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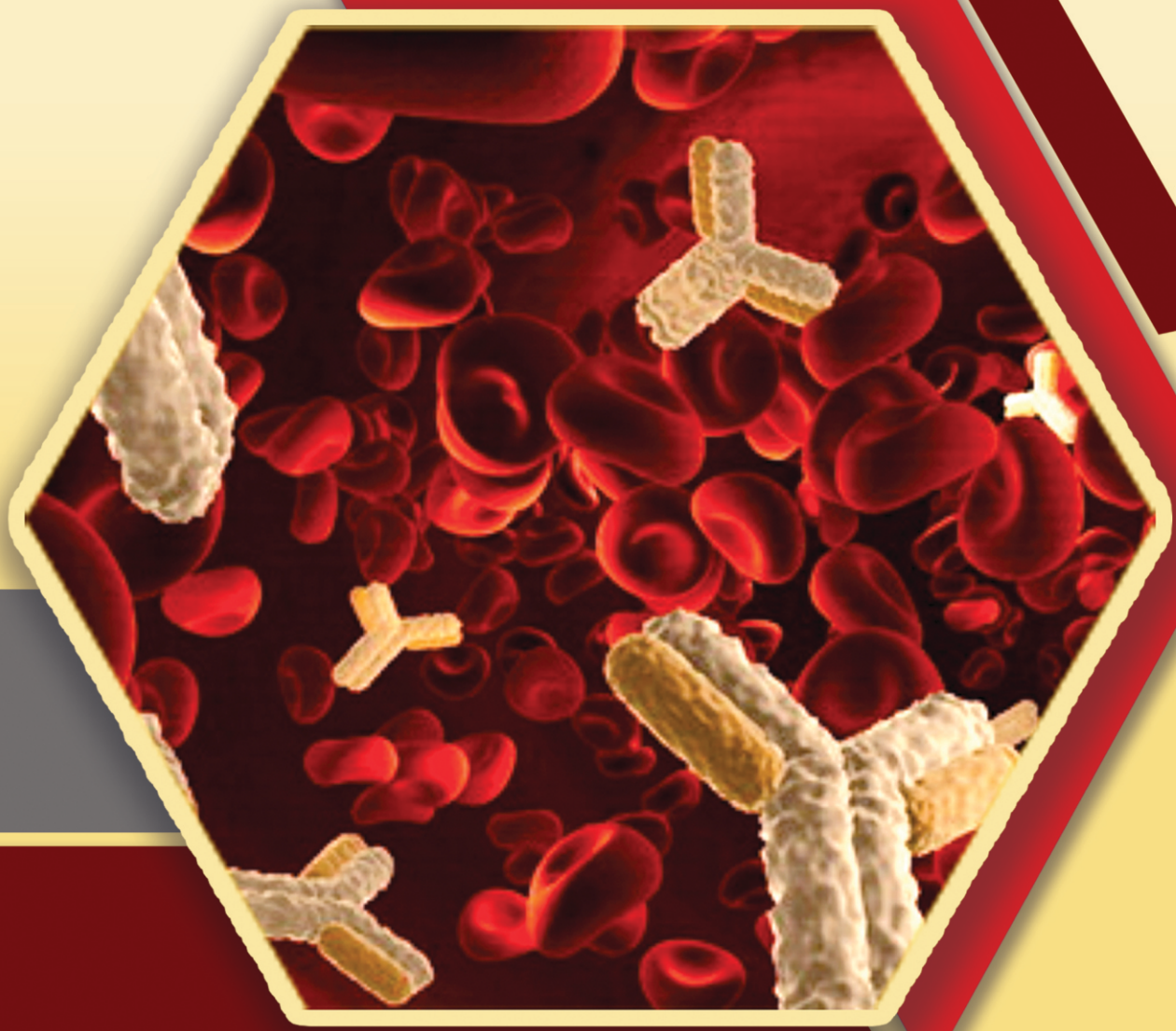

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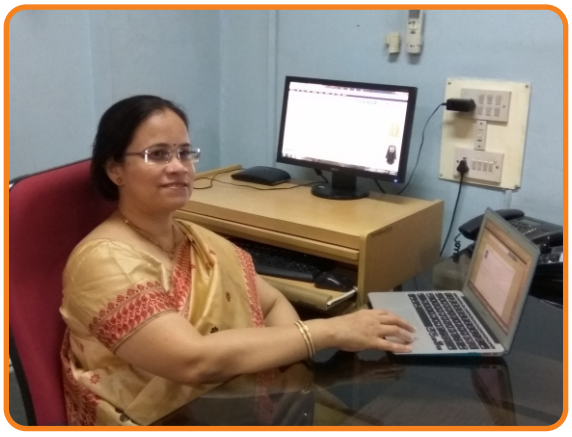

Dr. Manisha Madkaikar, was appointed as Director
of National Institute of Immunohaematology from 14 Dec, 2017

Dr. Babu Rao Vundinti, Scientist E, received BGRC Silver Jubilee Award for the year 2016 on 11th October 2017 from Indian Council of Medical Research, New Delhi for the research work on "Understanding the molecular basis of Hematological diseases". The award was given for his significant contribution to the field of Human Genetics of Hematological diseases in the past 20 years. He carried out extensive research on diagnosis, molecular pathology of Fanconi anemia and identified several novel mutations and also established its prenatal diagnosis.

Dr. Prabhakar Kedar, Scientist D, received ICMR Prize for Biomedical Research for Scientists belonging to Underprivileged Communities for the year 2013 on 11th October 2017 from Indian Council of Medical Research, New Delhi for his research work on "Studies on hereditary hemolytic anemia due to red cell enzymopathies". The award was given for his significant contribution to the field of Enzymopathies for the past 20 years. He has reported several novel mutations in the human PKLR gene in Indian patients and has developed a new test for diagnosis of red cell membrane protein defects.

