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Association of Xmn1 −158 γ^G variant with severity and HbF levels in β-thalassemia major and sickle cell anaemia

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Abstract Haemoglobinopathies including β-thalassemia and sickle cell anaemia (SCA) are considered to be classical monogenic diseases. There is considerable clinical variability between patients inheriting identical β-globin mutations. The reasons for this variability are not well understood. Previous studies have suggested that a variety of genetic determents influence different clinical phenotypes. The genetic variants that modulate HbF levels have a very strong impact on ameliorating the clinical phenotype. In the present study 6,500 blood samples from suspected cases were analysed using HPLC, ARMS-PCR, RDB techniques. Patients with β-thalassemia and SCA were classified into mild, moderate, severe according to the severity score based on Hb levels, age of onset, age at which patients received their first blood transfusion, the degree of growth retardation and splenectomy. Patients with β-thalassemia and SCA were analysed for Xmn1 polymorphism and association between this polymorphism and severity of β-thalassemia and SCA was evaluated. We found a significant difference in genotypic and allelic frequencies of Xmn1 polymorphism between mild and moderate and mild and severe cases. There was a significant difference in high and low percentage of HbF in CC, CT and TT bearing individuals. The TT bearing individuals were found to have a high percentage of HbF in β-thalassemia as well as SCA. This study confirms that increased γ^G-globin expression associated with Xmn1 polymorphism ameliorates the clinical severity in β-thalassemia as well as SCA in the study population.

Keywords β-Thalassemia · Sickle cell anaemia · Modifier genes · Xmn1 polymorphism · Clinical severity

Introduction

The haemoglobinopathies are a very heterogeneous group of congenital hemolytic anemias. Among the inherited disorders of blood β-thalassemia constitutes the major bulk of monogenic disorders in India. β-Thalassemia is seen practically in every population group in India. There are nearly 30 million carriers in India with a wide geographic variation in the frequency. Three main structural variants of haemoglobin seen in Indian population are HbS (Codon 6 A→T), HbE (Codon 26 G→A) and HbD (Codon121 G→C). HbS causes sickle cell anaemia (SCA) which is seen in the scheduled tribes and scheduled castes of Central, Southern and Western parts of India. The Sickle Cell gene is mainly concentrated in Madhya Pradesh, Orissa, Chhattisgarh, Jharkhand, Gujarat and Andhra Pradesh, where the carrier frequencies range from 5 to 40 %. Clinical manifestations of β-thalassemia and SCA are extremely variable. Despite seemingly genotypes the patients with β-thalassemia and SCA have a remarkable variability in anemia, growth, development, hepatosplenomegaly and transfusion requirement. However, what is not well understood is the marked variability of the clinical manifestation. Possible factors that influence the severity

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of anaemia in the patients may be inherited or non-inherited.

Previous studies have shown that the co-inheritance of α-thalassemia or the presence of genetic variants that sustain fetal haemoglobin (HbF $\alpha_2\gamma_2$) production has a strong impact on ameliorating the clinical phenotype. Increased levels of HbF are of no consequences in healthy adults but have been shown to confer major clinical benefits in patients with β-thalassemia and SCA. A shift from $\gamma \rightarrow \beta$ globin expression occurs around birth and underlies the switch from fetal to adult Hb production. The major haemoglobin is HbA ($\alpha_2\beta_2$) by the sixth month of age [1]. Individuals with SCA have been reported to have HbF ranging from 1 to 30 % [2]. Residual amounts of HbF also continue to be Synthesised such that the majority of the adults have <1 % HbF. High HbF levels are known to reduce the severity of symptoms by minimising the degree of imbalance of α and β-globin chains. Among genetic factors, Xmn1 polymorphism, a common variant (C→T) at position −158 upstream of the γ^G globin gene has been associated with increased HbF in normal individuals, β-thalassemia and SCA [3–5]. HbF can strongly influence the heterogeneity of β-thalassemia and SCA. The frequency of Xmn1 polymorphism and its association with HbF levels in β-thalassemia major and SCA patients have not been investigated in the South Indian population from Andhra Pradesh. Therefore, the present study was carried out with an aim to investigate the frequency of Xmn1 polymorphism and evaluate its association with HbF levels and severity of the disease in β-thalassemia and SCA patients in this ethnic group.

