG_000006.1:g.34247_38050del + NG_000006.1:g.19464_43064del23601) | -α3.7 / SA | 5 * | Hypochromic microcytic anemia, with intermittent blood transfusion, mild hepatosplenomegaly † | [24] |
| 42    | 4.2 kb deletion + α23601 bp deletion (NC_000016.10:g.169818_174075del + NG_000006.1:g.19464_43064del23601) | -α4.2 / SA | 2 * | Hypochromic microcytic anemia $ | |
| 43    | 3.7 kb (type I) deletion α-2 + Novel α0 deletion/Large deletion involving α1 and α2 (NC_000016.10:g.169818_174075del + Novel α0 deletion/Large deletion involving α1 and α2) | -α3.7 / – | 1 *** | Hypochromic microcytic anemia with pallor | [25] |

*: HGVS nomenclature is not available; †: Non deletional mutation was not available; ‡: The patient also had a partial deletion of the HBA2 gene; §: Indian origin residing in Durban, South Africa; †: Indian origin residing in Spain; ***: Indian origin residing in New Zealand; HGVS: Human Genome Variation Society; SA: South Africa; SEA: South East Asia; MED: Mediterranean; Kol: Kolkata; Kb: Kilo base pair; CD: Codon, H/O: History of; LDH: Lactate Dehydrogenase; UT: Union Territory; $: Other clinical findings are not mentioned; †: Clinical presentation of the indexcase.
A large study from Chandigarh, a Union Territory in north India reported 34 Hb H disease cases, all of them having moderate to severe anemia. Hb Sallanches in homozygous form was identified in six cases, Poly A (-AA) homozygous in three and Codon 19 (-G) homozygous in one of the cases. Compound heterozygosity for \(-\alpha^{3.7}/-\alpha^{sA}\) was observed in three cases while other compound heterozygous forms such as \(-\alpha^{3.7}/\alpha^{HB\text{Sallanches}}\), \(\alpha^{76+T}/\alpha^{HB\text{Sallanches}}\), \(\alpha^{Poly\ A}/\alpha^{HB\ Sun\ Prairie}\), \(\alpha^{Poly\ A}/\alpha^{\alpha^{\alpha}}\), \(\alpha^{76+T}/\alpha^{\alpha^{\alpha}}\) were identified in one case each. In 16 cases (\(\alpha^{Poly\ A}/\alpha^{\alpha^{\alpha}}\)), only one mutation was characterized, whereas the other large deletion involving \(\alpha 1\) and \(\alpha 2\) genes remained uncharacterized [15]. Another study from Chandigarh reported HbH disease due to compound heterozygosity for Hb Zürich-Albisrieden and Hb Sallanches (\(\alpha\alpha/\alpha^{Zurich,\ Albisrieden}\)), a rare combination. The patient had recurrent anemia with jaundice, reticulocytosis, intermittent blood transfusion requirements and splenomegaly [16].

Hall et al. reported the poly A (-AA) mutation in a homozygous condition or in a compound heterozygous state along with two gene deletions in an Asian Indian with hypochromic microcytic anemia originating from Gujarat in western India who was residing in Spain [17]. Another series of 17 HbH disease cases have been reported from Vellore, Tamil Nadu in south India where the cases originated from different regions of India, mainly from the states of West Bengal, Orissa, Punjab and Madhya Pradesh. The mutations characterized were \(-\alpha^{3.7}/-\alpha^{SAlanches}\), \(-\alpha^{3.7}/-\alpha^{MED}\), \(-\alpha^{3.7}/-\alpha^{3.7}\), Poly A (-AA) homozygous \(\rightarrow -\alpha^{3.7}/-\alpha^{3.7}\). All the patients had hypochromic microcytic anemia, however other clinical details were not mentioned [18]. A case of Hb H disease with homozygosity for Hb Sallanches was reported by Warang et al. in 2010. The proband had a history of recurrent jaundice and hemolytic anemia with reticulocytosis along with hepatosplenomegaly [19]. Four Hb H disease cases having the poly A (-AA) mutation in homozygous form have been reported in another study from Maharashtra. Three of them originated from Maharashtra in the west and one was from Uttar Pradesh in the north. Of the four cases, the one from Uttar Pradesh had a severe presentation with splenomegaly (spleen 9 cm), and the patient presented at 2.5 years, required regular blood transfusions every month and had then undergone splenectomy at the age of three years. Another case presented with severe anemia and splenomegaly and the remaining two cases were clinically milder with hypochromic microcytic anemia and a history of jaundice on two occasions [11].

