Single nucleotide polymorphisms (SNPs) in causing β-thalassemia

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ABSTRACT - In a densely populated country like India, the most commonly inherited disorders are hemoglobinopathiesincludes β-thalassemia and sickle cell anemia. The root cause for these disorders can understand by studying the gene disease association of the beta globin gene (HBB) of Hemoglobin protein, any change in the expression of the HBB gene may lead to phenotypic changes known as hemoglobinopathies. One important reason for the variability in the expression pattern of the genes is due to change in the protein sequence caused by a type of mutations known as SNPs; mutations that can be defined as the change in the single nucleotide base pair that may or may not lead to a phenotypic change. Understanding of SNPs can help in identifying the cause of β-thalassemia; a blood disorder in which the production of hemoglobin reduces lead to the reduction in oxygen supply to various parts of the body. The present review paper offers the readers information about the various SNPs in the HBB gene that are responsible for the traits of β-thalassemia.

Keywords: Gene disease association, HBB gene, β-thalassemia, single nucleotide polymorphisms (SNPs).

1. INTRODUCTION

Gene disease association is a kind of study involves the understanding of how any modifications in the gene expression may lead to a certainchanges in the phenotypic response of an individual. Gene disease association studies are carried based on the principle that the phenotypic change can be directly associated with the genotype expression. The concept of gene disease associations is used to understand how any change in the expression of the HBB gene leads to the disorders such as β-thalassemia; a blood disorder in which the production of hemoglobin reduces in turn leads to the reduction in oxygen supply to various parts of the body. Hemoglobin consists of four protein subunits i.e. two alpha-globin units produced by the HBA gene and two beta-globin units produced by the HBB gene [1]. The HBB is located on the short (p) arm of the 11^{th} chromosome at a position 15.5[2]. Mutation in the HBB gene which leads to the decreased production of beta-globin protein results acondition called beta-plus (β^{+}) thalassemia and also which leads to the complete absence of beta-globin protein causes a condition called beta-zero (β^{0}) thalassemia [3].Majority of these mutations occur at a single base pair level known as single nucleotide polymorphisms (SNPs). Genomic variation such as SNPs in the HBB gene is one of the major reasons for β-thalassemia. Based on the intensity of the symptoms, β-thalassemia is categorized into thalassemia major, thalassemia intermediate and thalassemia minor [4]. Analysis of various SNPsinvolved in the gene sequence variations helps in discovering newerengineered methods which can be successfully used to reduce the severity of hemoglobinopathies includes β-thalassemia and sickle cell anemia

2. SINGLE NUCLEOTIDE POLYMORPHISM

Single Nucleotide Polymorphism (SNP) can be defined as a nucleotide variation in which a single base change occurs that may or may not lead to a phenotypic change. It is a variation in the DNA sequence in which a single nucleotide (A, T, G or C) differs between members of a biological species or paired chromosomes in an individual. In humansthese variations occur at a frequency of more than 1% i.e. occurs in about every 3000 base pairs of the genome. SNPs occur both in the coding as well as the non-coding region of the DNA as depicted in the Figure 1.

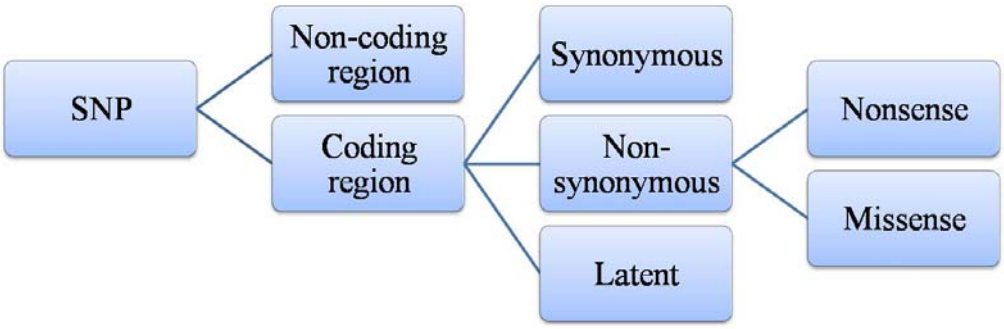

Figure 1 Types of SNP based on their occurrence

SNPs in the non-coding region do not hinder the translation of standardproteinshence report normal functional protein but SNPs in the coding region may alter the functionalities of the translated proteins which may lead toa noticeable phenotypic change. Synonymous SNPs are the third base pair changes in the codon which does not lead to any change in the amino acid;asper the Wobble hypothesis, a single amino acid can be coded by multiple codons, any change in the third base pair does not lead to any change in the protein structure. Non-synonymous SNPs are the mutations leads to the formation of abnormal protein sequences occurs due to either missense mutation or nonsense mutation. In case of missense type, the change in a base pair of the amino acid leads to the change in the codon,forms different amino acids which inturn change the entire protein sequence and the functionality of the protein may be altered [5]. In case of nonsense type of mutation, the change in the single base pair leads to the formation of a stop codon yields an incomplete non functional proteinupon translation.Latent SNPs are the variations which occur in the coding and regulatory region, do not cause any harm at the present state but may transform from harmless to harmful under certain stress conditions.