Materials and methods

Study population

The Institute of Genetics and Hospital for Genetic diseases is a premier institute in the country involved deeply in multidisciplinary research in the area of human genetics. Patients are generally referred by practicing physicians or get registered themselves. A total of 6,500 individuals who visited this institute during January 2005 to December 2012 were included in the study. The majority of cases come from different districts of Andhra Pradesh with Hyderabad as its capital. The patient population is representative of the disease pattern of this region. Information on clinical characteristics of the patients was collected by using a structured questionnaire.

All the cases were analyzed by HPLC for detection of β-thalassemia, SCA, hemoglobin variants, and HPFH. We used the HPLC technique with the β-Thalassemia Short Program of Variant Bio-Rad.

Clinical severity

The patients who had two β-thalassemia alleles and two Hbs (HbSS) alleles were categorized into mild, moderate, and severe groups according to the scoring system proposed by Sripichai et al. [6]. Parameters that showed correlation with disease severity at $r > 0.3$ were included in the multiple logistic regression analysis. These were hemoglobin level, age of onset, age at which patients received their first blood transfusion, age at thalassemia presentation, the degree of growth retardation, and splenectomy (Table 1). The scoring system consisting of these six clinical criteria scored 0, 0.5, 1, or 2 according to clinical presentation. Patients whose severity score was <3.5, 3.5–7.5, and >7.5 were considered as mild, moderate, and severe cases, respectively.

Molecular studies

Genomic DNA was extracted from the whole blood samples using the phenol–chloroform method. Amplification refractory mutation system (ARMS) PCR and reverse dot blot (RDB) hybridization were used to screen the specific mutation in patients with β-thalassemia as reported previously [7]. Specific mutation in the SCA was confirmed by PCR–RFLP technique. The 110 bp product was amplified using the primers: Forward 5′-ACACAACTGTGTTCACT AGC-3′ and Reverse primer 5′-CAACTTCATCCACGTT CACC-3′. The amplified fragment (110 bp) was digested with MsII restriction enzymes (Fermentas fast digest) by incubating at 37 °C for 5 min. The mutant T (T20) allele lacks the MsII restriction site, and remains uncleaved as 100 bp, while it is present in the A (A20) allele. The wild type A allele was detected as two fragments of 56 and 54 bp followed by separation of fragments by electrophoresis in non-denaturing polyacrylamide gel and visualized after silver staining. Xmn1 polymorphism −158 (C→T) was detected by PCR–RFLP technique. A 650 bp fragment was amplified using the primers 5′-AACTGTTGCTTTAT AGGATTTT-3′and 5′-AGGAGCTTATTGATAACTCAG AC-3. The 650 bp product was digested with PdmI restriction enzyme (Fermentas fast digest) by incubating at 37 °C for 5 min followed by separation of fragments on 3 % agarose gel. The C allele lacks the PdmI restriction site, while it is present in the T allele. The T allele was detected as two fragments of 450 and 200 bp.

Statistical analysis

Hardy–Weinberg equilibrium was tested for Xmn1 polymorphism. The association between the genotype and severity was examined by odds ratio with 95 % confidence

Table 1 Clinical severity criteria and scoring for classifying β-thalassemia and SCA patients

11 Criteria	Status	Score	Status	Score	Status	Score
Age at onset (years)	>10	0	2–10	0.5	<2	1
Hb at steady state	> 7.5	0	6.0–7.5	1	<6	2
Age at first transfusion	>10	0	4–10	1	<4	2
Growth and development	Normal	0	±	0.5	Retarded	1
Splenectomy	No	0	Yes	2		
Age at thalassemia presentation (years)	>10	0	3–10	0.5	<3	1

Severity category: Mild 0–3.5, Moderate 3.5–7.5, Severe 7.5–10

The scores were obtained by dividing the coefficients of the selected significant parameters by the smallest significant coefficient by rounding the resulting number to the nearest integer

interval (CI) and Chi squared analysis using Open Epi software (Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA). Allelic frequencies were calculated according to the number of alleles observed and the total number of alleles examined. The association of different genotypes with HbF percentage was evaluated by Two-way ANOVA. Statistical significance was defined as $p < 0.05$.