One review article from Maharashtra described different genotypes of Hb H disease cases encountered at a referral centre over 15 years. There were a few overlapping cases with other earlier case reports which have not been included here to avoid duplication. Several mutations were reported mainly as compound heterozygotes (Table 1), but the clinical presentation of these cases was not mentioned in the study [20]. Hemoglobin H disease due to homozygous Hb Sallanches was first reported by Dash et al. in 2006 in India. The proband was a nine years old from Punjab who had presented with pallor, jaundice, thalassemic bony changes and hepatosplenomegaly (liver 2.5 cm, spleen 5 cm) with no blood transfusion requirements [21]. Nineteen cases having the poly A (-AA) mutation in homozygous and compound heterozygous form along with other \(\alpha\) globin gene mutations resulting in Hb H disease were reported from Tamil Nadu in southern India. The different genotypes and clinical presentations of these cases is shown in Table 1. They also found a partial deletion of the HBA2 gene in the cases with \(\alpha^{Poly\ A}/\alpha^{HB\ Sun\ Prairie}\) \(\alpha\) mutation [22]. One Hb H disease case originating from West Bengal had the Hb Sallanches mutation in homozygous form and presented with hypochromic microcytic anemia and intermittent blood transfusion needs [23].

Few cases with Hb H disease were identified in persons of Indian origin residing in other countries. Fei et al. in 1992 described combinations of three different forms of \(\alpha\)-thalassemia in a large Indian family from Durban, South Africa. They reported the \(-\alpha^{3.7}/-\alpha^{sA}\) mutation in five individuals and the \(-\alpha^{4.2}/-\alpha^{sA}\) mutation in two individuals of the family. The index case had hypochromic microcytic anemia with intermittent blood transfusion requirements [24]. Recently, a novel \(\alpha^{0}\) thalassemia large deletion removing both HBA2
and HBA1 along with the 3.7 kb deletion has been identified in a three month old Indian infant residing in New Zealand, which led to Hb H disease. The clinical presentation was similar to most other cases with hypochromic microcytic anemia and pallor [25].

4. Discussion

Hemoglobin H disease has an extremely variable clinical presentation with considerable molecular heterogeneity which is more commonly seen in some regions of Southeast Asia, the Middle East and the Mediterranean countries [26]. The phenotypic presentation of Hb H disease ranges from mild anemia to a more severe hemolytic anemia requiring periodic blood transfusions [4]. It is usually associated with the presence of Hb H with a significant number of Hb H inclusion bodies in red blood cells. Various deletional and non-deletional, rare and novel mutations in homozygous and compound heterozygous forms have been identified among the Indian population with variable clinical phenotypes. Many individuals presented early, but a few were not diagnosed until adulthood (in the second or third decade) or occasionally during family screening.

The most common deletional gene defect in Hb H disease cases identified was the 23 kb South African (–SA) deletion. This deletion removes both α2 and α1 genes causing α-thalassemia-1 -SA. This mutation was originally found in the "cape" colored population of South Africa and was also detected in an individual from Gujarat in combination with the sickle cell trait [27,28]. As it was the common two gene deletion, it is co-inherited with different deletional (-α3.7 and -α4.2) and non-deletional mutations [poly A(-AA), Hb Sallanches and IVS 1-1(G→A)] in the α-globin gene with different genotypes [14,18,20,21]. The clinical presentation of these patients varied from moderate hypochromic microcytic anemia to severe hemolytic anemia requiring blood transfusions, with the majority of them having hepatosplenomegaly. Two cases had co-inherited three α-globin gene mutations, –SA, -α3.7 and Hb Sallanches, and were clinically severe. The Hb Sallanches mutation was present in cis on the -α3.7 chromosome [14].

