Visual and Retinal Abnormalities in Patients with Beta Thalassemia

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Abstract:

The current genetic investigation was conducted to characterize the molecular basis of variable ocular abnormalities observed in a consanguineous Pakistani family affected by β-thalassemia. Peripheral blood samples were collected from affected and unaffected members of the family, followed by targeted Sanger sequencing of the *HBB* gene to identify potential pathogenic variants. Comprehensive in silico analyses were carried out was also done. Clinical and laboratory findings were consistent with a severe transfusion-dependent anemia. Genetic screening revealed a homozygous insertion mutation, c.27_28insG, resulting in a frameshift and premature stop codon at position p.Ser10Valfs*14. This mutation was found to be associated with the β^{+}/β^{+} thalassemia major phenotype in the two affected siblings. Interestingly, both individuals exhibited diverse and progressive ocular manifestations, including congenital cataract, high-grade progressive myopia, involuntary eye movements (nystagmus), and bilateral strabismus, suggesting a possible correlation between the thalassemic genotype and retinal dysfunction. The computational analyses demonstrated significant structural perturbations in the mutated β-globin protein, supporting the pathogenic nature of the frameshift variant. Furthermore, ophthalmological evaluation, particularly fundoscopic examination, confirmed the presence of structural retinal abnormalities, which may be a secondary consequence of chronic hypoxia or iron overload commonly seen in thalassemia major. This study not only expands the phenotypic spectrum associated with β-thalassemia but also highlights the potential link between thalassemic mutations and extra-hematological complications such as ocular pathology. Early molecular diagnosis and clinical

intervention could significantly improve disease management and reduce the burden of preventable complications in future generations.

Keywords: Mutation, Pakistan, Retinal, Visual, Phenotype, *HBB* gene.

1. Introduction:

Thalassemia is a heritable blood disorder caused by mutations in the genes responsible for producing hemoglobin, the oxygen-carrying component of red blood cells, and is primarily classified into alpha-thalassemia and beta-thalassemia depending on whether the alpha or beta globin chains are affected (Chen et al., 2024). These mutations result in reduced or absent synthesis of the respective globin chains, leading to imbalanced hemoglobin formation, chronic hemolytic anemia, and compensatory bone marrow hyperplasia. In beta-thalassemia major, patients typically present within the first two years of life with severe anemia, hepatosplenomegaly, growth failure, and skeletal deformities due to bone marrow expansion, and they often become transfusion-dependent throughout life (Angastiniotis and Lobitz, 2019). Iron overload from repeated transfusions, unless controlled with chelation therapy, can result in life-threatening complications involving the heart, liver, and endocrine organs. In contrast, thalassemia minor (carrier state) is often asymptomatic and may go undiagnosed without specific testing, yet carriers can transmit the disorder to offspring if both parents are affected. Globally, thalassemia poses a significant health burden, particularly in the "thalassemia belt," which spans the Mediterranean region, Middle East, Indian subcontinent, and Southeast Asia, where the carrier frequency can be as high as 10%–15% in some populations. According to the World Health Organization, an estimated 5%–7% of the global population are carriers of a hemoglobin disorder, and approximately 300,000 to 400,000 infants are born each year with a major hemoglobinopathy such as thalassemia (Hokland et al., 2023). In Pakistan, studies indicate that around 5%–8% of the population are beta-thalassemia carriers, and over 100,000 patients are living with beta-thalassemia major, with 5,000–9,000 new cases born each year, often due to lack of awareness and prevalence of consanguineous marriages (Weatherall and Clegg, 2001). Management of thalassemia includes regular blood transfusions, iron chelation therapy with agents such as deferoxamine or deferasirox, and in some cases, hematopoietic stem cell transplantation (HSCT), which remains the only curative option but is limited by donor availability and high costs (Hashim et al., 2018). Gene therapy, although still