Genetic modulators of fetal hemoglobin

Priya Hariharan & Anita Nadkarni

Summary :

The clinical heterogeneity of sickle cell disease and β-thalassemia is so variable that it prompted the researchers to identify the genetic modulators of the disease. Though the primary modulator of the disease are the type of β-globin mutations that affects the degree of β-globin chain synthesis, the co-inheritance of α-thalassemia and the fetal hemoglobin levels also act as a potent secondary genetic modifier of hemoglobinopathies. During the course of development the human body sequentially expresses three different forms of hemoglobin: embryonic, fetal and adult hemoglobin in the maturing erythroblasts. Since increased production of the fetal hemoglobin ameliorates the severity of hemoglobinopathies, the study of genetic factors that play a role in the regulatory network of fetal to adult hemoglobin switch will facilitate development of new therapeutic strategies for hemoglobinopathies. In this review we have tried to summarise various mechanisms of hemoglobin switching and the genetic modulators lying within and outside the β-globin gene cluster with their plausible role in governing the fetal hemoglobin (HbF) levels.

Introduction:

Way back in 1866, Korber et al., discovered that the hemoglobin obtained from umbilical cord was far more resistant to alkali denaturation than the normal adult hemoglobin (HbA, $\alpha_2\beta_2$)[1]. After almost 60 years of this discovery, in 1934 Brinkman et al., proved that the variation in the alkali resistant characteristic of hemoglobin was due to the globin portion of the molecule and thus alkali resistant hemoglobin was termed as fetal hemoglobin (HbF, $\propto^2\Upsilon^2$) since it formed the major hemoglobin fraction of the growing fetus[2]. A string of experiments showed that the fetal hemoglobin had an altered physio-chemical properties as compared to the adult hemoglobin, like having increased resistance to elution from the RBCs under acid conditions, decreased solubility in phosphate buffer, increased resistance to heat and higher oxygen affinity[3]. In the same time period, Drescher - Kiinzer and Huehns et al., identified that the embryonic hemoglobin in small human embryos (gestational age:7-12 weeks) had an alkali denaturation between HbA and HbF[4]. These series of discoveries left behind a perplexing question; does the hemoglobin in humans during the ontogeny undergo successive switches? and if yes, is there a perspective for molecular cure by reactivating fetal globin genes in hemoglobinopathy patients?

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Association of elevated HbF and hemoglobinopathies :

The possibility that hemoglobinopathies can be cured by induction of fetal hemoglobin was realized as the symptoms are seen in six months of age after the birth when there is gradual reduction in the HbF levels. In late 1950s, it was observed that patients with Cooleys anemia survived beyond the period of the fetal to adult hemoglobin switch because of the prolonged synthesis of fetal hemoglobin in the erythroid cells[5]. In ß-thalassemia, the main cause of the disease is imbalance in the globin chain ratio, wherein the excess unbound α globin chain precipitates, inhibiting the maturation of the erythroid precursors, ultimately leading to ineffective erythropoiesis and anemia[5]. The clinical pathogenicity of ß-thalassemia develops during the first year of life when the γ-globin genes are gradually silenced with the activation of δ and ß- globin genes. Thus continued γ-globin gene expression in ß-thalassemics, may lead to reduction in the globin chain imbalance as γ-globin synthesized will combine with the excess unbound α-globin chain[6]. Similarly appreciating the role of fetal hemoglobin (HbF;α2γ2) in sickle cell disease started more than 60 years ago when Janet Watson confirmed that infants with sickle cell disease had few symptoms and that their deoxygenated erythrocytes took longer time to sickle and did not deform as extensively as did their sickle cell trait-carrying mother's cells[7]. Sickle cell disease is caused by the mutation in codon 6th position in the ß-globin gene by substitution of glutamic acid by valine, which affects the biophysical properties of the adult hemoglobin[5]. The pathophysiology of sickle cell disease is dependent on the polymerization of deoxy sickle hemoglobin under low oxygen condition, which is retarded with increased HbF concentration. Both HbF and its mixed hybrid tetramer ($\alpha_2\beta^S\gamma$) cannot enter the deoxy sickle hemoglobin polymer phase, thus circumventing the cellular damage evoked by HbS polymers[8]. In both the hemoglobinopathy conditions, increased γ-globin gene expression acts as a well-known disease modifier. Hence understanding the molecular mechanism of hemoglobin switching and identification of molecular targets, for reversing this switch, is a subject of intense research[5].

The hemoglobin switch :

During the ontogeny, globin genes (ε, $^G\gamma$, $^A\gamma$, δ, ß) in the ß-globin cluster are sequentially expressed, leading to production of different hemoglobin molecules with distinct physiological properties. The site for primary hemoglobin synthesis is the embryonic yolk sac and the first wave of hemoglobin switch (primitive to definitive) occurs in the fetal liver after 5 weeks of gestation, wherein there is a switch from embryonic ($\zeta_2\varepsilon_2$) to fetal hemoglobin ($\propto^2\Upsilon^2$)[9]. Towards the end of 3rd trimester, there is a gradual change from fetal ($\propto^2\Upsilon^2$) to adult hemoglobin ($\alpha_2\beta_2$). This second major hemoglobin switch occurs in the bone marrow and lasts until 6 months of age after birth[9]. Figure 1 shows a schematic representation of different hemoglobin progressively expressed during distinct stages of development. With the dawn of molecular era, models for gene switching suggested, that this process involved complex interaction between the cis-acting elements that includes the locus control region of the beta globin gene cluster and trans acting transcription factors which co-ordinately carry out chromatin remodelling activities[10].