3. THALASSEMIA

Thalassemia is agenetic blood disorder inherited from the parent with unusual production of hemoglobin which may or may not have any symptoms. If both the parents are carriers of thalassemia, then there is high chance that the offspring will develop severe thalassemia condition. If either one of the parent is a carrier, even theoffspring becomes carrier but is asymptomatic [6]. Since thalassemia deals with abnormalhemoglobin it always leads to anemia, although the severity depends on the kind of thalassemia. Other symptoms apart from anemia includefatigue, pale skin, enlarged spleen, dark urine and skeletal disorders.

The various forms of hemoglobin which can lead to hemoglobinopathies are –

1. Hemoglobin D-Punjab ($\alpha_2\beta_2^D$) – Is prevalent in Punjab region, Northwest Indian, with an estimated frequency of 2.0% [7].
2. Hemoglobin S ($\alpha_2\beta_2^S$) – Observed when there is a variation in β chain and leads to sickle cell anemia [8].
3. Hemoglobin C ($\alpha_2\beta_2^C$) – HbC is a variant caused by a mutation in the HBB gene in which the lysine is replaced by Glutamic acid at position 6 [9].
4. Hemoglobin E ($\alpha_2\beta_2^E$) -HbE is a variant caused by a mutation in the HBB gene in which the lysine is replaced by Glutamic acid at position 26 [10].
5. Hemoglobin H (β_4) – This form consists of tetramer of β chains and mainly found in α thalassemia [11].
6. Hemoglobin F ($\alpha_2\gamma_2$) – Called as the fetal hemoglobin consisting of two alpha and two gamma subunits [12].