Results

A total of 6,500 individuals were tested using HPLC, ARMS–PCR and RDB techniques for one of the heterogeneous group of congenital anaemia's which include Hb variants (D-thalassemia, E-thalassemia, and SCA) and β-thalassemia. The break of the analysed cases was in agreement with our previous reports [7, 8]. Only the patients possessing two alleles (n = 420) of β-thalassemia gene (β-thalassemia major) were genotyped for Xnm1 polymorphism. 200 SCA patients were also genotyped for Xmn1 polymorphism. These patients were grouped into sub-groups (mild, moderate, and severe) based on the severity of the disease determined by the severity score as reported previously (8) (Table 1). The clinical and biochemical characteristics of β-thalassemia and SCA patients have been given in (Tables 2, 3), respectively. A significant difference was observed in age, weight, baseline Hb levels, hematocrit (%), HbF (%) between mild, moderate, and severe groups in β-thalassemia as well as SCA patients. Genotypic frequencies and allele frequencies of Xmn1 polymorphism and mean HbF levels in β-thalassemia and SCA patients have been given in Table 4. There was a statistical significant difference in the mean HbF levels between the three genotypes in both the groups ($p < 0.001$). HbF levels were found to be higher in individuals with the TT genotype followed by CT and CC genotype in β-thalassemia as well as SCA patients. There was a significant difference in the genotypic distribution and allele frequency between mild and moderate as well as mild and severe cases in both the groups (Tables 5, 6, 7, 8). However, there was no significant difference in genotypic distribution and allelic frequencies of Xmn1 polymorphism between moderate and severe cases in β-thalassemia as well as SCA (Tables 6, 8). With regard to Xmn1 polymorphism there was a significant difference in the high and low percentage HbF levels between the three genotypes in both the groups (Tables 9, 10). The TT bearing individuals were found to have a high percentage of HbF in both the groups. However, in SCA patients the frequency of TT genotype individuals bearing high percentage of HbF was significantly higher in comparison with β-thalassemia patients indicating that the influence of the Xmn1 site on expression of HbF is more in case of SCA than in β-thalassemia.

Discussion

The clinical manifestations of SCA and β-thalassemia are extremely variable in severity. The patients show a remarkable variability in anaemia, growth development, hepatosplenomegaly and transfusion requirements increased. The reasons for this are not well understood. A number of factors like age, sex, co-inheritance of α-thalassemia and genetic factors such as DNA sequence variations within the β-globin gene cluster and also falling outside the HB genes have been identified to ameliorate the disease phenotype [9–13]. HbF levels or F cell (Hb containing erythrocytes) numbers have been shown to ameliorate the disease severity of β-thalassemia major as well as SCA. A sequence variation C→T −158 Hb γ^G, later termed as Xmn1 (rs 7842144) has been shown to promote the expression of Hb γ^G and to contribute to HbF variability [9, 14]. Independent studies have confirmed the association between Xmn1 T allele and increased HbF and

Table 2 Clinical data and biochemical characteristics of β-thalassemia patients according to the severity (data expressed as mean ± SD)

Parameter	Mild (n = 103)	Moderate (n = 204)	Severe (n = 113)	*p* value
Age (years)	17.95 ± 11.53	19.23 ± 14.26	14.89 ± 10.03	0.009
Male: female	65/38	73/131	40/73	ns
Weight (kg)	37.78 ± 14.82	33.78 ± 14.10	28.56 ± 10.15	<0.001
Baseline Hb level (g/dL)	8.01 ± 1.16	7.12 ± 1.17	6.20 ± 1.15	<0.001
Hematocrit (%)	26.03 ± 3.78	24.25 ± 4.05	22.16 ± 4.01	<0.001
Hemoglobin F (%)	49.40 ± 18.32	23.91 ± 3.79	18.36 ± 11.75	<0.001