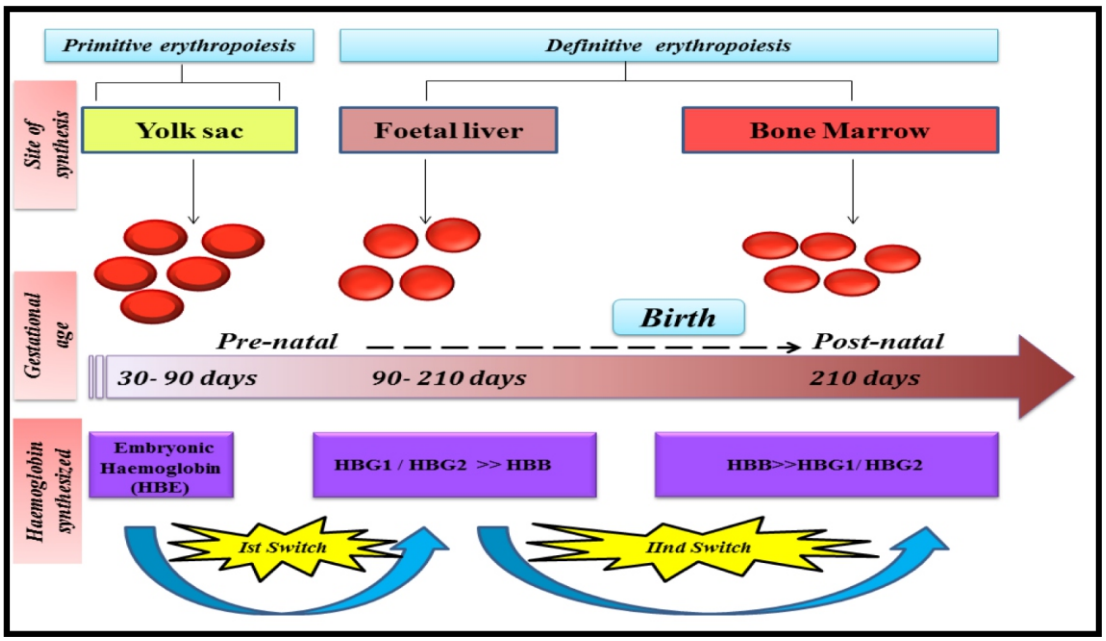

Figure 1: *Expression of globin genes during the ontogeny. The figure describes the site of hemoglobin synthesis and 2 major hemoglobin switches with respect to the gestational age of the fetus. Keys: HBG1 and HBG2 : A γ-globin and G γ-globin gene . HBB : ß globin gene .*

The cis- acting elements :

The ß- globin gene cluster contains a 5' distal regulatory element known as Locus Control Region (LCR), which has five active DNAase I hypersensitive sites (HS) 1-5, that is required for appropriate globin gene expression. This is followed with the series of globin genes (ε, ^{G}γ, ^{A}γ, δ, ß) arranged sequentially in their order of expression. To the downstream of the ß globin gene is another HS site 3' HS1 [Figure 2]. The ß-globin cluster is flanked with olfactory receptors at both 5' and 3' ends[11]. Wijgerde et al., proposed a competitive model for globin gene regulation, where in the LCR participates in the long range looping interaction, that leads to only one productive LCR-globin gene interaction[11]. The potent transcriptional activation of the LCR is also due to the clustering of various erythroid transcription factors that bind to the hypersensitivity sites which act in concert to facilitate long range looping activity. The cis- acting promoter sequences in the individual globin genes and the LCR serve as a template for binding of various transcription factors[11]. It is observed that naturally occurring mutations in γ–globin promoter region are associated with hereditary persistence of fetal hemoglobin phenotype (HPFH).The mechanisms underlying the continued expression of HbF with these mutations are thought to involve alterations in the protein binding motifs that ultimately ablates the binding of repressors or enhancers affecting the gene expression[12]. The most extensively studied variation in several population groups is the XmnI polymorphism (C→T) residing at -158 position (HBG2 c.-211 C→T) in the ^{G}γglobin promoter region. The T allele of XmnI polymorphism is linked to the raised HbF levels and milder hemoglobinopathy conditions[13]. Though T allele in its homozygous state seems to have little effect on HbF levels in

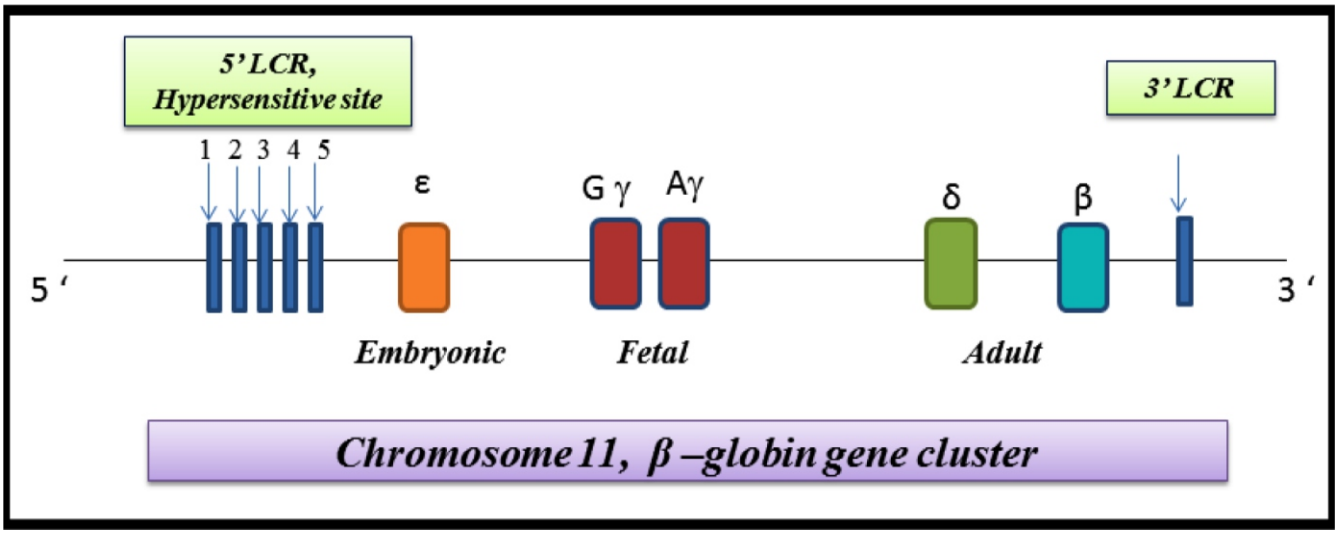

Figure 2: *Structure of* ß *-globin gene cluster showing embryonic (ε), fetal (* G gamma , A gamma *and adult (δ, ß) globin genes arranged according to the order of their expression that is controlled by locus control region (LCR).*

normal individuals, it has been associated with increase of HbF especially during the erythropoietic stress, suggesting its role in HbF induction [14]. In clinically milder patients where no mutation is detected in the γ–globin promoter region, the other genetic modulators [HBS1L-MYB intergenic region, BCL11A, KLF1] leading to raised HbF phenotype are screened.