The process of polyadenylation at the newly formed 3' end is important for the synthesis of normal messenger RNA. The Poly A site has the highly conserved sequence motif AATAAA, present 10-30 bp upstream of the majority of poly A addition sites. It is required for transcriptional termination. A mutation at the poly A site results in an extended transcript up to the new signal. The α2 globin gene with the poly A mutation downregulates the transcription of the α1 globin gene. The mutation in homozygous form or in compound heterozygous form along with a deletional α-thalassemia mutation produces a severe Hb H disease phenotype [29]. The poly A (-AA) mutation in homozygous form was also found to be very common in the Indian population [11,15,17,18,20,22]. The clinical presentation of these cases was variable; some had moderate anemia presenting in the second decade [15,17,18], while others had a severe clinical presentation with severe hemolytic anemia requiring intermittent blood transfusions, reticulocytosis, indirect hyperbilirubinemia and hepatosplenomegaly [11,20,22]. Compound heterozygosity of poly A along with variants of α-thalassemia, Hb Sun Prairie, Hb Iberia and Hb Koya Dora were also found in Hb H disease cases. They had a mild to moderate clinical presentation [15,22].

Homozygosity for Hb Sallanches is an uncommon cause of Hb H disease. However, among Indians, 9 Hb H disease cases were homozygous for this mutation. All of them were clinically severe [15,19,21,23]. The alpha Sallanches mutation does not produce abnormal αβ tetramers but produces free Sallanches chains which form dimers with β chains, which is why no abnormal hemoglobin fraction is detected in the patient and the relative excess β chains form tetramers (Hb H) [30]. The compound heterozygous condition of Hb Sallanches with the rare hemoglobin variant Hb Zürich-Albisrieden resulting in a severe phenotype has also been reported in an Indian [16]. The Southeast Asian (–SEA) deletion is the most common and severe form of α-thalassemia highly prevalent in Southeast Asia and South China. In association with milder mutations, it leads to Hb H disease [31]. Among 11 Hb H disease cases, this mutation was observed in 10 cases along with the –α3.7 kb deletion and in one case along with Hb Sallanches. All of them had a moderate to severe
clinical presentation [14,18,20]. The Mediterranean (−MED) deletion mainly occurs in the Mediterranean population. In India its occurrence is not common. Three cases of Hb H disease had this mutation along with a mild α-thalassemia mutation (−α^3.7^). One of them was clinically mild, while the clinical phenotypes of the other two cases were not mentioned [18,20]. Hemoglobin Seal Rock results from a mutation in the upstream α2 gene, reducing the expression of one of the α2-globin genes causing a more pronounced thalassemia effect than deletion or reduced expression of the α-globin gene [32]. This mutation was identified in 2 Hb H disease cases, one co-inheriting Hb Seal Rock along with a two gene deletion presenting severely and the other co-inherited with the −α^3.7^ deletion with a mild clinical presentation [15]. A novel α^0^ thalassemia mutation (−−KOL) and a novel frameshift mutation with the addition of a T at codon 76 were also reported in Indians [13,15]. The molecular spectrum of Hb H disease in India includes several deletional and non-deletional mutations from the most common ones [−α^3.7^, −α^4.2^, −SA, Poly A (AATAAA→AATA−), Hb Sallanches], to rare [−SEA, −MED, IVS 1nt 1 (G→A), Hb Koya Dora, Hb Sun Prairie], very rare [Hb Iberia, Hb Seal Rock, Hb Zürich-Albisrieden] and novel [Codon 76 (+T) and −Kol] α-globin gene mutations.

5. Conclusions

Hb H disease is relatively uncommon in India. The few reported cases confirmed by genotyping show considerable molecular as well as clinical heterogeneity. The clinical course is variable depending on the type of mutations that are inherited. Non-deletional Hb H disease is more severe than deletional Hb H disease. Some patients with milder phenotypes may remain undiagnosed, as they do not seek medical attention. In India, the frequency of α^0^ thalassemia is low. There are some common non deletional variants, like poly A and Hb Sallanches that, if co-inherited with another deletional or non deletional mutation, can give rise to severe Hb H disease. The molecular diagnosis of Hb H disease is important for genetic counseling and better management of the patients.

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