under investigation, offers promising future potential. Despite medical advances, the economic burden of long-term management and the psychosocial strain on families, especially in low- and middle-income countries, remains high. Prevention through public health initiatives, such as premarital carrier screening, genetic counseling, prenatal diagnosis via chorionic villus sampling or amniocentesis, and education campaigns has shown success in countries like Cyprus, Iran, and Saudi Arabia, where national thalassemia prevention programs have significantly reduced the incidence of new cases (Yutarti, 2023). However, in many countries, including Pakistan and India, the implementation of such programs remains fragmented, and cultural practices like cousin marriages continue to contribute to disease persistence. Therefore, thalassemia is not only a medical disorder but also a societal challenge that demands a comprehensive strategy integrating clinical care, public health policy, community engagement, and international collaboration to reduce disease incidence and improve patient outcomes (Khaliq, 2022). The main aim of the current study was to screen a Pakistani family suffering from variable phenotypes of ocular defects, including cataract, strabismus and myopia due to thalasemia.

2. Methods:

2.1. Family recruitment and Sample collection.

The thalassemia-affected family was Saraiki origin recruited from D I Khan city of Pakistan and blood was taken. DNA was then extracted using the salting out method (Gaaib et al., 2011; Miller et al., 1988). Moreover, to determine the status of the fundus, a fundoscopy was also performed.

2.2. Molecular Analysis.

Sanger Sequencing of the complete *HBB* was carried out to know about mutation and its segregation pattern in the whole family. BioEdit tool was accordingly used for comparing sequences (Muzammal et al., 2022).

2.3.Protein Tertiary Structure Prediction

To model 3D protein structure, Alphafold tool was used. The models with maximum confidence score (Cscore) were selected for further analysis (Yang et al., 2014).

2.4.Protein Secondary Structure prediction.

The protein 2D structure was predicted using the online tool PSIPRED.

2.5.Molecular Docking and Visualization

ClusPro tool was employed to understand interaction among wild-type, its mutated HBB protein, and HBA1 protein. Visualization of the structures was done with the help of LigPlot+ (Laskowski and Swindells, 2011) and Chimera 1.13.1 tools (Pettersen et al., 2004).

3. Results:

3.1. Medical Findings:

The family was enrolled from Dera Ismail Khan in Pakistan. Pedigree comprises three generations, having two affected females (III:1 and III:2) in the third generation. The family pedigree is shown in Figure 1a.

Both the affected individuals were about 14 and 13 years old, respectively, and previously diagnosed with Thalassemia Major with 10-12 transfusions per year. Besides thalassemia, both the affected individuals were showing different retinal abnormalities, i.e., cataract, nystagmus and bilateral strabismus. Both affected individuals had severe myopia and night vision problems but no photophobia was present in either patient. Ophthalmic analysis showed retinal Pigment Epithelium degeneration, diminished retinal blood vessels, retinal pigmentary deposits, and pallor of the optic disc in both patients. Patients' fundoscopy results are shown in figure 1b. Medical descriptions of the patients are shown in Table 1.

3.2. Genetic Finding:

Genetically, both the patients (III:1 & III:2) had homozygous severe frameshift mutation c.27_28insG, where insertion of a single nucleotide (G) took place between positions 27 and 28 in the first exon of the *HBB* gene which resulting in a frameshift of the protein (i.e., p. Ser10Valfs14Term). This mutation has been reported in the Pakistani population. Both parents were (II:1 & II:2, Figure 1c) were heterozygous carrier, while the normal sibling (III:3) was wild-type for the mutation.

3.3.Protein Modeling:

To check the similarity index (SI) between the wild-type and mutant HBB proteins, the 3D (tertiary) structure was predicted and superimposed. The SI of 3D structure of the mutation (Ser10Valfs14Term) with the wild-type protein was found to be 12.24% due to very early truncation of the protein. 3D structures are shown in Figure 2. In the case of the secondary structure of the HBB wild-type and mutant protein. The mutant protein showed complete deletion of all the halicales after the mutation; moreover, mutant protein showed both the upstream and downstream changes in the structure due to the mutation of the protein (Figure 3a).

3.4.Interactional Studies

The study of the interaction of wild-type and mutant HBB protein with the closely interacting protein HBA1 (illustrated in Figure 3b) showed strong changes in dockage site and bonding as a result of the mutation. Normal HBB was interacting with HBA1 by 2 hydrogen bonds and another salt bridge that was made through the 2 residues, e.g., Glu102 and Trp38. As the HBA1 protein was docking on the wild-type protein through amino acids, Arg32 and His123. That is mutant (p. Ser10Valfs14Term) protein was interacting via two Hydrogen bonds through the amino acids Cys14 and Gly17 whereas, HBA1 protein was interacting with mutant protein through two amino acids Val108 and Ser36. The mutation not only resulted in the loss of the normal docking site but also in the loss of a salt bridge. Figure 4 in addition demonstrates a 3D representation of protein-protein interaction.

4. Discussion:

In the present investigation, a single Pakistani family having variable ocular abnormalities due to thalassemia was investigated. Fundoscopy of the patient's eyes showed retinal Pigment Epithelium degeneration, attenuated retinal blood vessels and retinal pigmentary deposits, while genetic analysis revealed c.27_28insG mutation in *HBB* gene. *In silico* analysis of the mutation showed the pathogenic effect of the mutation on protein structure and function. Schematic representation of *HBB* gene mutation identified in this study is in figure 5.

β-thalassemia occurs in three forms, namely Thalassemia Major (TM), commonly referred to as Cooley anaemia and Mediterranean anaemia (Gibson et al., 1983; Ketner et al., 2015), Thalassemia Intermediate (TI), and thalassemia minor. TM and severe TI are treated with regular red blood cell

(RBC) transfusions, iron depletion and folic acid supplements. In certain conditions, it may need a splenectomy to reduce symptoms of the disease. At present, bone marrow transplantation is the unique declared therapy, although scientists currently study a genetic cure, and its results are encouraging to search among potential methods of disease treatment (Jafari et al., 2015; Karponi and Zogas, 2019). Beta thalassemia may also cause ocular symptoms through the disease, iron overload, or chelating medication. Well-known optic complications, such as tear functioning parameters that suggest the presence of ocular surface disease, and lens opacities, occur in 9 to 44 percent of all cases. There is a positive correlation between desferrioxamine and lenticular opacities and between deferriprone and retinal pigment epithelium (RPE) degeneration (Barteselli et al., 2014; Finger et al., 2009). Some have identified ocular fundus anomalies that include peau d'orange, angioid streaks, pattern dystrophies-like changes, and the optic disc drusen that is consistent with pseudoxanthoma elasticum (PXE). The age of the PXE-like fundus changes is mostly older, and the splenectomy is equally strongly connected with the development of such changes. In 11%-17.9% of cases, unaffiliated PXE-like retinal vascular tortuosity has been identified and is linked to aspartate aminotransferase, haemoglobin and ferritin values. In addition to fundus inspection, other procedures such as fundus self-fluorescence and electrophysiological testing (electro-oculogram and electroretinogram) may provide evidence about early stages damage in the eyes of people suffering from beta thalassaemia (Barteselli et al., 2014; Finger et al., 2009; Liaska et al., 2016).

The patients with beta-thalassemia may develop a wide range of complications that involve every organ of the organism including eyes. The ocular disorders entail retinal pigment epithelium degeneration, angioid streaks, venous tortuosity, night blindness, and visual fields defects, visual acuity, colour vision distortion, and sudden visual loss. The β-TM patients have to be transfused and their permanent iron overload requires iron chelation treatment (ICT) (Karponi and Zogas, 2019). The patients of 0-TM (thalassemia major) and 0-TI (thalassemia intermedia) can develop retinal disease over time. These retinal pathologies are distributed into two categories: Two kinds of retinal abnormalities exist: Pseudoxanthoma (PXE)-like and non-PXE-like. PXE is an inherited disease that is an outcome of the mutation in the ABCC6 gene that can be found on the 16th chromosome. The gene coding multidrug resistance-associated protein 6 is known as ABCC6, is expressed in liver, stomach, kidney, skin, eyes, and blood vessels (Barteselli et al., 2014; Finger et

al., 2009). PXE stands out because of calcium mineralisation fibres of elastic (elastin). Elastin is one of the storage sites of calcium and other minerals in blood vessels, epidermis, and beneath the retina pigment epithelium cells (Bruch membrane). Investigations that were carried out in the early 90s discovered that the ocular symptoms of β-thalassemia and PXE were similar. Abnormalities of the retina such as the angioid streaks, peau d'orange, and the formation of the optic disc drusen, characterize PXE-like syndrome. It was reported by Barteselli et al. that the most common non-PXE-like pathology affecting the retina in 88 beta-thalassemia patients was the retinal venous tortuosity (RVT) (Barteselli et al., 2014).

Peau d'orange is the small yellowish dark lesion on the retinal pigment epithelium. Such a finding is often followed by angioid streaks. Initially, the Bruch membrane is calcified in a centrifugal way with the center at the posterior pole. It has to do with peau d'orange appearance. Barteselli et al 2014., confirms that peau d orange is the most widespread symptom in patients of 3-thalassemic besides some other complications of the problem (Barteselli et al., 2014). Angioid streak is a retinal disease developed as a result of structural changes in Bruch membrane below RPE. Histopathologic features indicate the presence of jagged crack-like dehiscences of Bruch membrane that are connected to atrophies that affect overlying RPE (Lopes et al., 2011). Angioid streaks in some conditions are linked to pseudoxanthoma elasticum, Paget disease of bone, acromegaly, Ehlers-Danlos syndrome, diabetes type 2, sickle cell anaemia and beta-thalassemia. Its streak to the foveola or development of complications such as macular choroidal neovascularisation (CNV) or traumatic rupture of Bruch membrane (GB), is an issue that has to be corrected (Gibson et al., 1983). Those who have angioid streaks and traumatic brain injury have higher chances of experiencing visual deficiency since the angioid streaks damage the Bruch membrane(Bhoiwala and Dunaief, 2016). Lastly, CNV is the most injurious form of angioid streaks that has the capability of causing disastrous loss of vision. The presence of drusen in the optic nerve head is more common among the PXE patients. It has been also found in the cases of β-thalassemia (Georgalas et al., 2009; Ketner et al., 2015).

Conclusion:

Based on the result, it has been concluded that families having a history of Thalassemia should go for clinical and genetic testing to control the inheritance of Thalassemia into the next generation. Moreover, due to the high prevalence of Thalassemia in the southern area of the country, genetic

counseling and genetic testing facilities should be provided to overcome this disorder. This study not only expands the phenotypic spectrum associated with β-thalassemia but also highlights the potential link between thalassemic mutations and extra-hematological complications such as ocular pathology. Early molecular diagnosis and clinical intervention could significantly improve disease management and reduce the burden of preventable complications in future generations.

Statements and Declarations:

Acknowledgement

We are thankful to the individuals for donating their blood samples for scientific research.

Declaration of generative AI in scientific writing

No AI tool was used for writing this article.

Conflict of Interest

The authors declare no conflict of interests.

Funding:

None.

Author Contribution

N.A composed the manuscript. F.A and M.Y enrolled the patients and collected patients' samples. N.A and W.S performed the WES analysis, bioinformatics analyses reviewed and revised the manuscript. W.S conceived and supervised the study. All authors participated in the preparation and review of the manuscript and read and approved the final manuscript.

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Table 1: Clinical Features of the patients

Patient ID	III:1	III:2	Reference Range
Gender	Female		-
Age	~14	~13	-
Mutation	c.27_28insG		-
Protein Change	Ser10Valfs14Term		-
Zygosity	Homozygous		-
Thalassemia	Major		-
Cataract	Yes	Yes	-
Myopia	Yes	yes	-
Strabismus	Yes	Yes	-
Nystagmus	Yes	Yes	-
Transfusion per Year	10-12	10-12	-
Hb	5.3	5.6	12 to 16 g/dl

Figures:

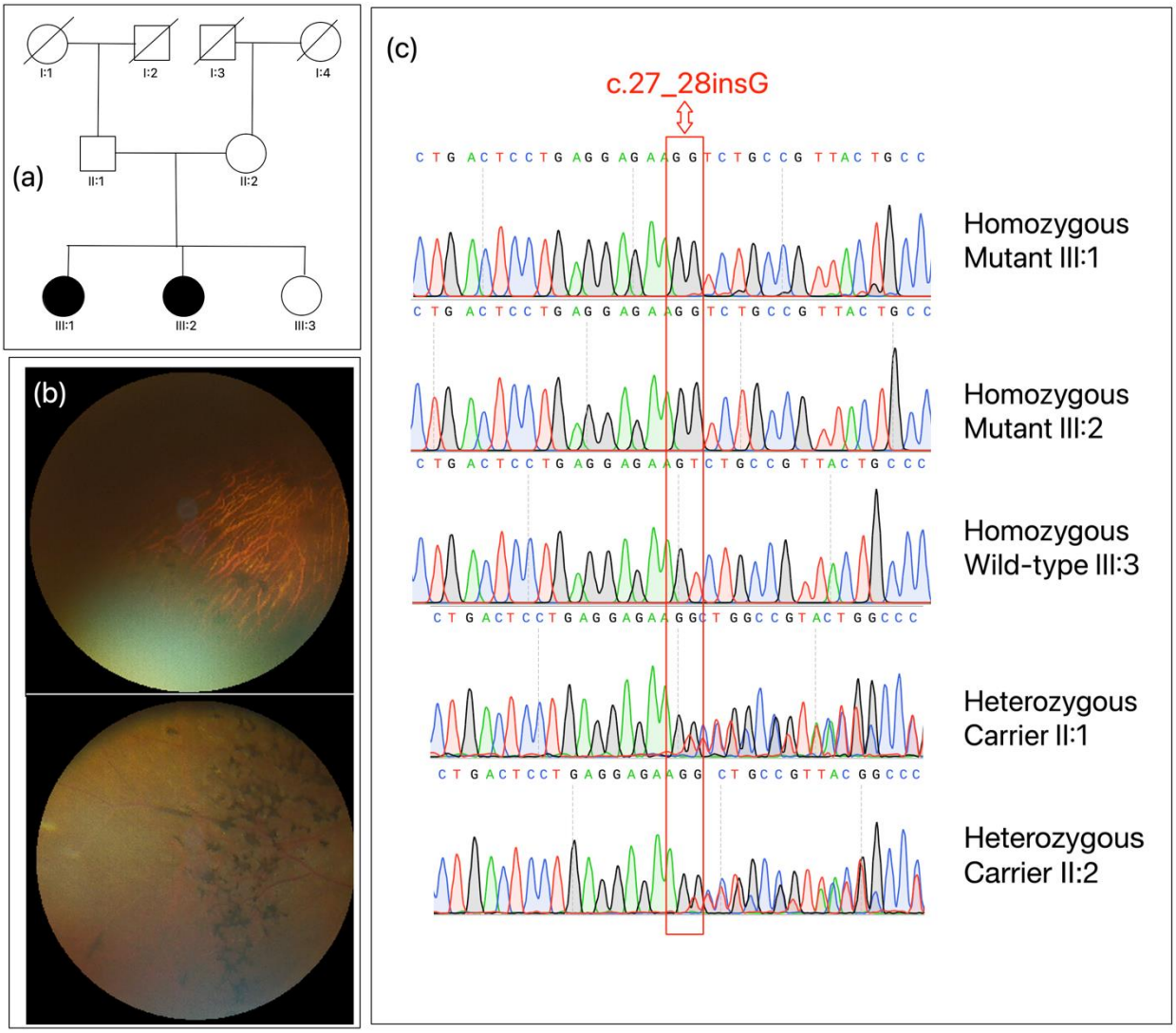

Figure 1: (a) Figure showing the pedigree of the family and segregation and inheritance of the affected alleles. (b) Fundoscopy results showing the attenuated blood vessels and hyper pigmentation (c) Sanger sequencing chromatograms of the affected family.