Role of factors involved in the hemoglobin switch and new therapeutic targets:

More recently recognised variability in HbF expression both in non anemic individuals and patients with ß-hemoglobin disorders stimulated efforts to detect the genetic factors that lead to this deviation. Genome wide association mapping studies have identified quantitative trait loci in the nuclear factors that play a role in the globin gene regulation and switching. The role of major transcription factors that are involved in the process of hemoglobin switching are as follows: [Figure 3].

- ***B- cell CLL / lymphoma 11 A [BCL 11 A]:***

B- cell CLL / lymphoma 11 A is a zinc finger transcription protein which is encoded by *BCL11A* gene located in chromosome 2p16 . This protein expressed in 4 different iso-forms (XS, S, L, XL) plays an important role in lymphopoiesis and neurogenesis[15]. The pioneering role of *BCL11A* polymorphisms in intron 2 in association with fetal hemoglobin levels was first demonstrated by Uda et al., 2008 after conducting genome wide association studies in the Sardanian population, where in variants in *BCL11A* were found to be significantly associated with increase in F-cell numbers in hemoglobinopathy patients[16].This suggested that the 3kb second intronic region of *BCL11A* had regulatory elements or functional motifs responsible for modulating the γ–globin gene expression. Further Sankaran et al., proved that *BCL11A* may function as a potent repressor of the γ– globin gene as lower levels of *BCL11A* m-RNA is expressed in variants causing higher levels of HbF[17]. This was validated by performing gene knockdown of *BCL11A* using short-hairpin RNA (shRNA)

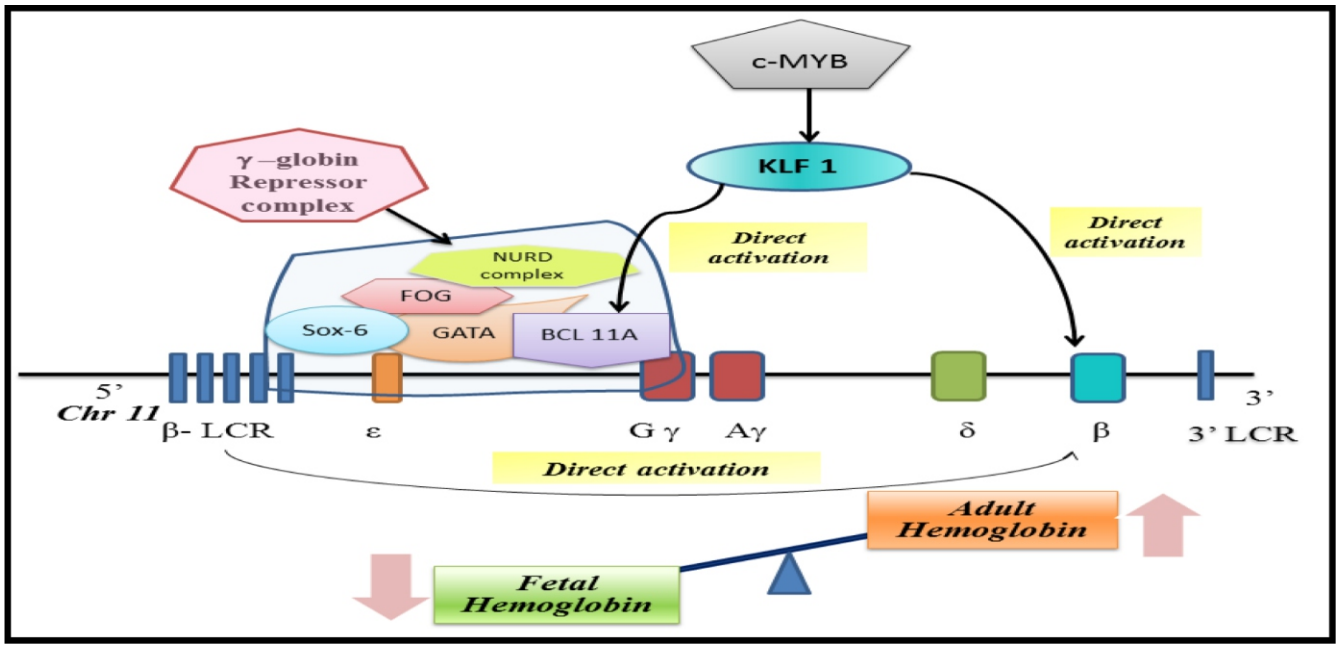

Figure 3: *Schematic representation of hemoglobin switching in presence of various transcription factors. During the switch process from fetal to adult hemoglobin , the γ - globin repressor complex occupies the γ– globin promoter region, thus blocking the γ - promoter interaction with LCR and enhancing its interaction with the ß-globin gene in presence of KLF1, thus leading to increase in adult hemoglobin.*

approaches in primary adult erythroid progenitors[17].Chromatin Immuno Precipitation (ChIP) technology demonstrated that *BCL11* A, interacts with NuRD chromatin remodelling and repressor complex, erythroid transcription factors – GATA -1, FOG -1, SOX6 and Matrin-3 to silence the gamma globin gene, suggesting the pivotal role of *BCL11A* in gamma globin silencing [17].

- ***Kruppel like factor 1 [KLF 1]*:**

Erythroid Kruppel like factor (EKLF) or *KLF1*, a erythroid specific transcription factor plays a critical role in erythropoiesis[18]. This founding member of Kruppel like factor family was first identified by Ira Miller and James Bieker in 1992 and named it as *KLF1*/ EKLF due to its zinc finger homology with that of Drosophila body pattern determining Kruppel-gap gene[18]. This master erythroid gene regulator, co-ordinates three important processes that includes erythroid lineage commitment, the γ to ß globin gene switch and activation or suppression of certain erythroid specific genes (red cell metabolism and structure proteins) by formation of chromatin remodelling complex[19]. *KLF1* transcription factor was thought to take part in ß-globin gene activation by binding to the CACCC box of the beta-globin promoter, as ablating this region lead to ß-thalassemia intermedia condition. This phenomenon was validated by knockdown of *KLF1* gene in mouse embryo that succumbed to severe beta thalassemia[19]. Though Singleton et al., 2008 primarily reported *KLF1* gene mutations leading to clinically benign In (Lu) blood group, the first event of raised HbF phenotype was mentioned by Borg et al., in an extended Maltese family where in the proposita inheriting two *KLF1* gene variants p.K288X

and p.M39L showed a HPFH phenotype with HbF levels of 19.5%[20,21]. The mechanism that *KLF1* participates in repressing the gamma globin levels via *BCL11A* activation was proved by Zhou et al by conducting gene knock down assay, in which reduction of *KLF1* gene markedly decreases the *BCL11A* levels and indirectly increasing the gamma ß-globin mRNA levels[22]. Thus established the bipartite role of *KLF1* gene, in indirectly reducing the ϒ globin gene expression by *BCL11A* activation and directly activating ß-globin gene by favouring looping interactions between the LCR and the ß-globin gene against γ-globin gene promoter [22].

