Using PCR to Identify the Mutation of α-Thalassemia

Chia-Chia Yu

Department of Biology, Fordham University, Bronx, New York 10458
cyu@fordham.edu

Abstract

Mutations in HBA1 and HBA2 genes cause α-thalassemia. Mutation in these two genes may produce deficient α subunit chains in hemoglobin in the red blood cells, and lead to anemia or even death. Since I was suspect of thalassemia, the objective of this study was to identify if there is any mutation in HBA1 and HBA2 genes in my genomic DNA. Using primers specific to the HBA1 and HBA2 genes, these two genes were amplified by PCR and then sequenced. Dr. Rubin's genomic DNA was used as a wild-type control. SNPs (single nucleotide polymorphism) were both found in Dr. Rubin's DNA and my DNA. Almost all of the DNA sequences of my two genes are identical as those published on the NCBI, except SNPs and several deletions in the 5' untranslated region in both of my two genes.

Introduction

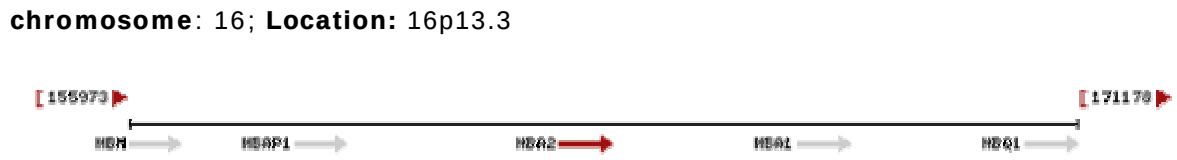
Thalassemia is a recessively trait inherited disease of the red blood cells. In thalassemia, the genetic defect results in reduced presence of the normal hemoglobin subunit chains. The thalassemias are classified according to which subunit chain of the hemoglobin molecule is affected. In α-thalassemia, the production of α subunit chain is deficient, whereas in β-thalassemia the production of β subunit chain is defective.

The estimated prevalence of the disease-causing mutation is 16% in people from Cyprus, 3-14% in Thailand, and 3-8% in populations from India, Pakistan, Bangladesh, and China. A lower prevalence has been reported from black people in Africa (0.9%) and northern Europe (0.1%).[1] In Southeast Asia, compound heterozygotes and homozygotes may have anemia which is mild to severe (hemoglobin HB H disease) or lethal (Hb Bart's hydrops fetalis).[2,3] In Taiwan, a high incidence of the deletion and point mutation has been reported.[4,5,6] Unlike β-thalassemia, in which nondeletional mutations predominate, over 95% of recognized α-thalassemia involves deletion of 1 or both α-globin genes from

chromosome 16p13.3[7,8] (Figure 1).

The α-thalassemia involve the genes HBA1 (Online Mendelian Inheritance in Man, OMIM, 141800) and HBA2 (Online Mendelian Inheritance in Man, OMIM, 141850). In this paper, the identification of mutation in HBA1 and HBA2 genes which lead to α-thalassemia was analyzed by PCR and using specific primers to these two genes. My genomic DNA was used as a suspect sample for thalassemia, whereas Dr. Rubin's genomic DNA was used as a wild-type control.

(A) Relative location of HBA2 and HBA1 genes on chromosome 16p13.3



(B) Structure of HBA2 gene

(C) Structure of HBA1 gene

Figure 1. The location and structure of HBA2 and HBA1 genes on chromosome 16p13.3.

(A) Schematic representation of HBA2 and HBA1genes on chromosome 16p13.3. (B) and (C) present schematic representations of the structure of HBA2 and HBA1genes, respectively. The alpha-2 (HBA2) and alpha-1 (HBA1) coding sequences are identical. These genes differ slightly in the 5' untranslated regions and the introns, and they differ significantly in the 3' untranslated regions. Two alpha chains plus two beta chains constitute HbA, which in normal adult life comprises about 97% of the total hemoglobin; alpha chains combine with delta chains to constitute HbA-2, which with HbF (fetal hemoglobin) makes up the remaining 3% of adult hemoglobin. Alpha thalassemias result from deletions of each of the alpha genes as well as deletions of both HBA2 and HBA1. (adapted from

NCBI)

Materials and Methods

Genomic DNA Extraction

Dr. Rubin's DNA and my DNA were both extracted by using QIAamp DNA Blood Mini Kit and stored at -20℃.

Primers

Primers that are specific for the HBA2 and HBA1 genes were generated and presented in Table 1. The relative location of each primer set on HBA2 and HBA1 gene are shown in Fig 2. Each gene was flanked by four pairs of primer sets. All primers were diluted to10 pmol/μl.