Data are expressed as mean ± SD, *ns* not significant

Table 3 Clinical data and biochemical characteristics of SCA patients according to the severity (data expressed as mean ± SD)

Parameter	Mild (n = 117)	Moderate (n = 63)	Severe (n = 20)	*p* value
Age (years)	16.85 ± 10.63	20.23 ± 11.26	15.88 ± 10.12	0.009
Male: female	60/57	37/26	9/11	ns
Weight (kg)	36.88 ± 13.72	32.98 ± 13.11	29.66 ± 11.16	<0.001
Baseline Hb level (g/dL)	8.71 ± 1.06	7.21 ± 1.07	5.99 ± 1.11	<0.001
Hematocrit (%)	25.03 ± 1.78	23.23 ± 3.05	20.16 ± 3.01	<0.001
Hemoglobin F (%)	45.40 ± 19.32	22.91 ± 3.16	17.36 ± 11.55	<0.001

Data are expressed as mean ± SD, *ns* not significant

Table 4 Distribution of genotype frequencies and allele frequencies of Xmn1 polymorphism and mean ± SD of HbF levels in β-thalassemia and SCA patients

Genotype	CC	CT	TT	Total	C	T	Total
β-Thalassemia patients	225	138	57	420	588	252	840
	(53.5 %)	(32.8 %)	(13.5 %)		(0.7)	(0.3)	
Mean ± SD of HbF levels	3.7 ± 1.6	6.7 ± 4.7	16.1 ± 9.4	$p < 0.001$			
SCA patients	21	82	99	200	124	276	400
	(10.5 %)	(41.0 %)	(49.5 %)		(0.31)	(0.69)	
Mean ± SD of HbF levels	3.8 ± 1.0	7.6 ± 4.7	19.3 ± 8.4	$p < 0.001$			

FC in patients affected with different hemoglobinopathies [15–18]. Negal et al. studied the association of XmnI polymorphism with increased levels of HbF in hematologically and genetically distinct forms of SCA in Africa. They found that the individuals bearing the Senegal haplotype and Arab Indian characteristics exhibited the XmnI polymorphism which associated strongly with increased levels of HbF and therefore more minimal clinical manifestations [19]. In another study by Lettere et al. showed that XmnI polymorphism in homozygotes accounted for 2.2 % of the variation in levels of HbF in 1,275 SCA patients from the African American Cooperative Study of Sickle Cell Disease [20].

A study carried out by Pandey et al. in 60 sickle homozygous and 75 sickle β-thalassemia patients found extremely significant differences of haematological parameters among patients with and without XmnI polymorphism and concluded that high HbF levels were found in XmnI carriers and therefore sickle cell patients were greatly influenced by this polymorphism [21]. Another study from Western Iran in β-thalassemia patients reported similar results [14].

T allele has also been associated with milder disease in the SCA and β-thalassemia [22, 23]. A genetic interaction between the Xmn1 and γ^G and a locus on chromosome 8q has also been reported to influence the adult F cell levels [24]. There is no evidence for dominance at the locus based on statistical analysis, which suggests an additive effect of the T allele of Xmn1 polymorphism [25]. The Xmn1 genotype has been shown to account for 13–32 % of the total F cell phenotypic variation in a non anaemic European population [26]. However, presence of T allele does not always dictate the presence of high HbF phenotype. High HbF has also been associated with β-haplotypes that do not include this gene [27–29]. The quantative trait is characterised by genetic heterogeneity. Therefore, to produce a full high HbF phenotype, the Xmn1 must exist on a genetic background requiring the presence of additional factors. The high HbF expression has clearly been shown to ameliorate the disease severity in β-thalassemia and SCA.

Table 5 Distribution of genotype frequencies and allele frequencies of Xmn1 Polymorphism in mild, moderate and severe cases with β-thalassemia

Genotype	Mild (n = 103)	Moderate (n = 204)	Severe (n = 113)
CC (%)	27 (27)	78 (38)	46 (41.0)
CT (%)	34 (33.0)	71 (35)	43 (38.0)
TT (%)	42 (40)	55 (28)	25 (22)
C	88 (0.4)	227 (0.5)	135 (0.6)
T	118 (0.6)	181 (0.5)	93 (0.4)

Table 6 Odds ratio and *p* values of mild, moderate and severe cases with β-thalassemia

	Genotypes	χ^2	Odds ratio	95 % CI	*p* value
Mild cases versus moderate cases	TT versus CC	6.8	2.2	1.2–3.9	0.01
	TT versus CC+CT	6.0	1.8	1.1–3.0	0.01
	T versus C	9.13	1.6	1.1–2.3	0.002
Mild cases versus severe cases	TT versus CC	9.1	2.8	1.4–5.6	0.002
	TT versus CC+CT	8.9	2.4	1.3–4.4	0.002
	T versus C	11.7	1.9	1.3–2.8	0.001
Moderate versus severe cases	TT versus CC	0.7	1.2	0.7–2.3	0.3
	TT versus CC+CT	0.9	1.3	0.7–22	0.3
	T versus C	0.7	1.1	0.8–1.6	0.3

Table 7 Distribution of genotype frequencies and allele frequencies of Xmn1 Polymorphism in mild, moderate and severe cases with SCA

Genotype	Mild (n = 117)	Moderate (n = 63)	Severe (n = 20)
CC (%)	30 (25.7)	26 (42)	10 (53)
CT (%)	34 (29)	15 (24)	4 (22)
TT (%)	53 (45.3)	22 (35)	6 (25.0)
C	94 (0.4)	67 (0.5)	44 (0.7)
T	140 (0.6)	59 (0.5)	16 (0.3)

However, it is not known if the effects translate to a selective advantage for carriers of HbF alleles in a region with a high incidence of these diseases. Genetic studies have shown that individuals with this disease concurrent with high HbF levels can maintain normal fitness levels that would otherwise be severely limited by the debilitating consequences of these haemoglobinopathies.

In the present study, we have evaluated the association of Xmn1 polymorphism with mild, moderate and severe groups of β-thalassemia as well as SCA. A significant association of TT genotype and T allele was observed with milder disease phenotype. In addition, we also evaluated

Table 8 Odds ratio and p values of mild, moderate and severe cases with SCA

	Genotypes	χ^2	Odds ratio	95 % CI	*p* value
Mild cases versus moderate cases	TT versus CC	4.0	2.0	1.0–4.3	0.04
	TT versus CC+CT	1.8	1.5	0.8–2.9	0.1
	T versus C	5.5	1.6	1.0–2.6	0.01
Mild cases versus severe cases	TT versus CC	3.8	2.9	0.9–8.9	0.05
	TT versus CC+CT	1.6	1.9	0.6–5.3	0.2
	T versus C	21.0	4.0	2.1–7.6	0.001
Moderate versus severe cases	TT versus CC	0.009	0.9	0.2–4.0	0.9
	TT versus CC+CT	0.1	1.2	0.4–3.7	0.6
	T versus C	6.8	2.4	1.2–4.7	0.001

Table 9 The influence of Xmn1 polymorphism and percentage of HbF in β-thalassemia patients

Genotype	CC (n = 21)	CT (n = 82)	TT (n = 99)	*p* value
High percentage of Hb F	5 (23.8 %) 5.5 ± 0.435	37 (44.0 %) 9.9 ± 4.50	72 (73.0 %) 19.6 ± 7.63	0.0001*
Low percentage of Hb F	16 (76.1 %) 3.8 ± 1.0	45 (56.0 %) 3.5 ± 0.691	27 (27.0 %) 1.4 ± 0.744	0.0001*