- ***HBS1L- MYB intergenic zone* [*HBS1L-MYB*]:**

With the aim of studying the genetic variants associated with the HbF regulation, genome mapping studies identified another loci located in chromosome 6, residing within 126-kb intergenic region between the *HBS1L* and *MYB* genes. This region is distributed in 3 disequilibrium blocks which are referred to as HPIM block 1, 2, 3 amongst which block 2 that spans 24 kb region is found to be associated with reduced disease severity in patients with hemoglobinopathy by increasing the HbF levels. Though the function of *HBS1L* in red blood cell development is uncharacterized, the *MYB* gene (encoding the c-Myb transcription factor [TF]) is a key regulator of hematopoiesis and erythropoiesis[23]. The functional importance of the intergenic region was first observed when transgene insertion within the murine *HBS1L-MYB* intergenic region completely disrupted the binding of core complex proteins LDB1, GATA1, TAL1, and ETO2 and abolished *MYB* transcription which resulted in severe anemia[23]. The most effective of these, are the variants residing within the core enhancer binding region in HMIP-2A and -2B [23]. A three -bp deletion (rs66650371) at HMIP-2A is suspected to directly cause HbF variability. Recent reports have described a transcriptional network within erythroid cells whereby *KLF1* and *MYB* activate each other by formation of an Active Chromatin Hub(ACH) structure that brings intergenic enhancer regions in proximity with the *MYB* promoter[23]. This same study showed that perturbations to the ACH structure, or to *KLF1* expression, do not affect gene expression of *HBS1L*, suggesting that the primary role of the GWAS-identified *HBS1L-MYB intergenic* region is to regulate the expression of *MYB*[23].

- ***GATA 1*:**

The GATA family of transcription factors (GATA 1 to GATA 6) are a group to zinc containing transcription regulators that bind to consensus (WGATAR) DNA motif present in the regulatory region of various erythroid development genes [24,25]. The role of GATA 1 as a lineage specific transcription factor and in regulating the HbF levels was first suggested by Philips et al., in a patient suffering from congenital erythropoietic porphyria with elevated HbF levels thus suggesting its paramount role in erythroid maturation and hemoglobin switching[26]. GATA 1 has 2 regions, the N-terminal transactivation that stabilizes DNA binding and a C terminal zinc finger domain that binds to the GATA 1 motifs[26]. Bottardi et al., proved that GATA 1 works in concert with other co-factors like friend of GATA 1 (FOG-1), histone acetyltransferase CBP/P300 / Mediator complex subunit MED-I and a chromatin modeller-BRG1 to bring about long range chromatin interactions between ß-LCR and the globin genes to

regulate their gene expressions[27]. Further Chromatin Immuno Precipitation assays showed that GATA 1 along with IKAROS : transcription factor, forms a repressome complex, that limits the recruitment of transcription activators to the γ-globin promoters, thus by silencing the γ-globin gene and progressively increasing the transcription of β-globin gene[27].

- ***SOX6*** **:**

SOX6 a member of the SOX transcription factor family is characterised by high mobility group (HMG) DNA binding domain. This gene codes for a 79 amino acid DNA binding protein that binds to the minor groove of the DNA, leading to conformational changes in the DNA structure[28]. SOX 6 thus acts as an architectural protein by dynamically remodelling the chromatin structure and assisting the binding of other transcription factors[28]. Its role in silencing the embryonic globin gene was first shown in a murine model, as SOX6 deficient mice persistently expressed the embryonic globin. This hypothesis was further proved by Zanhua et al., that 36-base pair region of the embryonic globin proximal promoter is a binding site for SOX6 mediated repression that leads to the silencing of the ε-globin gene[28]. Chromosome conformation capture (3C) assay, ChIP suggested that SOX6 co-occupies with BCL11 A and GATA1, in the β-globin gene cluster and conducts silencing of γ-globin transcription in the adult erythroid proginetors[29]. These results were further validated by carrying out combined knockdown experiments of SOX6 and BCL11A in primary human erythroblast culture which greatly enhanced the HbF production thus suggesting that both the transcription factors played a combined role in γ-globin gene regulation [29].

Targeted translational research for increasing the HbF levels: Opportunities and challenges!

The common transcription factors that govern the developmental switches execute their action by complex inter-dependent networks. The mechanisms underlying the function of these regulators are so stringent, that any deviations in their expression or protein-protein interaction may evoke disastrous phenotype leading to anemia, leukemia or lymphomas [30]. Hence it is pivotal to elucidate their mechanisms, with the basic step of placing these factors in a hierarchy in which they function. Despite of vigorous research for the search of HbF inducers, only Hydroxyurea (HU) – a ribonucleotide reductase inhibitor, remains the sole FDA approved medication for sickle cell disease. However the HU utility is limited by its unpredictable HbF induction that varies patient to patient and myelo-suppression. Thus there is a need for developing other potential strategies to elevate HbF levels in hemoglobinopathy patients. One such approach is to develop target based therapy which includes knocking down the target by RNA interference with high specificity and selectivity. The other way it could be achieved is by controlling small molecules by using therapeutic drugs. However in either technique, the greatest challenge is to selectively impair the activity of universally expressed transcription factors specifically in the erythroid lineage without affecting its universal function in the other cells[31].

Indian scenario and NIIH experience:

In India, the first study by Dadheech S. et al., showed that the C allele of rs 11886868 (C→T) in *BCL11A* gene is associated with amelioration of

the severity of ß-thalassemia as well as sickle cell anemia (SCA)[32]. Roy P. et al., showed that,A→G allele on the rs4895441 in the *HBS1L-MYB* intergenic region is associated with the raised HbF levels in the eastern population of India[33]. Previous study by Upadhye D et al., have tried to correlate the SNPs in the *BCL11A* and *HBS1L-MYB* gene with the HbF levels and clinical severity in paediatric sickle cell anemia patients. They identified A allele of the SNP rs4671393 in *BCL 11A* gene and G allele of rs9389268 in the *HBS1L-MYB* gene to be associated with raised HbF levels and with milder clinical symptoms[34]. In our recent study we have identified the C allele of rs11886868 and rs66650371 (-TAC) (3 base deletion) in the *MYB* gene to be associated with raised HbF levels in adult SCA and thalassemia intermedia patients. Similarly, the first event of *KLF1* variations associated with raised HbF phenotype was mentioned by Borg et al[22], however no such studies have been carried out in India to understand the prevalence and effect of *KLF1* variations in our population.We have identified 12 different *KLF1* gene mutations after screening 325 hemoglobinopathy patients, most of the mutations associated with mild increase in HbF levels. We found a novel *KLF1* gene variation codon 211 A→G (c.632 A>G)to be associated with increased HbF levels in thalassemia trait [HbA2: 4.1%, HbF: 8.6%, ß-globin gene mutation: codon 15 G→A heterozygous] and speculate that co-inheritance of this novel *KLF1* variant might have ameliorated the clinical manifestations of her six year old untransfused ß-thalassemia homozygous child [HbA_2 :1.6 %, HbF:97.9%, ß-globin gene mutation: codon 15 (G→A) homozygous][35]. Also we identified novel 5 bp deletion -ATAAG (-533 to -529) in the $^{G}\gamma$ globin promoter which when co-inherited with the ß-thalassemia trait condition lead to an elevated HbF levels [HbA_2: 3.6%, HbF: 31.7% and HbA_2 : 3.9%, HbF : 23.6%][36].

Conclusion :

Increase in HbF levels has potential therapeutic implications as it ameliorates the clinical severity of ß-hemoglobinopathies. With this review we conclude that SNPs located at various quantitative trait loci, are associated with meagre elevation of HbF levels in the range of 3-5%, nevertheless they can increase the HbF output to clinically beneficial levels when co-inherited with hemoglobinopathies. Identification of such variants are of importance as in future they can be used in genome editing to naturally increase the HbF levels in patients with ß-hemoglobinopathies. The study of transcription factors and their co-enzymes that play role in gamma globin gene silencing may help in determining new potential interacting surfaces and enzymatic mechanisms. Curtailing the activity of these proteins may thus lead to the development of novel therapies for treating hemoglobinopathies.

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सारांश

फिटल हिमोग्लोबीन के अनुवंशिक कारक

मनिषा पटवर्धन

सन 1866 में कोर्बेट और साथियो को वैज्ञानिक निरीक्षण में पता चला की नाभीरज्जू (umbilical cord) से रिसनेवाली हिमोग्लोबीन प्रौढ़ (adult) हिमोग्लोबीन से अलग है। जहाँ प्रौढ़ हिमोग्लोबीन अल्कली के संपर्क से तुरंत विघटीत हो जाती है (alkali denaturation), वहीं नाभी रज्जू रसित हिमोग्लोबीन काफी देर के बाद इस प्रक्रिया में हिस्सा लेती है। सन 1934 में ब्रिंकमन और साथियो ने उजागर किया की हिमोग्लोबीन में मौजूद 'ग्लोबीन' प्रोटीन इस प्रक्रिया का कारण है। 'ग्लोबीन' हिमोग्लोबीन रेणू (molecule) का महत्वपूर्ण हिस्सा हैं।

कई अभ्यास में सामने आया की प्रौढ़ हिमोग्लोबीन और फिटल हिमोग्लोबीन में मुलभूत भौतीक और रासायनिक (physical & chemical) अंतर होता है।

इसी बीच वैज्ञानिकों की जोडी (ड्रेझर-किनझर और हेन्स) ने प्रौढ़ हिमोग्लोबीन और फिटल हिमोग्लोबीन के बीच की कडी दर्शानेवाले भ्रूणीय हिमोग्लोबीन (embryonic hemoglobin) की खोज की। अब वैज्ञानिकों को कई सवालों के उत्तर ढुंढने थे। सबसे अहम सवाल था, 'क्या मानवी जीवन की अलग अलग अवस्था में हिमोग्लोबीन बनने की प्रक्रिया में बदलाव होते रहते है? अलग प्रोटीन बनने से हिमोग्लोबीन रुप बदल लेती है? ऐसा क्युं होता है? क्या इस बदलाव का उपयोग प्रौढ़ हिमोग्लोबीन से जुडे रोगों में किया जा सकता है?

जीव के भ्रूण अवस्था में शरीर के अलग अलग अंगों में हिमोग्लोबीन संश्लेषण (synthesis) होता है। प्राथमिक हिमोग्लोबीन की उत्पत्ती भ्रूण की थैली में (embryonic sac) में होती है। इस दौरान भ्रूणक हिमोग्लोबीन बनना काफी हद तक कम हो जाता हैं। और प्रौढ़ हिमोग्लोबीन बनने लगती हैं। शिशुअवस्था के ६ माह तक हिमोग्लोबीन कई अवस्थाओं से गुजरती रहती है।

हिमोग्लोबीन संबंधित बिमारियों में बीटा थेलेसेमिया / कुलीज अनेमिया एक जटील बिमारी है। बीटा ना होने से प्रौढ़ बननेवाली हिमोग्लोबीन साधारण रुप से काम नहीं करती। हिमाग्लोबीन आक्सिजन को उतकों तक पहुँचाने का महत्वपूर्ण कार्य करती है। इस बिमारी के रुग्णों मे वैज्ञानिकों ने काफी अनुसंधान किया। थॅलसेमिया रोग होने के कारण शरीर के हिमोग्लोबीन बनाने की प्रक्रिया में गडबडी हो जाती हैं, जिसके कारण रक्तक्षीणता (anaemia) के लक्षण प्रकट होते है। पीडित को बार-बार काफी मात्रा में खून चढाना पड़ता है। आम तौर पर थालेसेमिया के रुग्ण प्रौढ़ आयु तक जिंदगी नहीं जी सकते। अनुसंधान मे प्रयास किये गये की फिटल हिमोग्लोबीन को प्रवर्तित (induce) करवा के उसे शिशुअवस्था के बाद भी बनने की प्रक्रिया बरकरार रहें।