Gene	**Primer's sequence**	**Set**
HBA2	Forward-5' AGGGTGGAGACGTCCTGG 3' Reverse-5' AGAGAAGAGGGTCAGTGC 3'	A
HBA2	Forward-5' CCCAAGCATAAACCCTGG 3' Reverse-5' AGAGAAGAGGGTCAGTGC 3'	B
HBA2	Forward-5' ACAGGCCACCCTCAACCGTCC 3' Reverse-5' CCATTGTTGGCACATTCC 3'	C
HBA2	Forward-5' CACCACCAAGACCTACTTCC 3' Reverse-5' AGAGGTCCTTGGTCTGAGACAGG 3'	D
HBA1	Forward-5' AGGGTGGAGACGTCCTGG 3' Reverse-5' AGAAGAGGGTCAGTGGGGCCGAG 3'	E
HBA1	Forward-5' GCCCCAAGCATAAACCCTGG 3' Reverse-5' AGAAGAGGGTCAGTGGGGCCGAG 3'	F
HBA1	Forward-5' ACAGGCCACCCTCAACCGTCC 3' Reverse-5' ATGCCTGGCACGTTTGCTGAGG 3'	G
HBA1	Forward-5' CACCACCAAGACCTACTTCC 3' Reverse-5' ATGCCTGGCACGTTTGCTG 3'	H

Table 1. Primer designs for HBA2 and HBA1 genes.

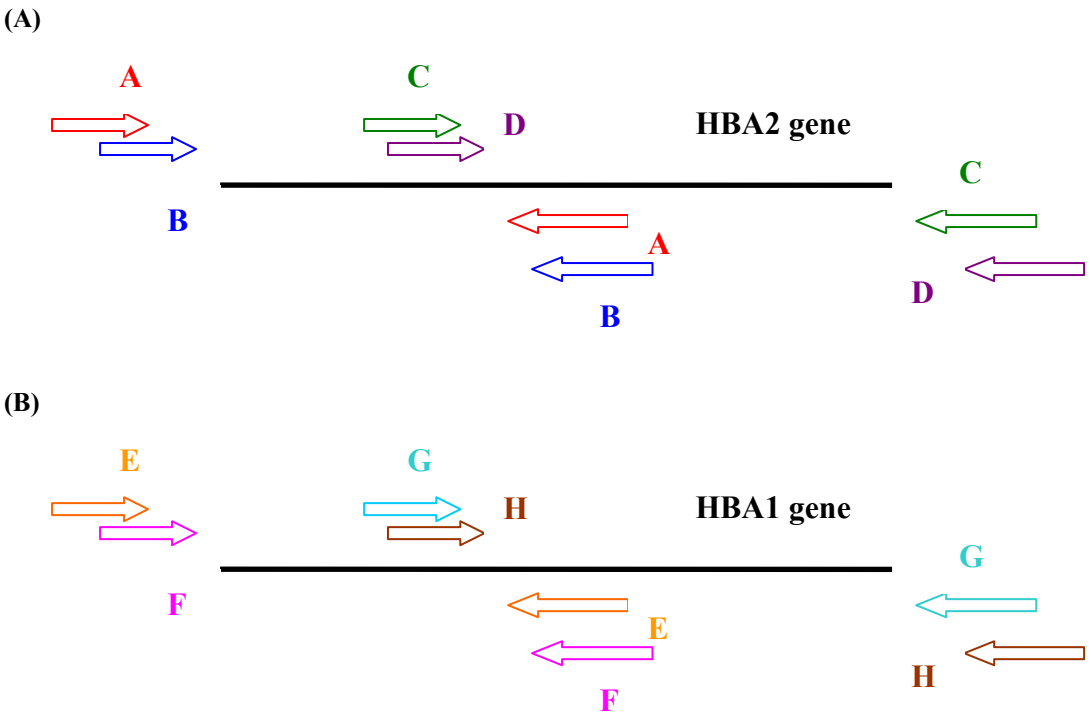


Figure 2. Schematic representation of different primer sets on (A) HBA2 gene (B) HBA1 gene. Different primer pairs are indicated in different colors.

PCR

PCR was performed using the Promega PCR Kit. For each set of reactions, 12.5 µl of GoTaq master mix was dispensed into each PCR tubes. 7.5 pM of forward primer and 7.5 pM of reverse primer were added to each tube. 25 ng of genomic DNA was added to each tube. Additional RNase-free water was added to make the reaction volume 25 µl. PCR was performed according to the following protocol: 95℃ for 5min, PCR—94℃ for 20sec, 60℃ for 30sec, 72℃ for 30sec, 50 cycles, followed by 72℃ for 2min.

PCR Purification

The PCR products were purified either from the PCR reaction tubes using the Marligen Papid PCR Purification System or after being fractionated on 1% agarose gels using the QIAquick Gel Extraction Kit.

DNA Sequencing

PCR products were sequenced by Genewiz. Inc.