TT versus CC: $\chi^2 = 22.8$, Odds ratio = 4.3 (2.3–8.1), $p < 0.01$; TT versus CC+CT: $\chi^2 = 16.9$, Odds ratio = 3.3 (1.8–6.0), $p < 0.01$

* *p* Value calculated by two way ANOVA

Table 10 The influence of Xmn1 polymorphism and percentage of HbF in SCA patients

Genotype	CC (n = 225)	CT (n = 138)	TT (n = 57)	*p* value
High percentage of Hb F±	79 (35.0 %) 7.2 ± 1.38	72 (52.0 %) 9.9 ± 5.37	40 (70.0 %) 18.0 ± 11.3	0.016*
Low percentage of Hb F±	146 (65.0 %) 3.1 ± 0.680	66 (48.0 %) 3.7 ± 0.708	17 (30.0 %) 4.9 ± 5.1	0.0001*

TT versus CC: $\chi^2 = 17.8$, Odds ratio = 8.5 (2.8–25.5), $p \leq 0.01$

TT versus CC+CT: $\chi^2 = 20.8$, Odds ratio = 3.8 (2.1–6.9), $p \leq 0.01$

* *p* Value calculated by two way ANOVA

Table 11 Xmn1 polymorphism and its association with HbF Variability in Indian population

Polymorphism	Region	Association	Reference	Year
−158 $^{G}\gamma$ (C→T) Xmn1 polymorphism in Indian population	Western	XmnI polymorphism modulating the severity of HbE-β-thalassemia	Panigrahi et al. [17]	2005
	Arab Indian	A strong association between the XmnI-$^{G}\gamma$ site and FC levels	Garner et al. [26]	2000
	New Delhi	The presence of Xmn-1 polymorphism and IVS 1-1 mutation lead to a milder phenotypic presentation and a delay in onset of blood transfusion	Aditya et al. [30]	2006
	Uttar Pradesh	The prevalence of Xmn1-$^{G}\gamma$ polymorphism was high in β-thalassemia Intermedia patients	Oberoi et al. [31]	2011
	Chhattisgarh	Xmn1 polymorphism was associated with Hb F levels in SCA	Das [32]	2012
	New Delhi	The phenotypes of Indian sickle cell patients were greatly influenced by Xmn1 polymorphism	Pandey et al. [21]	2012

the association of Xmn1 polymorphism with HbF levels to estimate whether this variant modifies the phenotype of homozygous β-thalassemia as well as SCA by modulating HbF levels. A significant difference in the high and low percentage of HbF in CC, CT and TT bearing individuals ($p < 0.01$) was observed. TT genotype associated significantly with high HbF in β-thalassemia as well as SCA. The association of Xmn1 polymorphism with disease severity has been studied in different parts of India (Table 11). However, this is the first study evaluating the association of this polymorphism with HbF levels and disease severity in β-thalassemia as well as SCA in a South Indian population from Andhra Pradesh. Our previous study shows that another genetic modifier ZHX2 G779A variant is not associated with disease severity and HbF levels in the same population [8]. This study confirms that increased γ^{G}-globin expression associated with the Xmn1 polymorphism ameliorates the clinical severity in β-thalassemia as well as SCA in the study population.

The patients were also assessed based on the age at diagnosis. 70 %of the TT bearing individuals were diagnosed after 1 year of age while 30 % were diagnosed at <1 year of age. In individuals with late diagnosis the mean HbF levels were found to be significantly higher in comparison with individuals with an early diagnosis ($p = 0.004$). The strength of the present study is that we have employed a large number of patients in the study and evaluated the association of this polymorphism with HbF levels in different severity groups.

This suggests that the screening of −158 Xmn1 γ^{G} polymorphism and HbF levels in early childhood may help in the management of β-thalassemia major and SCA patients and possibly prevent severe complication in our population. Understanding the roles played by genetic modifiers in haemoglobinopathies will revolutionize the diagnosis, treatment and prevention.