सिकल सेल रोग एक अनुवंशिक रक्तविकार हैं, जिसमें शरीर अलग प्रकार का हिमोग्लोबीन (HbS) बनता हैं। सिकल सेल में लाल रक्त कोशिका दरांती (Sickle) का आकार धारण करती है और कठोर (hard) बन जाती है। सिकल हिमोग्लोबीन बहुलीकरण (Polymerization of

deoxy sickle Hb under low oxygen) सिकल सेल रोग का प्रमुख कारण है। फिटल हिमोग्लोबीन की अधिक मात्रा सिकल सेल के लक्षणों को घटाती हैं। इस अनुसंधान से फिटल हिमोग्लोबीन की उपयुक्तता सिद्ध हुई।

फिटल हिमोग्लोबीन की मात्रा बढने में कई घटक कारण हैं। इनमें गॅमा ग्लोबीन प्रमोटर वेरिएशन, HBS1L-MYB, BCL11 A, आदि पर प्रकाश डाला गया है। गॅमा ग्लोबीन प्रमोटर जीन में परिवर्तन (Mutation) की वजह से प्रौढ आयु में भी ज्यादा मात्रा में फिटल हिमोग्लोबीन तैयार होता रहता है।

बीटा थेलेसेमिया में फिटल हिमोग्लोबीन की बढी हुई मात्रा का उपयोग

अलग अलग प्रकार के हिमोग्लोबीन एक कडी के तरह बदलते रहते है। यह एक जटील प्रक्रिया है। इस में छोटा बदलाव भी रुग्ण में अनेमिया या ल्युकेमिया का रूप धारण कर सकता हैं। अबतक हुए अनुसंधान में फिटल हिमोग्लोबीन को लंबे समय तक और अधिक मात्रा में बनते रहने से बीटा थेंलेसिमिया के लक्षणों में सुधार होता हैं।

विश्व में सबसे पहले 'गेलेनिलो'ने सारदानिया के लोगों में फिटल हिमोग्लोबीन पर अनुसंधान किया। उसने दिखा दिया की कई अलिल (allele) की मौजुदगी के कारण थेलेसिमीया इंटरमिडीया में थेलसिमिया मेजर से अधिक मात्रा में फिटल हिमोग्लोबीन पायी जाती है। इसके पश्चात उदा., बोर्ग आदि वैज्ञानिकों ने इस विषय पर विस्तृत अनुसंधान किए।

एन. आय. आय. एच. में अनुसंधान: उपाध्ये और सहयोगीओं ने 'सिकल सेल अनेमिया' मे अलग अलग जीन और फिटल हिमोग्लोबीन मात्रा के अनुपान (level) पर अनुसंधान किया। उन्हे BCL II A जीन और अलिल HBS1L-MYB जीन अलिल के साथ फिटल हिमोग्लोबीन का संबंध प्रस्थापित करने में कामयाबी मिली।

इसके अलावा, हालिया अध्ययन मे हमे 325 मरिजो में अब तक KLF1 जीन में 12 परिवर्तन (mutation) का पता चला। KLF I जीन में पाये गए परिवर्तनों मे (codon 211 A→G) का संबंध बढे हुए फिटल हिमोग्लोबीन से पाया गया।

निष्कर्ष : फिटल हिमोग्लोबीन की बढती मात्रा बीटा थेलेसिमिया रुग्णों के लक्षणों में सुधार लाती हैं। अनुसंधान के जरिए जीन 'थेरपी' द्वारा फिटल हिमोग्लोबीन में 3-5% इजाफा भी फायदेमंद शाबित हो सकता हैं। इस पर अनुसंधान कामियाबी की कगार पर हैं।

Award Winning Abstracts

1. Abstract awarded Harold Gunson fellowship at the 28th Regional conference of ISBT held at Guangzhou, China on 25-28 November, 2017

PHENOTYPIC CHARACTERISTICS OF NOVEL RHD VARIANTS IN INDIANS

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Background: The RhD antigen being unarguably the most clinically significant blood group antigen has led to it being studied in several comprehensive studies in Caucasians, Black Africans and East Asians. Several phenotypic studies have been carried out in Indians but an extensive study was lacking. India being a formidable heterogeneous population a study of the phenotypic variability would definitely add to the repertoire of RhD variants and a better understanding of the same.

Aims: To phenotype and study novel RhD variants in Indians.

Methods: Samples (n=223) presenting with a weak D phenotype by serological analysis using routine commercial anti-D reagents were genotyped for the RHD gene by conventional molecular approaches, i.e by melting curve analysis and sequencing the RHD coding regions. Quantitative multiplex PCR of short fluorescent fragments (QMPSF) assay was performed for assessment of RHD exon copy number variations. The novel D variant samples identified were further characterized by using the Advanced Partial RhD Typing Kit (Alba Bioscience, Edinburgh, UK). Serological data of D antigen epitope profile and antigenic sites on these D variant samples was also evaluated.

Results: Out of the 223 RhD variants identified in the study seven variants were found to be novel. They are as follows: Exon 3 duplication (n = 130), RHD(G63C) (3), RHD(A59T) (1), RHD(A237V) (1), RHD-CE(5:E223Q,V238M,V245L)-D (1), RHD(T201R)- CE(5)-D(I342T) (1), RHD(L214F)-CE(7)-D (1). Majority of the exon 3 duplication variants were detected in the IgM phase with weaker strength and showed presence of the 'C' antigen. They also showed a heterogeneous pattern of agglutination with varying strengths on testing with panel of anti-D reagents from ALBAclone Advanced Partial RhD Typing Kit (Alba Bioscience, Edinburgh, UK). Absence of epitopes 1.2, 8.1 and 6.3 in different combinations was observed. Additionally, flow cytometry analysis in a subset of samples showed a range of D antigenic sites (range: 1276-6050 D antigens/cell). Another novel weak D mutation RHD(A237V) predicted to be present on the top of transmembrane domain eight which is close to the extracellular domain showed absence of 1.1, 1.2, 6.3, 8.1 and 9.1 epitopes and was found to be responsible for the production of anti-D. For three samples showing weak D mutation RHD(G63C) predicted to be located in the second transmembrane domain the mean antigenic density was found to be in the

range of 5088-7406 D antigens/cell.

Summary / Conclusions: All the novel RhD variants gave weaker reaction at IgM phase of testing and did not require indirect antiglobulin test for detection. Overall we describe a major novel weak D allele resulting due to exon 3 duplication in Indians. The varying pattern of agglutination, strength and the antigenic density observed for this novel variant can be attributed to the altered transcript biosynthesis. Preliminary data also suggest inter individual variation in the relative amount of transcripts produced. The study contributes to improve Rh blood group diagnostics significantly in more than one billion Indians.

2. Best oral paper award first prize at the 6th National Conference of Indian Society of Transfusion Medicine held at Lucknow, on 3-5th November, 2017

NOVEL MOLECULAR MECHANISM PRODUCING WEAK D PHENOTYPE IN INDIANS

Swati Kulkarni[1], Disha Parchure[1], Harita Gogri[1], Vidhya G[1], Manisha Madkaikar[1], Kanjaksha Ghosh[1], Yann Fichou[2]. [1]National Institute of Immunohaematology ICMR, Mumbai, India, [2]EFS - Inserm UMR1078, BREST, France

The Rh blood group system is the most polymorphic system and is implicated in hemolytic transfusion reaction and hemolytic disease of the fetus and newborn. Molecular genetics of the *RH* genes has been extensively studied in Caucasians, Africans and East Asians and the variant alleles giving rise to weak and partial RhD phenotypes have been reported. Though the variability of Rh expression has been documented in Indian population, extensive genetic studies on Rh antigens have not been carried out. In the present study we aim to characterize the molecular bases of weak D expression in Indians.

Material & Methods: Samples (n=223) presenting with a weak D phenotype by serological analysis were genotyped in the *RHD* gene by conventional molecular approaches. Firstly, all samples were tested for the three common variant *RHD* alleles (weak *D*, type 1, 2, and 3) in the Caucasian population by melting curve analysis. Samples were then analyzed by sequencing the *RHD* coding regions for identification of base substitutions/ insertions /deletions. Quantitative multiplex PCR of short fluorescent fragments (QMPSF) assay was performed for assessment of *RHD* exon copy number variations. This test aims to calculate the number of both the *RHD* and the *RHCE* genes/exons in two separate tests and is performed mainly to look for partial *D* variants resulting by the formation of hybrid genes by rearrangement. Data of serological D epitope profile and antigenic site on these D variant samples was also evaluated.

Results: Three common variant *RHD* alleles (weak *D*, type 1, 2, and 3) were absent in study population and sequencing analysis identified only a limited number of variations, including novel single nucleotide substitutions. Conversely, QMPSF approach revealed a common duplication of exon 3 in a significant proportion of weak D samples (58.3%), suggesting a novel, predominant rare allele specific of the Indian population.

Further functional analysis by cloning / sequencing, minigene splicing assay showed that this genetic variation results in the expression of several transcripts, including a wild-type product. These results suggest that this allele quantitatively affects the expression of the normal transcript, and then subsequently the expression of the normal RhD protein, finally resulting in a weak RhD phenotype. Genomic characterization of the duplicated region finally identified a ˜12 kilobase region encompassing the very 3′-end of exon 2, full intron 3, exon 3, and a partial sequence of intron 3. Serological epitope profile in this novel variant samples showed a variable pattern. Flow cytometry analysis in a subset of duplication positive samples showed a varying D antigen density in range of 1276–6050 D antigens/cell. **Conclusion:** Overall, we have identified a novel, predominant *RHD* variant allele specific to the Indian population. As most of the weak D variants reported in literature are due to SNP and formation of hybrid RHD-CE genes, our study of genotyping of the RhD variants led us to discover a new molecular mechanism producing weak RhD variants in Indians. This discovery not only extends the current knowledge of *RH* molecular genetics but also extends the spectrum of mutational mechanisms involved in the variability of Rh expression. The study will also help us to develop Indian specific genotyping assay.

Dr Ajit Gorakshakar, Scientist F, superannuated on 30th Nov 2017
after completing his service for 42 years at Institute.

Hindi Pakhwada conducted at NIIH Seminar hall on 26-27th Sept 2017. Essay writing and elocution competition were conducted. Chief guest for this occasion were Mr. Vipul Lakhnavi and Mr. Vinod Kumar Sharma.

NIIH staff and students conducted Sadbhavna Diwas Oath at Seminar Hall on 24th Nov 2017

Program on Swachh Bharat Abhiyaan was conducted in N.P. High School, Lalbaug Mumbai- 21st Nov 2017. Drawing & Essay writing competition was held in which 30 students participated and in elocution competition five students took part. The topic for all the three competitions, were on Swachh Bharat Abhiyaan.

Dr. Swati Kulkarni, Scientist D from the Department of Transfusion Medicine, was awarded Best oral paper award first prize for presentation entitled "Novel molecular mechanism producing weak D phenotype in Indians" at the 6th National Conference of Indian Society of Transfusion Medicine held at Lucknow, on 3-5th November, 2017.

Miss. Disha Parchure, PhD student from Department of Transfusion Medicine, was awarded the prestigious Harold Gunson fellowship (for transfusion medicine students/researchers) at the 28th Regional conference of ISBT held at Guangzhou, China on 25-28 November, 2017. This fellowship is a travel grant exclusively for young researchers. She was awarded the fellowship for her abstract entitled "Phenotypic characteristics of novel RHD variants in Indians"

“Award of Excellence” was given to Dr. Swati Kulkarni, Scientist D for extraordinary contribution in the field of Transfusion Medicine by Indian Society of Blood Transfusion and Immunohaematology during 42nd Annual conference held at Kota from 8-10th Dec 2017.

Flowcytometry workshop on Diagnosis of Primary Immunodeficiency Disorders (PID) was organized by Pediatric Immunology and leukocyte biology department, on **7-8th Sept 2017** at NIIH.

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