Analysis of DNA Sequence Alignments

The DNA sequence alignments were generated using Geneious 2.5.4.

Results

PCR reactions were performed using different primer sets (A, B, C, D, E, F, G, H) which were specific for the HBA2 and HBA1 genes. PCR products were then analyzed by 1% agarose gel. The primers utilized were designed to generate PCR products of approximately 600 bp. Some primer sets do not work (e.g. A, E, data not shown). PCR products were prepared by PCR purification (e.g. C, D, G, H) or by gel extraction (e.g. B, F) (Figure 3).

PCR Amplification and Characterization of the PCR Products

(A)

(B)

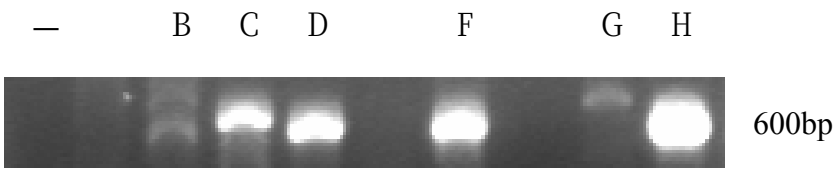
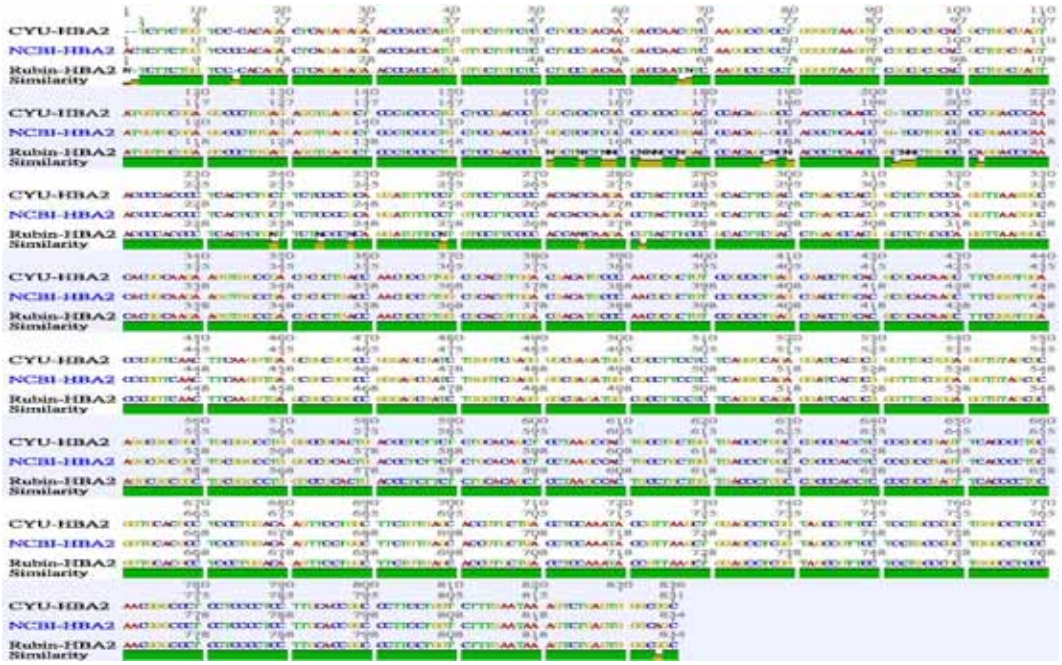


Figure 3. Gel analysis of PCR products. (A) My Genomic DNA. (B) Dr. Rubin's genomic DNA.
—: negative control. The letter above each well indicates which primer set was used for PCR.

DNA Sequencing and Analysis of DNA Sequence Alignments

PCR products were extracted by PCR purification or gel extraction and then sequenced by Genewiz. Inc. The DNA sequence alignments were generated by the software Geneious 2.5.4. Fig. 4 shows the DNA sequence alignments of my genomic DNA and Dr. Rubin's genomic DNA (as a wild type control) in reference with the DNA sequence published on the NCBI website.

(A)



(B)