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References

1. Stamatoyannopoulos G (2001) Molecular and cellular basis of hemoglobin switching. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL (eds) Disorders of hemoglobin: genetics, pathophysiology, and clinical management. Cambridge University Press, Cambridge, pp 131–145
2. Nagel RL, Platt OS (2001) General pathophysiology of sickle cell anemia. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL (eds) Disorders of hemoglobin: genetics, pathophysiology, and clinical management. Cambridge University Press, Cambridge, pp 494–526
3. Verma IC (2000) Burden of genetic disorders in India. Indian J Pediatr 67:893–898
4. International Committee for Standardization in Haemotology (1978) Recommendations for selected methods for quantitative estimation of HbA2 and HbA2 reference preparation. Br J Haematol 38:573–578
5. Gilman JG, Huisman THJ (1985) DNA sequence variation associated with elevated fetal Gg globin production. Blood 66:783–787
6. Sripichai O, Makarasara W, Munkongdee T, Kumkhaek C, Nuchprayoon I, Chuansumrit A, Chuncharunee S, Chantrakoon N, Boonmongkol P, Winichagoon P, Fucharoen S (2008) Scoring system for classification of β-thalassemia/HbE disease severity. Am J Hematol 83:484-4
7. Munshi A, Anandraj MPJS, Joseph J, Shafi G, Anila AN, Jyothy A (2009) Inherited haemoglobin disorders in Andhra Pradesh, India: a population study. Clin Chim Acta 400:117–119
8. Munshi A, Dadheech S, Jain S, Joseph J, Al-Hazzani A, Alshatwi AA, Babu MS, Rajeshwar K, Jyothy A (2011) Lack of association of G779A ZHX-2 gene variant with HbF levels in β-thalassemia major. Euro J Haematol 86:502–506
9. Thein SL, Menzel S, Peng X, Best S, Jiang J, Close J, Silver N, Gerovasilli A, Ping C, Yamaguchi M, Wahlberg K, Ulug P, Spector TD, Garner C, Matsuda F, Farrall M, Lathrop M (2007) Intergenic variants of HSSIL-MYB are responsible for a major quantitative trait locus on chromosome 6q23. Influence fetal haemoglobin levels in adults. Proc Natl Acad Sci USA 104:11346–11351
10. Menzel S, Garner C, Gut I, Matsuda F, Yamaguchi M, Heath S, Foglio M, Zelenika D, Boland A, Rooks H, Best S, Spector TD, Farrall M, Lathrop M, Thein SL (2007) A QLT influencing F cell