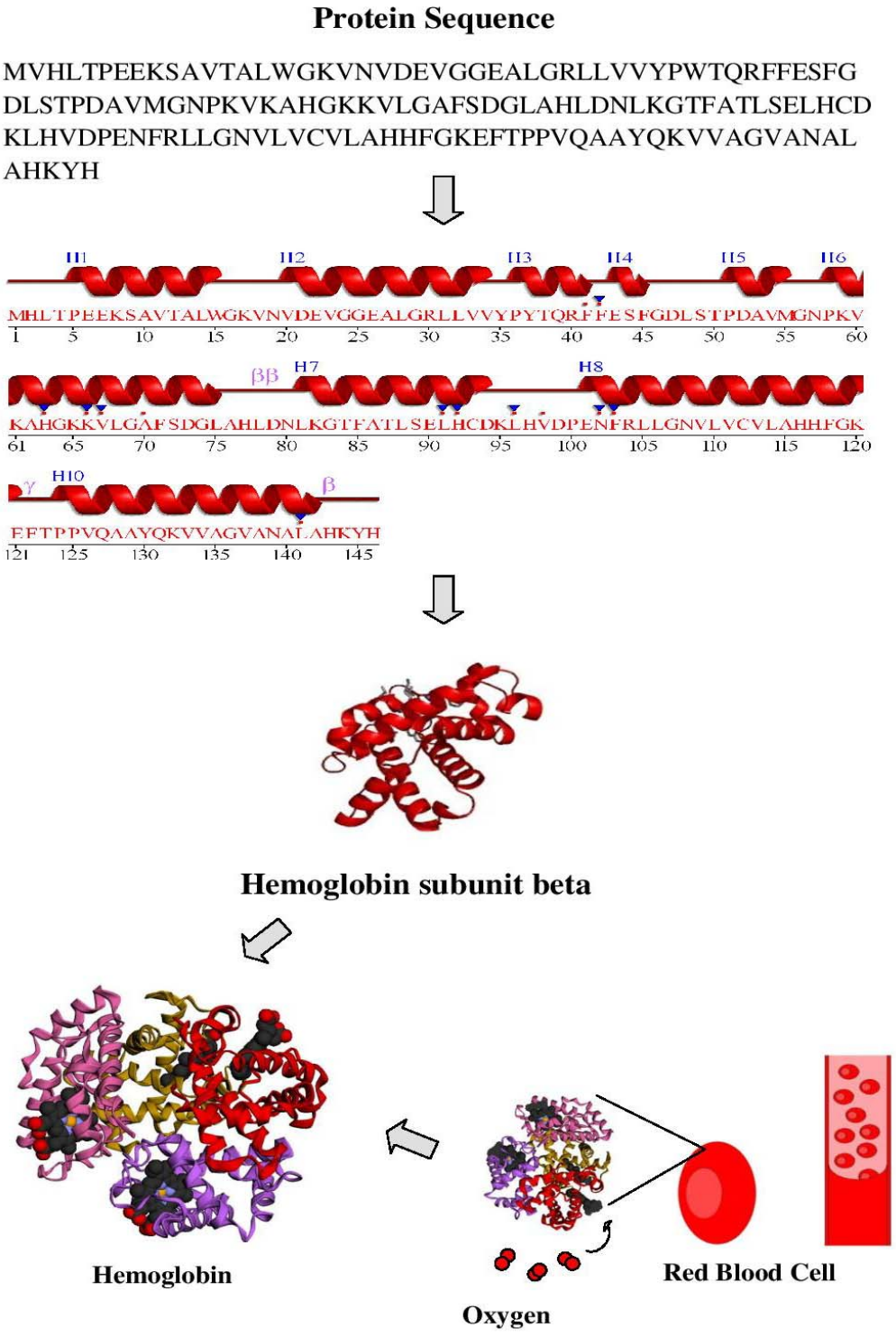

Figure 2 Mechanism of oxygen transport by Hemoglobin

The different forms of thalassemia are–α thalassemia and β thalassemia. Each of thisthalassemiahas different sub types. Hemoglobin is a metalloprotein present in RBC that carries oxygen from lungs to tissues and carbon dioxide from tissues to lungs. In humans,hemoglobin is a tetramer having four subunit proteins i.e. two α and two β subunits as shown in Figure 2. The four polypeptides are bound together by salt bridges, hydrogen bonds and hydrophobic interactions.The alpha subunit consists of 141 amino acid residues and β subunit consists of 146 amino acid residues [13]. Infant hemoglobin is made up of two α and two γ chains. The γ chain gets subsequently replaced by β chains as they grow [14]. Hemoglobin has an important role in giving shape to RBCs. The deficiency of hemoglobin leads to the distraught structure of RBCs reducing its functionality.

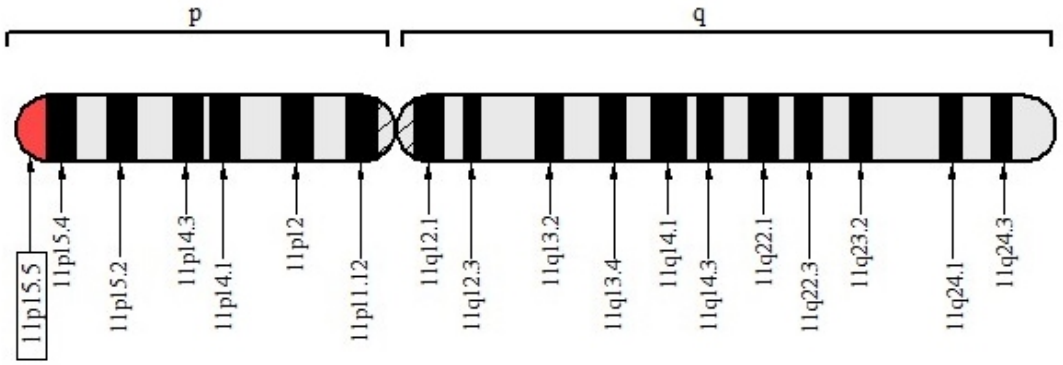

Figure 3 Location of HBB gene on the chromosome 11

Alpha chain, also known as HBA is coded by HBA gene while β chain, also known as HBB is coded by HBB gene[15]. HBB gene is present on chromosome 11 specifically on the short arm of the chromosome at position 15.5 (Figure 3). Mutation in this gene can lead to inherited blood disorders like β thalassemia and sickle cell anemia

Beta thalassemia is characterized by a partial or complete absence of β globin chains. There are different forms of β thalassemia depending on the production of β globin chains. Beta thalassemia major or beta-zero (β^0) thalassemia is the condition where there is complete absence of β globin chains; condition occurs when both the parents are carriers of the traits. As a result, the child acquires two mutated alleles from the parents due to which the beta chain is not synthesized in the offspring. Symptoms of β thalassemia major can be seen from as early as the time of birth or within 2 years of birth. The child develops a severe anemiaand high chances of cardiac arrest. The only treatment for β thalassemia major is lifelong blood transfusion or bone marrow transplant. Due to the continuous blood transfusion, there is a chance of iron overload in the body which leads to damage of heart, liver and endocrine system. Chelation therapy is one of the treatments given during such cases.

Beta thalassemia minor or beta-plus (β+) thalassemia is a condition where there is reduced production of β globin chain. This condition occurs when one of the parents is a carrier of β thalassemia. As the child inherits one normal and one mutated allele of HBB gene from the parents, there will be reduced production of the beta chain in the offspring. As β thalassemia minor is not a severe disorder, the symptoms often include minor anemia and sometimes may be prone to other diseases like asthma and liver disorders. Beta thalassemia intermediate is a condition where both the alleles inherited are mutated but they are not as severe as the alleles in β thalassemia major. Symptoms of β thalassemia intermediate mainly include moderately severe anemia and sometimes regular blood transfusion may also be required.

The thalassemia are among the most common genetic disorders worldwide, occurring more frequently in the Mediterranean region[16], the Indian subcontinent, Southeast Asia, and West Africa[17]. Hemoglobinopathies are one of the most prevalent genetic disorders seen in the Indian population, either as a carrier or as an affected individual. As of 2012, the overall prevalence of β-thalassemia trait was 2.78 % and varied from 1.48 to 3.64 % in different states [18]. This variation in the frequency of the disorder is due to the diversity in the geographic and ethnic origin.Analysis of the prevalence of β-thalassemia in various geographical regions of India helps in understanding the different traits that are seen in the Indian population and also aids in the development of effective treatment.Studying the gene frequency of the various alleles of β-thalassemia also helps in understanding the distribution across the six cities in India. However, it can be seen from the Table 1and 2 that there is an uneven distribution of the abnormal hemoglobin traits in the major cities of the India.

Table 1 Prevalence of β-thalassemia in major cities of India

Sl. No.	Name of the city	β-thalassemia trait (%)	HbS trait (%)	HbD trait (%)	HbE trait (%)
1	Bangalore	2.16	0.20	0.19	0.13
2	Kolkata	3.64	0.14	0.2	3.92
3	Dibrugarh	1.48	0.13	0.02	23.9
4	Ludhiana	3.96	0.06	1.09	0.04
5	Mumbai	2.55	0.53	0.21	0.13
6	Vadodara	2.68	2.94	0.34	0.12

Table 2 Gene frequency of β-thalassemia in major cities of India

Sl. No.	Name of the city	β-thalassemia allele	HbSallele	HbDallele	HbEallele
1	Bangalore	0.0112	0.0020	0.0012	0.0010
2	Kolkata	0.0187	0.0008	0.0012	0.0213
3	Dibrugarh	0.0148	0.0009	0.0000	0.1799
4	Ludhiana	0.0189	0.0003	0.0059	0.0003
5	Mumbai	0.0127	0.0026	0.0010	0.0007
6	Vadodara	0.0136	0.0161	0.0021	0.0007

4. SNPS CAUSING β-THALASSEMIA

SNP is a single nucleotide polymorphism that occurs at a single base pair level has afrequency of occurrence about one in 3000 nucleotides of the human genome. Beta thalassemia is caused due to SNPs in the hemoglobin beta globin gene (HBB) which is located in the chromosome 11 specifically on the short arm of the chromosome at position 15.5 [2]. The various types of SNPs seen in the HBB gene are:

- Nonsense or Stop gained
- Missense mutations
- Frameshift variant
- Intron variant
- Upstream variant 2kb
- Untranslated region (UTR) variant3'
- Untranslated region (UTR) variant 5'
- Coding DNA sequence (CDS) indel
- Splice acceptor variant

4.1 Nonsense or stop gain

Isa type of point mutation which leads to the formation of premature stop codon instead of the amino acid coding codon [5]. It is a kind of non-synonymous SNP; the polypeptide transcribed is shorter than the functional polypeptide as it lacks the necessary amino acid due to the occurrence of stop codon caused by mutation. For example, the 168th nucleotide of mRNA coding for beta chain of hemoglobin changes from CAG to UAG results in changing the amino acid in protein from Glutamine to stop codon causes the premature termination of the translation hence results in the loss of complete functional protein. Various types of Nonsense mutations causing β-thalassemia were discussed in Table 3.

Table 3 Nonsense type mutation causing β-thalassemia.

Sl. no.	Accession no.	Criteria	Normal	Mutated
1	rs11549407	Coding sequence	C A G	T A G
		mRNA	C A G	U A G
		amino acid	Glutamine	Stop
2	rs33986703	Coding sequence	A A G	T A G
		mRNA	A A G	U A G
		amino acid	Lysine	Stop
3	rs33959855	Coding sequence	G A A	T A A
		mRNA	G A A	U A A
		amino acid	Glutamate	Stop
4	rs34716011	Coding sequence	T G G	T A G
		mRNA	U G G	U A G
		amino acid	Tryptophan	Stop
5	rs63750783	Coding sequence	T G G	T A G
		mRNA	U G G	U A G
		amino acid	Tryptophan	Stop

4.2 Missense mutations

Missense mutations are referred to be a type of non-synonymous SNPs, occurs due to the point mutations, results in the formation of a codon coding for a different amino acid [5]. A polypeptide with different amino acid sequence further affects the functionality of the protein sequence. For example 414th nucleotide of the

mRNA coding for beta chain, changes from GAA to CAA causes the change of 122nd amino acid in the protein sequence from Glutamate to Glutamine results in impaired functional protein. Different types of Missense mutations involved in causing β-thalassemia were discussed in Table 4.

Table 4 Missense type mutation causing beta thalassemia

Sl. no.	Accession no.	Criteria	Normal Sequence	Mutated Sequence
1	rs28933077	Coding sequence	T G T	G G T
		mRNA	U G U	G G U
		Amino acid	Cysteine	Glycine
2	rs33986703	Coding sequence	A A G	C A G
		mRNA	A A G	C A G
		Amino acid	Lysine	Glutamine
3	rs33986703	Coding sequence	A A G	G A G
		mRNA	A A G	G A G
		Amino acid	Lysine	Glutamate
4	rs33959855	Coding sequence	G A A	A AA
		mRNA	G A A	A AA
		Amino acid	Glutamate	Lysine
5	rs33959855	Coding sequence	G A A	C A A
		mRNA	G A A	C A A
		Amino acid	Glutamate	Glutamine
6	rs33946267	Coding sequence	G A A	C A A
		mRNA	G A A	C A A
		Amino acid	Glutamate	Glutamine
7	rs33941844	Coding sequence	C T G	C A G
		mRNA	C U G	C A G
		Amino acid	Leucine	Glutamine
8	rs33941844	Coding sequence	C T G	C C G
		mRNA	C U G	C C G
		Amino acid	Leucine	Proline
9	rs33941844	Coding sequence	C T G	C G G
		mRNA	C U G	C G G
		Amino acid	Leucine	Arginine
10	rs33925391	Coding sequence	G T G	G C G
		mRNA	G U G	G C G
		Amino acid	Valine	Alanine
11	rs33930702	Coding sequence	A T G	A T A
		mRNA	A U G	A U A
		Amino acid	Methionine	Isoleucine
12	rs33930702	Coding sequence	A T G	A T C
		mRNA	A U G	A U C
		Amino acid	Methionine	Isoleucine
13	rs33930702	Coding sequence	A T G	A T T
		mRNA	A U G	A U U
		Amino acid	Methionine	Isoleucine
14	rs34407387	Coding sequence	A A T	A C T
		mRNA	A A U	A C U
		Amino acid	Asparagine	Threonine
15	rs34579351	Coding sequence	G A C	G G C
		mRNA	G A C	G G C
		Amino acid	Aspartate	Glycine

4.3 Frameshift variant

Frameshift variant are the mutations in the nucleotides due to indels (insertions or deletions). The numbers of mutations of the nucleotide are such that it is not divisible by three which affects the reading frame (the coupling of codons), in turn affects the gene expression [19]. The frame shift mutations can result in formation of abnormal long or short polypeptide due to alteration is the first stop codon (Table 5).

Table 5 Frameshift type mutation causing beta thalassemia

Sl. No.	Accession no.	Allele change	Amino acid position	Amino acid change
1	rs35497102	AAG ⇒	9	K [Lys] ⇒ V [Val]
2	rs35477349	⇒ GTA	27	E [Glu] ⇒ V [Val]
3	rs35894115	G ⇒ GAA	48	D [Asp] ⇒ E [Glu]
4	rs63750475	GTG ⇒	2	V [Val] ⇒ C [Cys]
5	rs63751201	GTG ⇒	110	V [Val] ⇒ C [Cys]
6	rs63751218	AGT ⇒ TTG	2	S [Ser] ⇒ L [Leu]
7	rs80356821	TTC ⇒	42	F [Phe] ⇒ L [Leu]

4.4 Intron variant

Intron variant are the mutations occurrence in the intron region, do not affect the gene expression but do affects the splicing mechanism [20].

4.5 Upstream variant 2kb

It is the sequence variant of 2kb usually occurs at the 5' region [20].

4.6 Untranslated region (UTR) variant 3'

It is the sequence variant occurs at the untranslated regions of the 3' UTR region. These regions are not translated during protein synthesis but contain regulatory genes which control the gene expression [21]. 3' UTR follows the translation termination codon but does not participate in translation.

4.7 Untranslated region (UTR) variant 5'

Is the sequence variant which occurs at the untranslated region at 5' end. The 5' UTR is present upstream of the initiation codon [21]. UTRs are not translated but contain regulatory genes which affect the translation mechanism in different organisms. In some organisms the UTRs are translated to form a protein product which then helps in translating the main coding sequence, but in some organisms this process does not take place.

4.8 Coding DNA sequence (CDS) indel

Indel is insertions or deletions of nucleotides in the genome [22]. Any indel in the CDS of the HBB gene may result in β-thalassemia.

Change in charge properties of amino acids due to mutation results in the change of overall integrity and 3 dimensional (3D) confirmations of the proteins and hence it altered functionality. The SNPs causing change in charge properties of amino acids results in β-thalassemia was discussed in the Table 6. Table 7 discuss about the impact of the change in isoelectric point (pI) of amino acids due to mutation involved in promoting malfunctions of the proteins hence β-thalassemia.

Table 6 Change in charge properties of amino acids due to mutation

Accession number	Position	Amino acid		Property	
		Normal sequence	Mutated sequence	Normal sequence	Mutated sequence
rs28933077	475	Cysteine	Glycine	Polar uncharged	Non polar
rs33986703	102	Lysine	Glutamine	Positive	Polar uncharged
rs33986703	102	Lysine	Glutamate	Positive	Negative
rs33959855	117	Glutamate	Lysine	Negative	Positive
rs33959855	117	Glutamate	Glutamine	Negative	Polar uncharged
rs33941844	370	Leucine	Glutamine	Non polar	Polar uncharged
rs33941844	370	Leucine	Arginine	Non polar	Positive
rs34579351	334	Aspartate	Glycine	Negative	Non polar

Table 7 Change in pI of amino acids due to mutation

Accession number	Position	Amino acid		pI	
		Normal sequence	Mutated sequence	Normal sequence	Mutated sequence
rs33986703	102	Lysine	Glutamine	9.74	5.65
rs33986703	102	Lysine	Glutamate	9.74	3.22
rs33959855	117	Glutamate	Lysine	3.22	9.74
rs33959855	117	Glutamate	Glutamine	3.22	5.65
rs33941844	370	Leucine	Arginine	5.98	10.76
rs34579351	334	Aspartate	Glycine	2.77	5.97

4.9 Splice acceptor variant

Splice acceptor variations are insertions, deletions or any other change in the nucleotide sequence that occur specifically at the splicing sites [20]. Such mutations results in malfunctioning of the RNA splicing process cause the alteration in translation mechanism and forms abnormal proteins.

5. DISCUSSION

The occurrence of inherited disorders such as hemoglobinopathies includes β-thalassemia and sickle cell anemia is found to be increasing in the hugely populated country like India. SNPs are one of the main causative mutations involved in promoting β-thalassemia. The various forms of SNPs seen in the HBB gene are the major reasons for the occurrence of β-thalassemia in an individual. The non synonymous SNPs are the reason for deviation of the properties of amino acids which in turn alters the protein conformation. These changes in the protein conformation are responsible for the defects in the protein. As mentioned in Table 6, various mutations lead to the variation in the charge properties of the amino acid which can have a major impact on the protein folding and directly related to its functionality. For example in rs33959855, glutamate, a negative amino acid gets mutated to form positive amino acid lysine, a change in the charge from a negative to a positive amino acid at position 117 can have a significant effect on the protein folding characteristics and result in altered protein functionality which causes majorly β-thalassemia. Similarly, from Table 7, significant effects on the isoelectric point (pI) of the amino acids due to SNPs can be seen. The considerable change in pI has a direct impact on the protein solubility. The proteins have their lowest solubility at the pI. Such changes in the physical and chemical properties of the proteins have a significant effect on the protein folding which has a direct impact on its functionality. There are various other complications associated with defect in the HBB gene.The various symptoms seen in an individual suffering from β-thalassemia are

- Fatigue and weakness
- Pale appearance
- Irritability
- Deformities of bones
- Slow growth
- Swollen abdomen
- Dark urine

Anemia is one of the common problems associated with β-thalassemia. Blood transfusion is one of the most preferred modes of treatment for the anemic condition. Due to prolonged cycles of blood transfusion, iron overload becomes one of the major problems seen. The iron overload in the body affects the vital organs of the system such as heart, liver and endocrine system. Cardiac dysfunction is the most common reason for death in cases of β-thalassemia [23]. Iron deposits on the cardiac tissue leads to its degeneration, fibrosis and dysfunction [24]. There is variation in the ventricular wall thickness due to iron deposition [25]. The mechanism of heart injury includes iron overload, chronic anemia, vascular damage, increased pulmonary vascular resistance, infections. Digoxin is prescribed to patients with atrial fibrillation resistant to conversion [26].

The extent of kidney damage due to iron overload depends on the patient's age, severity of anemia, frequency of blood transfusion and type of thalassemia [27]. Glomerular and tubular dysfunction is the major factor that leads to kidney failure. Prolonged tubular damage leads to tubulointerstitial fibrosis. The renal dysfunction may be partially explained by excessive oxidative stress as well as by deferoxamine toxicity[28]. Endocrine dysfunction is often seen in transfusion dependent thalassemia patients and is due to iron overload. One of the major observations was that the growth hormone responses to glucagon stimulation were significantly impaired in all of the patients with iron overload. Puberty delay is also common in patients with thalassemia, especially if iron chelation therapy is started late. Cases of hypothyroidism with variations in concentrations of plasma thyroxine and plasma thyroid stimulating hormone were also observed [29]. The spleen helps fight off infections and filters out unwanted materials such as dead or damaged blood cells from the

body. β-thalassemiacan cause red blood cells to die at a faster rate, making the spleen work harder, which makes it grow larger. A large spleen can make anemia worse and may need to be removed if it gets too big [30].

The various complications caused due to β-thalassemia can be tackled by methods such as blood transfusion, iron chelation, stem cell therapy, and bone marrow transplant.

- Transfusion therapy – Regular blood transfusion promotes normal growth and physical activities, suppressed erythropoiesis and prevents chronic hypoxia. The duration between transfusions is between 2-6 weeks depending on the weight, age and work of the individual. Pre transfusion hemoglobin is monitored routinely to maintain optimal pre-transfusion hemoglobin (Hb) of 9-10.5gm/dL [26].
- Chelation therapy – Chelation therapy is usually started after 10-20transfusions or when serum ferritin level reaches >1000g/l. Chelation therapy helps remove the excess iron deposits in the body and controls the iron overload condition. The various chelating agents used are
    - Desferrioxamine (DFO)
    - Deferiprone
    - Deferasirox – being the most widely used[26].
- Bone marrow transplantation - Till date, over 1000 bone marrow transplants have been performed inthalassemic patients at medical centres of excellence. Withadequate iron chelation therapy, the survival rate was about 80-90% with no damage to the liver[14].
- Natural agents – natural inducers that are used to improve the fetalhemoglobin production in β-thalassemia patients are
- Angelicin- Angelicin is a potent inducer of erythroid differentiation, gamma globin gene expression and fetalhemoglobin (HbF) production, thereby making it useful in the treatment of β-thalassemia.
- Rapamycin- Rapamycin is more efficient than hydroxyurea for stimulating the production of g globin mRNA and increasing HbF level. Rapamycin was found to increase HbF level in β-thalassemia patients.
- Wheatgrass- The pH of wheatgrass juice (7.4) is same as the human blood due to which it is rapidly absorbed in human blood. Wheatgrass increases the HbF level, expands the time period of repeated blood transfusions as well as reduces the amount of total blood transfused in β-thalassemia patients.
- Curcuma comosa- Curcuma comosa is a Thai herbal medicine and is known for its anti-inflammatory activity. It is reported that the n-hexane extract of the aerial parts of Curcuma comosa increases HbF production in K562 cell line [31].

Mutations in the HBB gene that is responsible for the β-thalassemia trait are inherited from the parent. These mutations are basically differentiated based on the geographic and cultural origin of the individual. Awareness plays a vital role when it comes to minimizing the number of β-thalassemia cases in India. More awareness programs need to be organized which should provide adequate information about the cause, symptoms and complications, diagnosis, treatment and preventive measures. The various strategies that can be employed for future prevention of beta thalassemia are:

- Population screening [32]
- Mass awareness program [33]
- Antenatal screening [34]
- Premarital screening [35]
- Cascade screening [36]
- Students' screening [37]

Positive family history and previous enrolment in thalassemia screening has significant impact on the knowledge of β-thalassemia. Prenatal diagnosis and prevention of births of β-thalassemia homozygotes is the most preferred approach adopted in India. Counseling in such cases can help create awareness. In India, there is urgent need to initiate large scale population carrier screening of high risk communities along with education and awareness.

6. RESEARCH GAP

Beta-thalassemia found to occur in two forms i.eβ-thalassemia major and minor. Around 7% of the world population is carriers of β-thalassemia and were survives with low hemoglobin production throughout their life. After discussing the prevalence of β-thalassemia in different cities of India and its frequency of occurrence in different geographical regions of the world from the reported research papers, it can be understood that different mutations in different geographical areas are responsible for β-thalassemia. When it comes to understanding the role of each SNP as a principle causative mutation of β-thalassemia, there is a limited research reported which in turn depicts the profound lacunae of information on the role of SNPs in promoting β-thalassemia. Through this review paper, an attempt has been made towards the understanding of the effect of various SNPs on the properties of the coding amino acids such as the pI and the charges of the amino acid promoting β-thalassemia.

Understanding these changes in the protein properties can help directly correlate the effect of SNPs to the severity of the disorder.

7. CONCLUSION

Hemoglobinopathies are known to be one of the most frequently occurring genetic disorders worldwide. In India, on an average the occurrence of β-thalassemiais about 2.5% of the total population. Beta thalassemia is caused due to a defect in the HBB gene. Hemoglobin consists of two alpha and two beta chains. The HBB gene which is located on the short arm of chromosome 11 codes for beta chains of hemoglobin. Any defect in the production of beta chain gives rise to defect in hemoglobin structure and function. As a result, the hemoglobin is unable to carry sufficient amount of oxygen required by the body and develop a condition known as β-thalassemia. SNPs are the single genetic variations that are responsible for the defects in HBB gene which in turn affects the production of beta chain, leading to β-thalassemia. Creating awareness about the disorder among the population, educating them on how the condition is inherited and can be treated, plays an important role in controlling the occurrence of the disease.

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CONFLICT OFINTEREST

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REFERENCES

[1] Bunn HF, Subunit assembly of hemoglobin: An important determinant of hematologic phenotype, Blood 69: 1 (1987) 1-6.
[2] NCBI, Map Viewer, Homo sapiens (human) Annotation Release 108 (Current)chromosome11.https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Homo_sapiens/108/, 2016, (accessed Jun 6 2016).
[3] Beta thalassemia, Genetics Home Reference, U.S National Library of Medicine. https://ghr.nlm.nih.gov/condition/beta-thalassemia, 2017, (accessed December 12 2017).
[4] RenzoGalanello and RaffaellaOriga, Beta-thalassemia, Galanello and Origa, Orphanet Journal of Rare Diseases 5:11 (2010) 1-15.
[5] Kaleigh Smith, Genetic Polymorphism and SNPs: Genotyping, Haplotype Assembly Problem, Haplotype Map, Functional Genomics and Proteomics. http://www.cs.mcgill.ca/~kaleigh/compbio/snp/snp_summary.html, 2002, (accessed February 19 2002).
[6] Antonio Cao and Renzo Galanello, Beta-thalassemia,Genetics IN Medicine12: 2 (2010) 61-76. doi: 10.1097/GIM.0b013e3181cd68ed.
[7] Lidiane de Souza Torres, JéssikaViviani Okumura, DaniloGrünigHumberto da Silva, Claudia Regina Bonini-Domingos,Hemoglobin D-Punjab: origin, distribution and laboratory diagnosis, RevistaBrasileira de Hematologia e Hemoterapia 37:2 (2015) 120-126.
[8] U.S. Department of Health & Human Services, National Heart, Lung and Blood Institute https://www.nhlbi.nih.gov/health/health-topics/topics/sca, 2017, (accessed on September 19 2017)
[9] Dora Bachir and FrédéricGalacteros, Hemoglobin C, Orphanet Encyclopedia.https://www.orpha.net/data/patho/GB/uk-HbC.pdf. 2004 (accessed November 2004).
[10] Dora Bachir and FrédéricGalacteros, Hemoglobin E, Orphanet Encyclopedia.https://www.orpha.net/data/patho/GB/uk-HbC.pdf. 2004 (accessed November 2004).
[11] Cornelis L Harteveld and Douglas R Higgs, α-thalassaemia, Harteveld and Higgs Orphanet Journal of Rare Diseases 5:13 (2010) 1-21. https://doi.org/10.1186/1750-1172-5-13
[12] Dominic Edoh, Charles Antwi- Bosaiko, Dominic Amuzu, Fetal hemoglobin during infancy and in sickle cell adults, African Health Sciences 6:1 (2006) 51–54.
[13] Haemoglobin, School of Biomedical Sciences, Newcastle University https://teaching.ncl.ac.uk/bms/wiki/index.php/Haemoglobin#cite_note-12, 2016 (accessed December 4 2016).
[14] Marengo-Rowe, Alain J. "The Thalassemias and Related Disorders. Proceedings Baylor University Medical Center 20:1 (2007) 27–31.
[15] HBB gene, Genetics Home Reference, U.S National Library of Medicine. https://ghr.nlm.nih.gov/gene/HBB, 2017, (accessed December 12 2017).
[16] Fawdry AL, Erythroblastic anaemia of childhood (Cooley's anaemia) in Cyprus, Lancet 1 (1944)171–176.
[17] Weatherall DJ, The thalassemias. In Beutler B, Lichtman MA, Coller BS, Kipps TJ, Seligsohn S, eds. Williams Hematology, 6th ed. New York, McGraw-Hill, 2001, pp. 562–564.
[18] D. Mohanty,R. B. Colah, A. C. Gorakshakar, R. Z. Patel, D. C. Master, J. Mahanta, S. K. Sharma, U. Chaudhari, M. Ghosh, S. Das, R. P. Britt, S. Singh, C. Ross, L. Jagannathan, R. Kaul, D. K. Shukla, V. Muthuswamy, Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study, Journal of Community Genetics4 (2013) 33–42.
[19] Losick, Richard; Watson, James D.; Baker, Tania A.; Bell, Stephen; Gann, Alexander; Levine, Michael W, Molecular biology of the gene, 6th ed., San Francisco: Pearson/Benjamin Cummings, 2008.
[20] Batch Genome Variation Server 138, SNP summary table values.http://gvs.gs.washington.edu/GVSBatch138/HelpSNPSummary.jsp
[21] Lucy W. Barrett, Sue Fletcher, Steve D. Wilton, Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements, Cellular and Molecular Life Sciences 69 (2012) 3613–3634. doi: 10.1007/s00018-012-0990-9
[22] Mills, R. E, An initial map of insertion and deletion (INDEL)variation in the human genome, Genome Research.16:9 (2006) 1182–1190.
[23] Modell B, Khan M, Darlison M, Survival in beta-thalassemia major in UK: data from the UK thalassemia register, Lancet 355 (2000) 2051–2.doi: 10.1016/S0140-6736(00)02357-6
[24] Olivieri NF, The β-thalassemias, The New England Journal ofMedicine 341(2) (1999) 99-109.
[25] Samira Z Sayed, Basma A Aly, Abd El-Hakim A Abd El-Hakim, Suzan M Omar, Amr S Amin, The early cardiac involvement in patients with β-thalassemia major, The Egyptian Heart Journal 65(2013)243–249.

[26] Dubey A.P., A. Parakh and S. Dublish, Current Trends in the Management of Beta Thalassemia, Indian Journal of Pediatrics 75 (7) 2008 739-743.
[27] Galanello, R., &Origa, R. Beta-thalassemia. Orphanet Journal of Rare Diseases5:11 (2010) 1-15. http://doi.org/10.1186/1750-1172-5-11
[28] Hamed, E. A., &ElMelegy, N. T.. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. Italian Journal of Pediatrics36 (2010) 39. http://doi.org/10.1186/1824-7288-36-39
[29] Grundy R G, K A Woods, M 0 Savage, J P M Evans, Relationship of endocrinopathy to iron chelationstatus in young patients with thalassaemia major, Archives of Disease in Childhood 71 (1994) 128-132.
[30] KidsHealth, The Nemours Foundation, Beta thalassemia, http://m.kidshealth.org/en/parents/beta-thalassemia.html?ref=search&WT.ac=msh-p-mob-en-search-clk 2015, (Reviewed July 2015).
[31] AayushKukreja, NehaWadhwa, ArchanaTiwari, Therapeutic role of natural agents in beta-thalassemia: A review, Journal of pharmacy research 6 (2013) 954-959.
[32] Patel AP, Naik MR, Shah NM, Sharma NP, Parmar PH. Prevalence of common hemoglobinopathies in Gujarat: an analysis of a large population Screening program. National Journal of Community Medicine 3 (2012) 112-116.
[33] United Kingdom Thalassemia Society. Thalassemia Asian Awareness Campaign Report of a three-year campaign (1997-2000). http://www.ukts.org/pdfs/awareness/asianaware.pdf
[34] Patel AP, Patel RB, Patel SA, Vaniawala SN, Patel DS, Shrivastava NS, et al. Beta Thalassemia Mutations in Western India: Outcome of Prenatal Diagnosis in a Hemoglobi-nopathies Project. Hemoglobin38 (2014) 329-334. doi: 10.3109/03630269.2014.951889. Epub 2014 Sep 15.
[35] Memish ZA, Saeedi MY.Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and Beta-thalassemia in Saudi Arabia.Annals of Saudi Medicine31 (2011) 229-235. doi: 10.4103/0256-4947.81527
[36] Gorakshakar AC, Colah RB. Cascade screening for Beta-thalassemia: A practical approach for identifying and counseling carriers in India. Indian Journal of Community Medicine34 (2009) 354-356. doi: 10.4103/0970-0218.58399
[37] Colah R, Thomas M, Mayekar P. Assessing the impact of screening and counselling high school children for b-thalassemia in India. Journal of Medical Screening14:3 (2007) 158.doi: 10.1258/096914107782066202