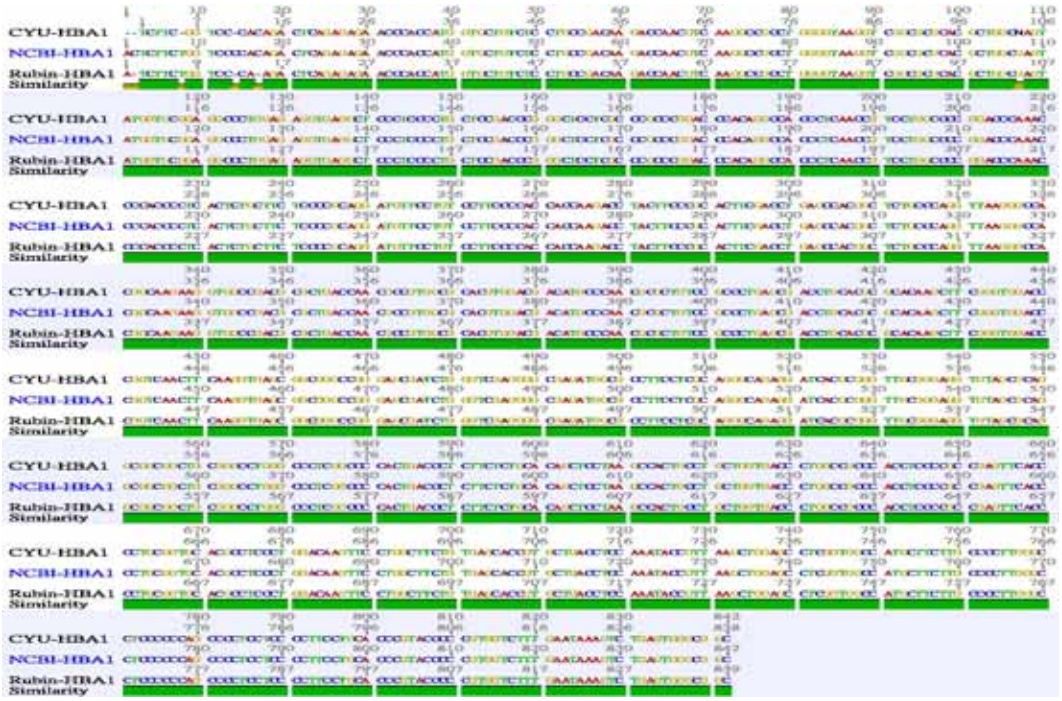


Figure 4. The DNA sequence alignments of my genomic DNA (CYU) and Dr. Rubin's genomic DNA (Rubin) in reference with the DNA sequence published on the NCBI website (NCBI).

(A) The DNA sequence alignments of HBA2 gene. (B) The DNA sequence alignments of HBA1 gene.

SNPs

SNP: rs2541640 was found in both Dr. Rubin's and my HBA2 genes. SNP:

rs33939620 was only found in my HBA1 gene. Figure 5 showed the SNP presented in my HBA1 gene.

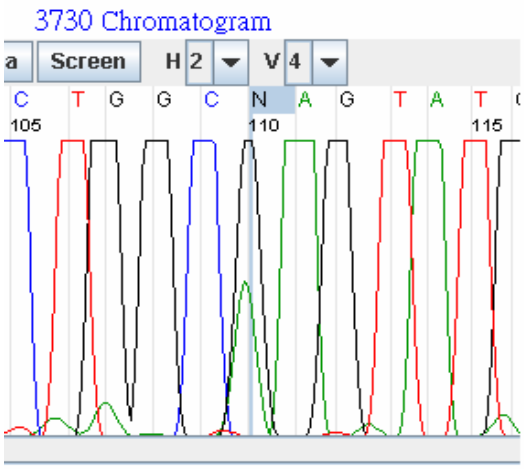


Figure 5. The chromatogram showed these two possibilities, G and A, at the 107th nucleotide of my HBA1 gene. This is a SNP (rs33939620) which has been reported on the NCBI website.

Discussion

Except two SNPs and several deletions were found in my HBA2 and HBA1 genes, the rest of the DNA sequences of my two genes are identical as those normal DNA sequences published on the NCBI. Deletions and point mutations in α-thalassemia have been reported in many research articles.[4,5,6,9]

Two SNPs were found in my genes. SNP: rs2541640 was found in my HBA2 gene, and SNP: rs33939620 was found in my HBA1 gene. The SNP: rs33939620 found in my HBA1 gene have been reported to cause α-thalassemia.[10] The 24th amino acid is Lys (AAG) in a α-thalassemia person instead of Glu (GAG) in a healthy person. However, this mutation in my case should be confirmed by repeating the DNA sequencing to make sure the mutation is real.

Three deletions were found in the 5' UTR in my HBA2 gene, whereas four deletions were found in my in the 5' UTR of my HBA1 genes. These deletions are located within 1-14 bp downstream from the transcription start site, which may have an influence on the stability of the transcripts and result in deficient production of α-subunit chain of hemoglobin. The next step could be to study if these deletions change the half-life of the transcripts. In addition, these deletions might destroy the

enhancer, which will also cause deficient expression of these genes.

To confirm if the expression of my HBA2 and HBA1 genes is different from the normal level is required. If so, future studies can focus on if any mutation happens in the promoters of these two genes. Other genes involved in β-thalassemia and δ-thalassemia haven't been examined in my case yet. Future research on identification if there is any mutation present in these genes is also necessary.

Acknowledgements

I would like to thank Dr. