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Prediction of hemoglobin protein structure and function of the $\alpha^{4.2}$ deletion mutation and αα/-$\alpha^{4.2}$/βCD8 (-AA)/β^N co-inheritance

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Abstract

The $\alpha^{4.2}$ deletion is typically characterized as a silent mutation. The clinical impact of this mutation can vary, particularly when inherited alongside other globin gene mutations, for example co-inheritance with β-thalassemia mutations, such as βCD8 (-AA)/β^N. This study aims to determine the $\alpha^{4.2}$ deletion breakpoint and analyze the structural and functional impact of α-thalassemia heterozygous mutation associated with $\alpha^{4.2}$ deletion and co-inheritance of αα / -$\alpha^{4.2}$/ βCD8 (-AA) / β^N on the resulting hemoglobin protein. DNA sample was sequenced using the Sanger method by 1st BASE laboratory. Sequence alignment was performed via Clustal Omega, while hemoglobin structure prediction and analysis were conducted using UCSF Chimera (Chimera-1.19) and BIOVIA Discovery Studio. The results identified the $\alpha^{4.2}$ deletion breakpoint between nucleotide 26 of the X2 box and nucleotide 347 of the X1 box. Despite the mutations, the $\alpha^{4.2}$ deletion and the αα / -$\alpha^{4.2}$/ βCD8 (-AA) / β^N co-inheritance resulted in a protein structure identical to normal residues, maintaining all stabilizing interactions. Therefore, we conclude that *in silico* predictions indicate no major structural deviations from wild-type hemoglobin A for both the $\alpha^{4.2}$ deletion and the co-inheritance of αα/-$\alpha^{4.2}$ with βCD8 (-AA)/βN. This is consistent with the molecular nature of α-thalassemia deletions, which primarily affect globin gene dosage rather than amino acid sequence, although this observation is limited to computational analysis and has not been experimentally confirmed.

Introduction

Hemoglobin is the primary protein in erythrocytes responsible for oxygen transport. Its structure consists of two α-globin and two β-globin subunits, which are homologous and share a similar three-dimensional protein fold. Each subunit is associated with a prosthetic heme group, where the iron atom is coordinated to four nitrogen atoms within a tetrapyrrole ring. To effectively bind, the iron in the heme group must remain in the reduced ferrous state (Fe^{2+}) (Ahmed *et al.*, 2020). In adult humans, the predominant form is Hemoglobin A (HbA), a heterotetramer composed of two α-globin and two β-globin polypeptides (α2β2). The α-globin chain consists of 141 amino acids and is encoded by the *HBA1* and *HBA2* genes located on chromosome 16 (16p13) (Kountouris *et al.*, 2022).

Mutations in DNA sequences lead to amino acid substitutions that fundamentally alter primary, secondary, tertiary, and quaternary protein structures (Nagasundaram *et al.*, 2015). Since a protein's structure is essential to its function, changes in structural conformation can impair protein expression and result in pathological conditions (Bhattacharya *et al.*, 2017). α-thalassemia, for instance, is a disorder caused by mutations in the gene, which encodes the α-globin subunit. One of the most prevalent causes is the $\alpha^{4.2}$ deletion, which is widespread across tropical and subtropical regions, including Melanesia, India, Southeast Asia, and South China, with gene frequencies ranging from 2% to 60% (Taher *et al.*, 2018).

Previous studies in Indonesia on individuals with double deletions ($-\alpha^{3.7}/-\alpha^{4.2}$) identified the deletion breakpoint between nucleotides 169,498 and 174,076 within the homologous X1/X2 box sequences (Husna & Handayani, 2021). This deletion results in the loss of a functional gene on one of the paternal chromosomes. Given that the gene is critical for producing the α-globin chains required for hemoglobin conformation (α2β2), its absence disrupts the interaction with β-globin subunits, thereby compromising oxygen transport (Harewood & Azevedo, 2019).

The $\alpha^{4.2}$ deletion is generally classified as an α-thalassemia silent trait, which generally does not present clinical symptoms in carriers (Viprakasit *et al.*, 2022). However, the clinical impact of this mutation can vary, particularly when inherited alongside other globin gene mutations. For example, co-inheritance with β-thalassemia mutations, such as βCD8 (-AA)/β^N, has been reported to significantly reduce hemoglobin levels, Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin (MCH) values (Khatri *et al.*, 2021).

While most thalassemia research focuses primarily on mutation detection (Handayani *et al.*, 2017), the relationship between specific genetic variations, changes in protein structure, and their physiological consequences remains largely unexplored. In this study, subjects co-inherited αα /$-\alpha^{4.2}$/ βCD8 (-AA)/β^N exhibit hematological parameters below the normal reference values, indicating a phenotype that deviates from the typical silent characteristics.

Therefore, this study aimed to determine the precise breakpoint of the $\alpha^{4.2}$ deletion and predict its structural impact on hemoglobin in the context of co-inheritance αα /$-\alpha^{4.2}$/ βCD8 (-AA)/β^N. By integrating molecular characterization and protein structure analysis, this study is expected to bridge the link between genetic variation and clinical manifestations.

Materials and methods

1. Research subject

This study uses sample code F3 as an 18-year-old male who participated in thalassemia screening in the study by Husna & Handayani (2021). Ethical approval was granted by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (Ref. No.: KE/FK/1320/EC/2019). The subject was identified as carrying the $\alpha^{4.2}$ deletion based on genotyping analysis using the gap-PCR method. However, based on hematological examination, the sample showed lower MCV and MCH values compared to the reference ranges (**Table 1**).

Table 1. Hematological Profile of the Subject

Parameter	Subject	Reference range (Dean, 2005)
Sex-age	Male-18	
Hb (g/dL)	13.10	13.5−17.5
MCV (fl)	73.5*	80−100
MCH (pg)	23.9*	25.4−34.6
Eritrocyte (10^6/µL)	5.48	4.3−5.9
Morphology	Anisocytosis, hypochromic, microcytic, and pencil cell	

*lower or higher value than reference

2. DNA and Sequencing

Genomic DNA from sample F3 was isolated using the Geneaid Genomic DNA Mini Kit for Blood or Cultured Cells (Geneaid, Taipei, Taiwan), followed by PCR and Sanger sequencing to confirm thalassemia variants. Amplification was performed using a Bio-Rad T100 Thermal Cycler in a final volume of 25 µL, containing ≤200 ng of DNA template, 12.5 µL of Master Mix (Bioline), and 0.5 µM primers (**Table 2**). The PCR conditions were as follows: pre-denaturation at 95°C for 1 minute 40 seconds; 35 cycles consisting of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. The PCR products were subsequently subjected to Sanger sequencing at the 1st BASE laboratory.

Table. 2 Sequence and size of the primers used for DNA amplification

Varian	Primer Name	Sequens	Size	Reference
$\alpha^{4.2}$ deletion	4.2 F	GGTTTACCCATGTGGTGCCTC	1628 bp	(Chong *et al.*, 2000; Husna & Handayani, 2021)
	4.2 R	CCCGTTGGATCTTCTCATTTCCC		
HBB gene	F	CCAAGGACAGGTACGGCTGTCATC	704 bp	(Gupta & Agarwal, 2003)
	R	CCTTCCTATGACATGAACTTAACCAT		

3. Alignment

The Sanger sequencing results were aligned with the normal sequences obtained from NCBI (National Center for Biotechnology Information) with accession number NC_000016.10 for the α-globin template sequence and reference sequence U01317.1 for the β-globin template sequence. Alignment was subsequently performed using Clustal Omega to characterize the breakpoint regions.

4. Prediction and Analysis of Protein Structure

Protein structure prediction was initiated by translating DNA sequences into amino acid sequences using the ExPASy Translate tool to convert nucleotides sequence into corresponding amino acid sequence. *In silico* mutagenesis was subsequently performed using the 1HGA deoxyhemoglobin crystal structure, retrieved from the RCSB Protein Data Bank (PDB), as a template. Structural modeling and visualization were conducted using UCSF Chimera (Chimera-1.19) and BIOVIA Discovery Studio. Furthermore, the protein-protein interactions between hemoglobin subunits were analyzed using Discovery Studio, following the protocol described by Wang *et al.* (2015).

Results and discussion

1. The breakpoint of $\alpha^{4.2}$ deletion

The structure of the α-globin gene cluster on chromosome 16 features two primary genes, *HBA1* and *HBA2*, on each allele. The α globin gene cluster has three highly homologous regions (X-box, Y-box, and Z-box) and *HBA2* and *HBA1* are located in Z-boxes. The unequal crossing-over events between the highly homologous X boxes result a $\alpha^{4.2}$ deletion. This recombination error leads to the deletion of the Y2 and Z2 boxes (**Figure 1**), as described by Harteveld & Higgs (2010).

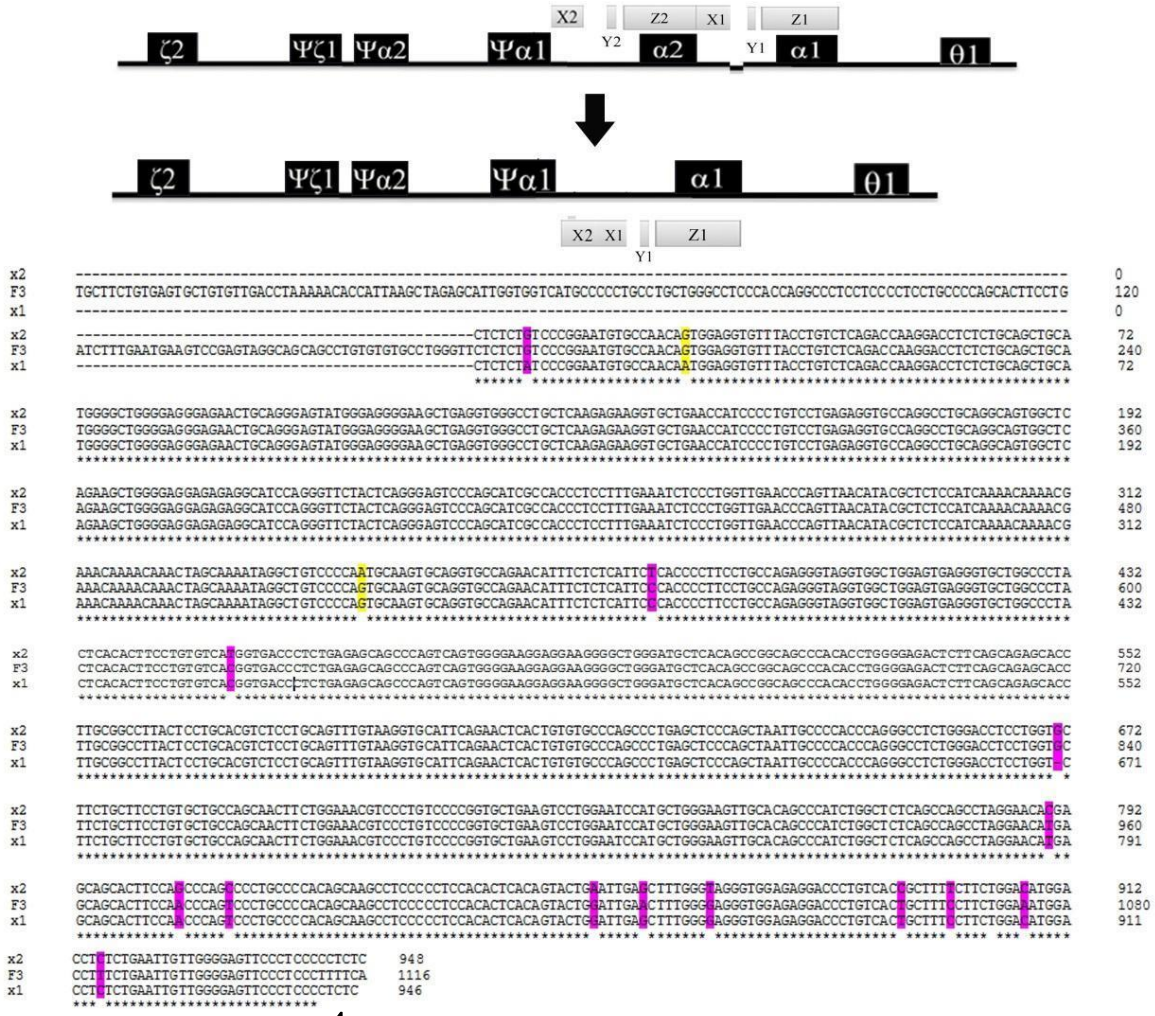


Figure 1. Delineation of the $\alpha^{4.2}$ deletion breakpoint within the α-globin gene cluster. (a) Schematic representation of the α-globin locus, including ζ2, ψζ1, ψα2, ψα1, α2, α1, and θ1 genes, and the homologous X, Y, and Z repeat regions that mediate recombination. (b) Model of unequal homologous recombination between the X2 and X1 boxes, resulting in the $\alpha^{4.2}$ deletion allele through loss of the intervening genomic segment and formation of a hybrid sequence at the recombination junction. (c) Sequence alignment of the F3 fragment, defined as a PCR-amplified region spanning the predicted breakpoint, with X2 and X1 reference sequences. The alignment demonstrates a sequence transition from X2-derived to X1-derived regions, allowing precise delineation of the breakpoint junction. Highlighted nucleotides represent informative mismatches and conserved positions used to localize the recombination site.

The sequences of Box X2 and X1 exhibit high homology, complicating the precise determination of the $\alpha^{4.2}$ deletion breakpoint, as illustrated in the alignment results (**Figure 1c**). Polymorphic sites 1 and 2 reveal that the deletion sequence matches the Box X2 sequence, indicating that this segment originates from Box X2. Conversely, polymorphic sites 3 and 4 align with the Box X1 sequence. However, at polymorphic site 5, the $\alpha^{4.2}$ deletion sequence deviates from Box X1, before returning to match the Box X1 sequence at sites 6, 7, 8, and 9. Due to the high homology between Box X2 and X1, the breakpoint can only be defined within a specific range. This range is determined by the first divergent polymorphic site and the subsequent consistency of the nucleotide bases with Box X1 or X2, as indicated by the yellow-highlighted regions in **Figure 1**. This range spans from base 26 of Box X2 (position 169,498 on NC_000016.10) to base 347 of Box X1 (position 174,076 on NC_000016.10). The identified breakpoint range is consistent with previous findings for $\alpha^{4.2}$ (Husna & Handayani, 2021).

2. Prediction of hemoglobin protein structure and function

Human somatic cells are diploid, containing two sets of 23 chromosomes inherited from each parent. Consequently, every cell possesses two homologous copies of chromosome 16. Within the α-globin gene

cluster, the functional genes and exist as dual copies, with one set originating from the maternal lineage and the other from the paternal lineage (Alberts *et al.*, 2022).

In the $\alpha^{4.2}$ deletion, the loss of one functional gene on one of the homologous copies of chromosome 16 results in an α-thalassemia carrier state. These carriers retain a functional allele from the unaffected chromosome (Taher *et al.*, 2018). The $\alpha^{4.2}$ deletion does not result in abnormal hemoglobin variants because the remaining and genes encode an identical and indistinguishable α-globin chain, which is essential for the quaternary structure of hemoglobin (**Figures 2** and **3**). This complete gene deletion differs significantly from partial deletions, such as the GAC deletion in codons 19-21 (Hb Boyle Heights).

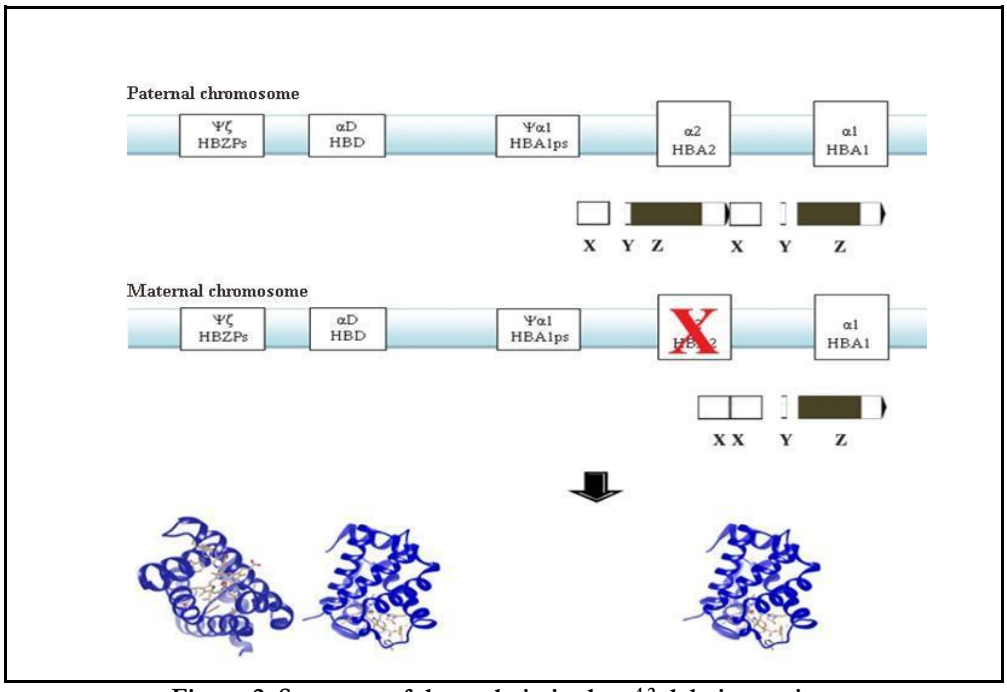
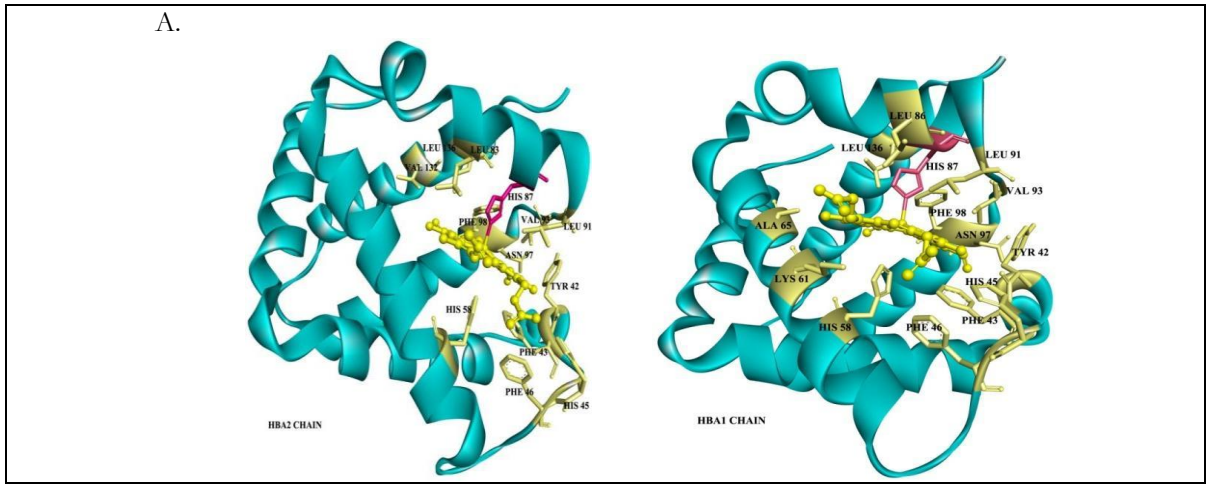


Figure 2. Structure of the α-chain in the $\alpha^{4.2}$ deletion variant

Both the *HBA2* and *HBA1* subunits consist of 141 amino acid residues. Structural analysis identified 13 interface residues within the subunit and 14 residues within the *HBA1* subunit (**Figure 3**)**.** A total of 8 intermolecular and 17 intramolecular salt bridges were predicted between the protein subunits. These salt bridge interactions are formed between the carboxylate (CO^{2-}) and ammonium (NH^{3+}) groups (**Table 3**). Furthermore, 201 intermolecular interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces, were predicted between the hemoglobin subunits in the $\alpha^{4.2}$ deletion.



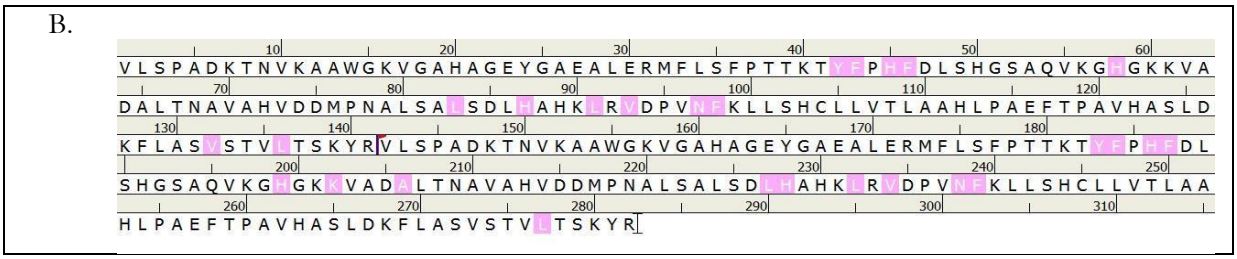


Figure 3. Interface residues and protein sequence of the *α-globin* subunit. (a) Structural visualization of the α2- and α1-globin chains; (b) Amino acid sequence of the α-subunit. Residues highlighted in purple indicate the specific amino acids involved in inter-subunit interactions (interface residues).

Table 3. Predicted inter-subunit salt bridge interactions in the $\alpha^{4.2}$ deletion hemoglobin variant.

Category	Type	H-Donor	H-Acceptor	Distance (Å)
Hydrogen Bond;Electrostatic	Salt Bridge	A:Lys40:NZ	D:His146:OXT	2.2929
Hydrogen Bond;Electrostatic	Salt Bridge	A:Arg92:NH2	D:Glu43:OE2	3.999
Hydrogen Bond;Electrostatic	Salt Bridge	A:Lys127:NZ	C:Arg141:O	2.74357
Hydrogen Bond;Electrostatic	Salt Bridge	A:Arg141:NH1	C:Asp126:OD2	2.58119
Hydrogen Bond;Electrostatic	Salt Bridge	C:Lys40:NZ	B:His146:OXT	2.61265
Hydrogen Bond;Electrostatic	Salt Bridge	C:Lys127:NZ	A:Arg141:O	2.9797
Hydrogen Bond;Electrostatic	Salt Bridge	C:ARG141:NH1	A:Asp126:OD2	2.73447
Hydrogen Bond;Electrostatic	Salt Bridge	D:LYS66:NZ	D:Hem147:O2D	3.29006

* A: HBA2 chain; B: HBB1 chain; C: HBA1 chain; D: HBB2 chain

Salt bridges are non-covalent interactions formed between two oppositely charged amino acid residues situated within a distance sufficient to exert electrostatic forces. Mutations that result in the loss of these interactions, such as the βCD8 (-AA) deletion in β-thalassemia, are expected to destabilize the protein and increase its susceptibility to denaturation. This is due to the disruption of inter-residue interactions and subsequent loss of conformational stability (Ban *et al.*, 2019). In contrast, as shown in **Table 3**, the number of inter-subunit salt bridges in T-state deoxyhemoglobin with the $\alpha^{4.2}$ deletion remains identical to the wild-type (8 interactions). Similarly, the intramolecular salt bridges (17 interactions) are conserved. This stability is attributed to the fact that the $\alpha^{4.2}$ deletion does not alter the amino acid sequence; thus, the H-donor and H-acceptor sites for hydrogen bonding and electrostatic interactions remain unchanged. Furthermore, hydrophobic interactions involving alkyl groups, π−π stacking, π− π T−shaped, π−alkyl, and π−orbitals involve the same residues and bond distances as normal hemoglobin. Consequently, the $\alpha^{4.2}$ hemoglobin variant is predicted to possess folding stability comparable to normal hemoglobin, as the essential stabilizing interactions are fully preserved.

The βCD8 (-AA)/β^{N} genotype represents a β-thalassemia trait that is frequently co-inherited with the $\alpha^{4.2}$ deletion. This β-thalassemia mutation is caused by a dinucleotide (AA) deletion at the 8th codon, which induces a frameshift leading to a premature stop codon at position 21. This early termination significantly alters the amino acid sequence of the C-terminal and truncates the β-globin chain. Unlike the $\alpha^{4.2}$ deletion, which produces normal subunits, the βCD8 (-AA) mutation drastically disrupts the protein's primary structure, often leading to the absence of functional hemoglobin complexes due to subunit instability (**Figure 4**).

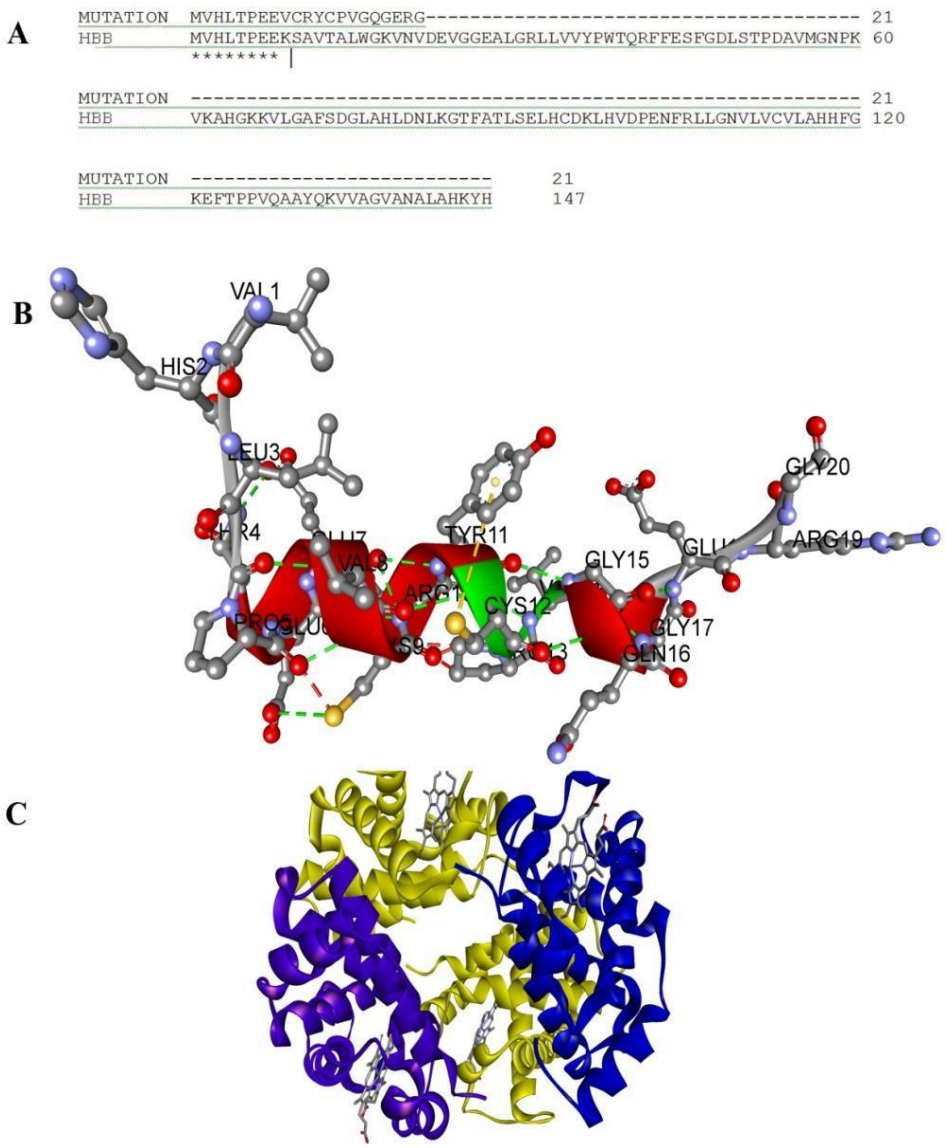


Figure 4. Structural analysis of the mutant β-globin subunit and the α- and β-thalassemia co-inheritance protein complex. (a) Altered amino acid sequence resulting from the dinucleotide (AA) deletion at codon 8; (b) Modeled protein structure derived from the βCD8 (-AA) mutation. The secondary structures are color-coded: red for α-helices, green for turns, and gray for coils. Interaction markers include green dotted lines for hydrogen bonds, red/pink lines for steric clashes (unfavorable bumps), and orange lines for alkyl-hydrophobic and sulfur-π interactions; (c) Structural visualization of the hemoglobin complex in α/β-thalassemia co-inheritance.

Table 4. Predicted overall intramolecular interactions within the truncated β-globin subunit resulting from the βCD8 (-AA) mutation.

Category	Type	Distances (Å)	From	From chemistry	To	To chemistry
Hydrogen Bond	Hydrogen Bond	2,76815	THR4:N	H-Donor	GLU7:OE1	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,8434	VAL8:N	H-Donor	THR4:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,96896	CYS9:N	H-Donor	PRO5:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	3,6043	CYS9:SG	H-Donor	GLU6:OE1	H-Acceptor

Category	Type	Distances (Å)	From	From chemistry	To	To chemistry
Hydrogen Bond	Hydrogen Bond	2,82338	ARG10:N	H-Donor	GLU6:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,83906	ARG10:N	H-Donor	GLU7:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,76671	TYR11:N	H-Donor	GLU7:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	3,26961	CYS12:N	H-Donor	VAL8:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	3,34514	VAL14:N	H-Donor	ARG10:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,89844	GLY15:N	H-Donor	TYR11:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	3,28287	GLN16:N	H-Donor	CYS12:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	2,74362	GLY17:N	H-Donor	VAL14:O	H-Acceptor
Hydrogen Bond	Hydrogen Bond	3,36667	GLU18:N	H-Donor	GLY15:O	H-Acceptor
Other	Pi-Sulfur	5,64705	CYS12:SG	Sulfur	TYR11	Pi-Orbitals
Hydrophobic	Alkyl	5,39504	VAL1	Alkyl	LEU3	Alkyl
Hydrophobic	Alkyl	4,40937	VAL8	Alkyl	LEU3	Alkyl
Hydrophobic	Alkyl	5,2252	CYS12	Alkyl	VAL8	Alkyl
Unfavorable	Unfavorable Bump	2,41841	PRO5:O	Steric	CYS9:SG	Steric
Unfavorable	Unfavorable Bump	2,32373	CYS9:C	Steric	PRO13:CD	Steric
Unfavorable	Unfavorable Bump	1,59461	CYS9:O	Steric	PRO13:CG	Steric
Unfavorable	Unfavorable Bump	1,26203	CYS9:O	Steric	PRO13:CD	Steric

The protein produced by the βCD8 (-AA) deletion, as shown in **Figure 4**, undergoes premature translation termination, resulting in a truncated polypeptide of only 21 amino acids, compared to the 146 residues in a normal β-globin subunit (**Figure 4A**). This truncated protein is unable to form essential interactions with other hemoglobin subunits. For instance, key residues such as His146, which forms critical salt bridges with Lys40 of the subunit, are absent (**Table 4** and **Figure 4b**). Furthermore, inter-subunit interactions are not only vital for complex formation but also for the cooperative oxygen-binding mechanism (Nagai *et al.*, 2017). The hydrogen bond between Tyr42 in the α-subunit and Asp99 in the β-subunit, known as the 'switch region', stabilizes the T-state and facilitates the quaternary structural transition required for effective oxygen release to tissues (Nagai *et al.*, 2017; Hub *et al.*, 2021). In addition to the lack of subunit assembly, this truncated protein lacks the heme-binding residues. Consequently, the defective mRNA transcript is primarily targeted for degradation by nonsense-mediated mRNA decay (NMD). NMD acts as a quality control mechanism that eliminates erroneous transcripts, specifically those containing premature termination codons (PTC), thereby preventing the synthesis of potentially cytotoxic truncated proteins (He & Jacobson, 2015).

In the formation of the hemoglobin tetramer (α2β2), individuals with the βCD8 (-AA) mutation still produce normal hemoglobin complexes (**Figure 4C**). Similar to the $\alpha^{4.2}$ deletion, the βCD8 (-AA) mutation affects only one of the two *HBB* loci on chromosome 11. Although the mutated allele produces a non-functional truncated protein, the wild-type *HBB* allele on the homologous chromosome continues to synthesize normal β-globin chains. Consequently, the resulting quaternary structure of the hemoglobin complex remains structurally normal. The formation of these subunits is independent of which specific α-globin gene (*HBA1* or *HBA2*) is utilized, as they encode identical proteins. Therefore, the hemoglobin

produced in both $\alpha^{4.2}$ and α/β-thalassemia co-inheritance is predicted to maintain a standard oxygen-binding function, albeit at a reduced quantitative level.

3. Correlation Between Mutations and Physiological Disorders in Thalassemia

Previous studies have indicated that individuals with heterozygous α-thalassemia (α+/αα) often exhibit hematological parameters within the reference range (Taher *et al.*, 2018). However, heterozygous α-thalassemia resulting from the $\alpha^{4.2}$ deletion typically presents with MCH and MCV values below the reference thresholds. Hemoglobin is the primary protein located in the cytosol of red blood cells (Caulier *et al.*, 2022), providing the characteristic red pigment to erythrocytes (Sae-Lee *et al.*, 2022). A reduction in MCH values results in hypochromic erythrocytes, characterized by a pale or faded appearance (**Table 1**). The $\alpha^{4.2}$ deletion leads to diminished hemoglobin synthesis, thereby reducing the hemoglobin concentration within the cytosol and decreasing the MCV. As MCV reflects the average volume or size of red blood cells (Sadiq *et al.*, 2024), a decrease in this value manifests as microcytosis (cell size <7.5 μm). Furthermore, morphological abnormalities such as pencil cells, elongated, pale erythrocytes, are frequently observed in these cases (**Table 1**) (Strati *et al.*, 2014; Matthews *et al.*, 2022; Singh *et al.*, 2025).

Reduced hematological values in α-thalassemia are primarily driven by the diminished production of hemoglobin subunits, which correlates directly with the number of deleted genes (Keikhaei *et al.*, 2024). It is therefore inferred that the sub-reference MCV and MCH values observed in heterozygous $\alpha^{4.2}$ thalassemia patients ($\alpha\alpha/\alpha^{4.2}$) result from decreased *HBA2* subunit production, following the loss of one functional *HBA2* gene on one of the homologous copies of chromosome 16. Despite this reduction, individuals with heterozygous $\alpha^{4.2}$ deletions do not typically exhibit severe anemia or significant oxygen transport impairment, unlike those with HbH disease. This stability is attributed to the fact that the synthesized hemoglobin maintains a normal structure and function, ensuring that oxygen delivery remains effective (Harewood & Azevedo, 2019).

A comparison of hematological indices reveals that MCH and MCV values in individuals with the single-gene $\alpha^{4.2}$ deletion are higher than those observed in cases of α/β-thalassemia co-inheritance ($\alpha\alpha/\alpha^{4.2}/\beta CD8(-AA)/\beta^{N}$) (**Table 1**). Although the co-inheritance state involves a reduction in the functional output of two globin genes (one α and one β), both genotypes ultimately result in the synthesis of structurally normal hemoglobin. Consequently, despite the lower erythrocyte indices in co-inherited cases, there is no significant impairment in oxygen transport, as the functional integrity of the synthesized hemoglobin remains intact.

Hemoglobin is a heterotetramer consisting of two α and two β subunits, each associated with a heme group. The iron atom within the heme is axially coordinated to the globin protein via the proximal histidine (His F8) residue (**Figure 3**). While a reduction in MCH values is often clinically associated with iron deficiency (Piel *et al.*, 2021), this was not observed in heterozygous $\alpha^{4.2}$ thalassemia patients. Previous research suggests that even a single α-gene deletion triggers increased erythropoietic activity and a concomitant decrease in hepcidin levels—the primary regulator of iron homeostasis. This mechanism enhances iron absorption and mobilization to balance hemoglobin synthesis with iron status (Yang *et al.*, 2024). Consequently, $\alpha^{4.2}$ patients maintain sufficient iron levels despite low MCH. Hematological data further support this compensatory mechanism; the erythrocyte count was 5.48 10^{6}/μL, nearly reaching the upper limit of the normal range. This elevated erythropoiesis, combined with *HBA2* production from the remaining normal alleles, ensures functional hemoglobin complex formation. However, this compensatory pressure results in anisocytosis, manifesting as a mixed population of microcytes, pencil cells, and normocytes, the ratio of which depends on the degree of gene deletion (Sadiq *et al.*, 2024). Unfortunately, this study includes only a single subject, which limits the ability to generalize the findings to the broader population.

Conclusion

In silico predictions indicate no major structural deviations from wild-type hemoglobin A for both the $\alpha^{4.2}$ deletion and the co-inheritance of $\alpha\alpha/-\alpha^{4.2}$ with βCD8 (−AA)/βN. This is consistent with the molecular nature of α-thalassemia deletions, which primarily affect globin gene dosage rather than amino acid sequence, although this observation is limited to computational analysis and has not been experimentally confirmed.

Author Statements

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Author's contributions: Niken S. N. Handayani served as the corresponding author, supervised the study, and contributed to the research, as well as reviewed and revised the manuscript. Nurul Fitri T Tagunu conducted the research, performed data analysis, and wrote the manuscript. Tri Ratnaningsih contributed to the research and discussion. Indra Lesmana contributed to the research and data analysis. Nur Imma Harahap contributed to the research and data analysis. All authors participated in proofreading and approved the final version of the manuscript.

Generative AI: Not applicable.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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