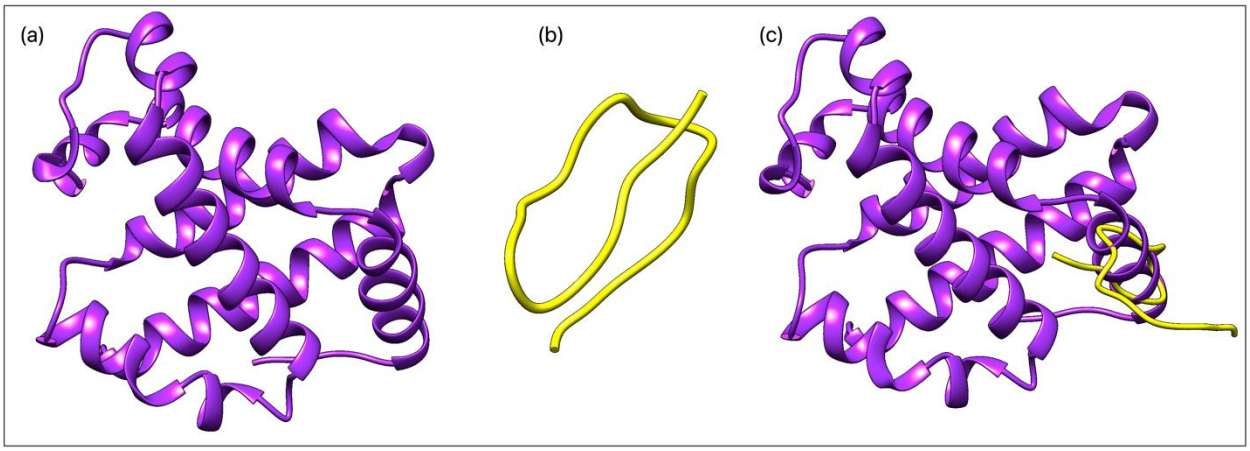

Figure 2: (a) Tertiary (3D) structure of wild-type, (b) mutant (c) superimposed structure of wild-type and mutant HBB protein

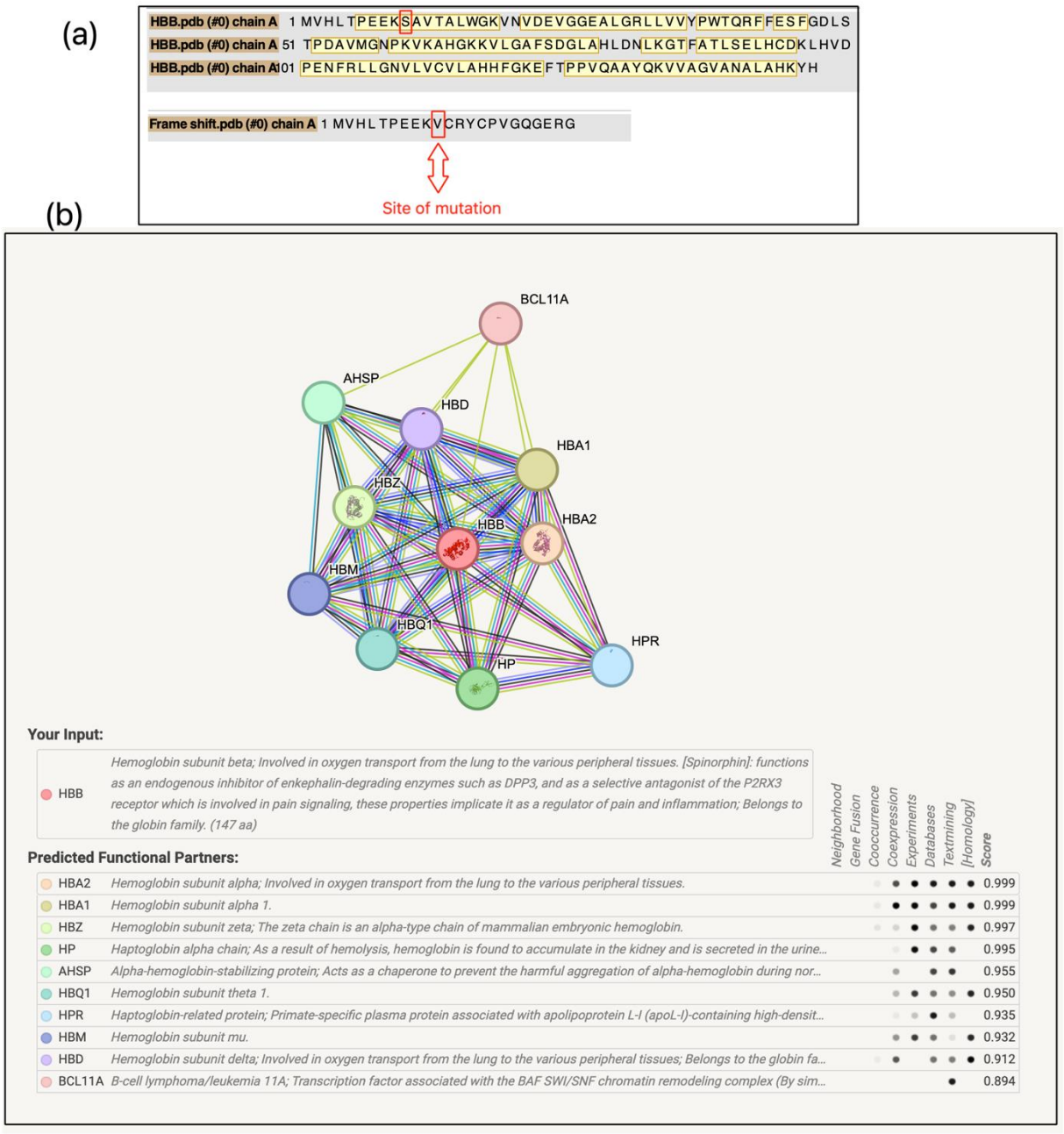

Figure 3: (a) 2D structure of wild-type and mutant HBB protein showing site of mutation in red box. (b) Close functional interactor HBA1 protein predicted through string data base

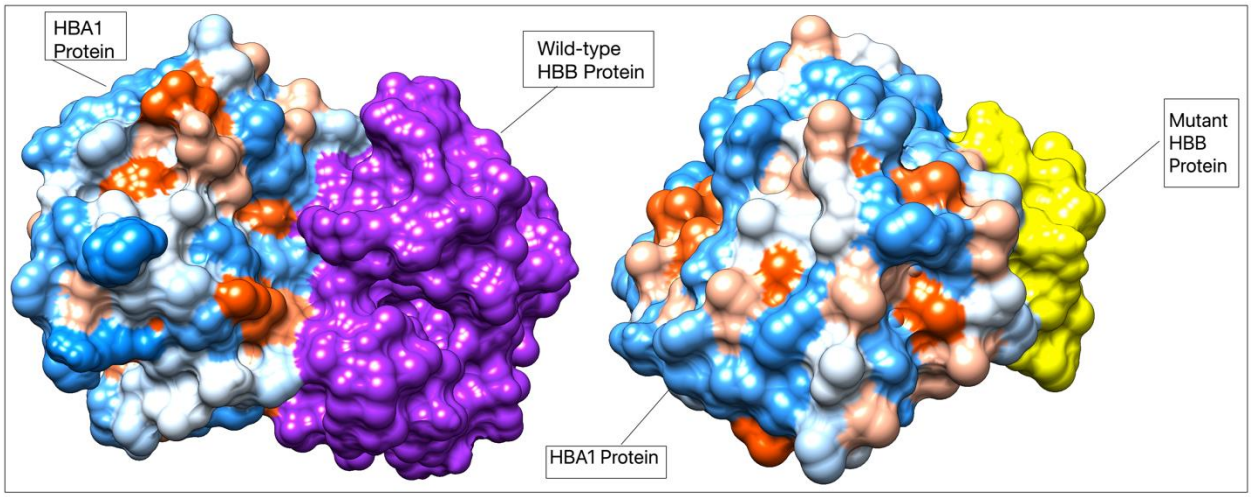

Figure 4: (a) Protein-Protein docking of wild-type HBB and close interactor HBA1 protein, (b) Protein-Protein docking of mutant HBB protein with close interactor HBA1 protein.

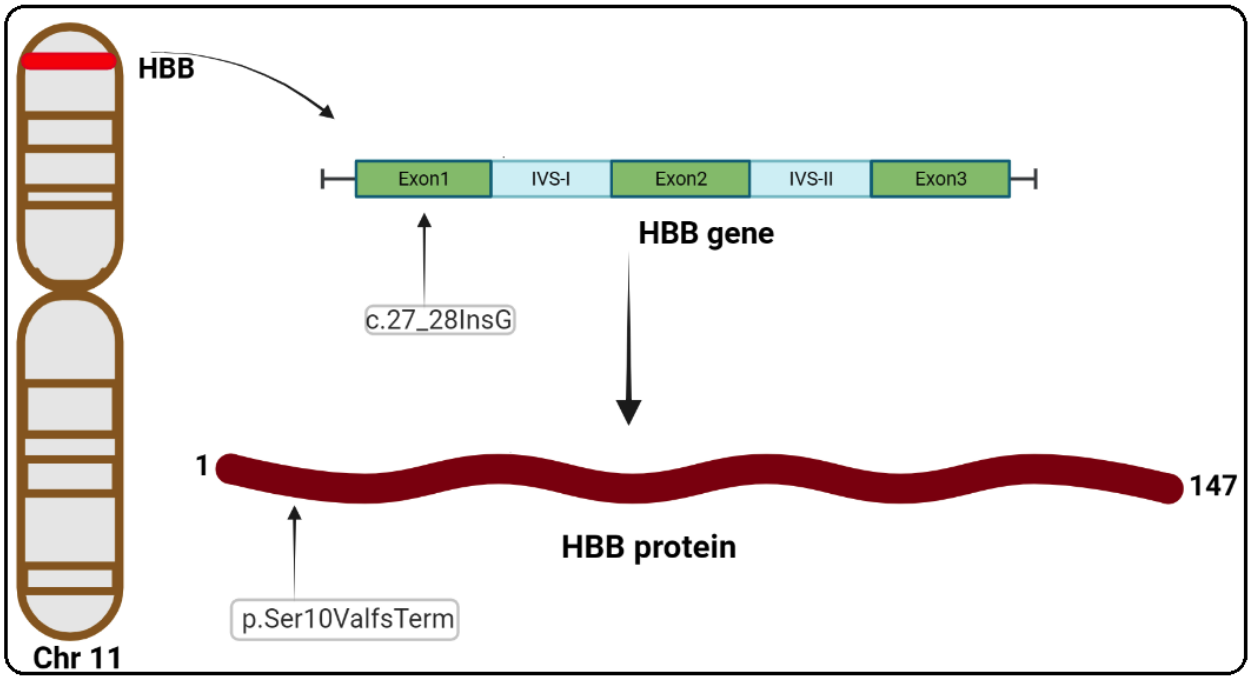

Figure 5: Schematic representation of *HBB* gene mutation identified in this study.