production maps to a gene encoding a zinc-finger protein on chromosome 2p15. Nat Genet 39:1197–1199
11. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A, Albai G, Piras MG, Sestu N, Lai S, Dei M, Mulas A, Crisponi L, Naitza S, Asunis I, Deiana M, Nagaraja R, Perseu L, Satta S, Cipollina MD, Sollaino C, Moi P, Hirschhorn JN, Orkin SH, Abecasis GR, Schlessinger D, Cao A (2008) Genome-wide association study shows BCL11A associated with persistent fetal haemoglobin and amelioration of the phenotype of β-thalassemia. Proc Natl Acad Sci USA 105:1620–1625
12. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, Barella S, Uda M, Usala G, Abecasis GR, Cao A (2009) Amelioration of Sardinian β0 thalassemia by genetic modifier. Blood 114:3935–3937
13. Rochette J, Craig JE, Thein SL (1994) Fetal haemoglobin levels in adults. Blood Rev 8:213–224
14. Hooshang N, Zohreh R, Gholamreza B (2010) The Xmn1 polymorphic site 5′ to the $^{G}\gamma$ gene and its correlation to the $^{G}\gamma$: $^{A}\gamma$ ratio, age at first blood transfusion and clinical features in β-thalassemia patients from Western Iran. Mol Biol Rep 37:159–164
15. Pandey S, Mishra RM, Suhail A, Rahul S, Ravi K, Pandey SW, Seth T, Saxena R (2011) Association of low serum iron with alpha globin gene deletions and high level of HbF with Xmn-1 polymorphism in sickle cell traits. Indian J Clin Biochem 27(3):270–273
16. Serjeant GR (2001) Sickle cell-bthalassemia. In: Serjeant GR (ed) Sickle cell disease, 3rd edn. Oxford University Press, Oxford
17. Panigrahi I, Agarwal S, Gupta T, Singhal P, Pradhan M (2005) Hemoglobin E-beta thalassemia: factors affecting phenotype. Indian Pediatr 42(4):357–362
18. Shimmoto MM, Vicari P, Fernandes AC, Guimaraes GS, Figueiredo MS (2006) XmnI polymorphism is associated with fetal hemoglobin levels in hypoplastic syndromes. Sao Paulo Med J 124(2):110–111
19. Nagel RL, Fabry ME, Pagnier J, Zohoun I, Wajcman H, Baudin V, Labie D (1985) Hematologically and genetically distinct forms of sickle cell anemia in Africa: the Senegal type and the Benin type. N Engl J Med 312:880–884
20. Lettre G, Sankaran VG, Bezerra MA, Araújo AS, Uda M, Sanna S, Cao A, Schlessinger D, Costa FF, Hirschhorn JN, Orkin SH (2008) DNA polymorphisms at the BCL11A, HBS1L-MYB, and β-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci USA 105:11869–11874
21. Pandey S, Pandey S, Mishra RM, Saxena R (2012) Modulating effect of the −158 γ (C→T) Xmn1 polymorphism in Indian sickle cell patients. Mediterr J Hematol Infect Dis 4(1):e2012001
22. Thein SL, Wainscoat JS, Sampietro M, Old JM, Cappellini D, Fiorelli G, Modell B, Weatherall DJ (1987) Association of thalassaemia intermedia with a beta-globin gene haplotype. Br J Haematol 65:367–373
23. Labie D, Dunda-Belkhodja O, Rouabhi F, Pagnier J, Ragusa A, Nagel RL (1985) The −158 site 50 to the Gg gene and Gg expression. Blood 66:1463–1465
24. Garner C, Tatu T, Best S, Creary C, Thein S (2002) Evidence of genetic interaction between the β-globin complex and chromosome 8q in the expression of fetal haemoglobin. Am J Genet 70:793–799
25. Thein SL, Menzel S, Lathrop M, Garner C (2009). Control of fetal hemoglobin: new insights emerging from genomics and clinical implications. Hum Mol Genet 18(2):R216–R223
26. Garner C, Tatu T, Game L, Cardon LR, Spector TD, Farrall M, Thein SL (2000) A candidate gene study of F cell levels in sibling pairs using a joint linkage and association analysis. Gene Screen 1:9–14
27. Efremov GD, Gjorgovski I, Stojanovski N, Diaz-Chico JC, Harano T, Kutlar F, Huisman THJ (1987) One haplotype is associated with the Swiss type of hereditary persistence of fetal hemoglobin in the Yugoslavian population. Hum Genet 77:132–136
28. Kulozik AE, Kar BC, Satapathy RK, Serjeant BE, Serjeant GR, Weatherall DJ (1987) Fetal hemoglobin levels and β-globin haplotypes in an Indian population with sickle cell disease. Blood 69:1742–1746
29. Pissard S, Beuzard Y (1994) A potential regulatory region for the expression of fetal hemoglobin in sickle cell disease. Blood 84:331–338
30. Aditya R, Verma IC, Saxena R, Kaul D, Khanna VK (2006) Relation of Xmn-1 polymorphism & five common Indian mutations of thalassaemia with phenotypic presentation in β-thalassaemia. JK Sci 8(3):139–143
31. Oberoi S, Das R, Panigrahi I, Kaur J, Marwaha RK (2011) Xmn1-Gγ polymorphism and clinical predictors of severity of disease in β-thalassemia intermedia. Pediatr Blood Cancer 57(6):1025–1028
32. Das S (2012) A study to understand the relation beween fetal haemoglobin, the hematological parameters and Xmn i gene polymorphism. Ind J Med Healthc 1(9):2278–2266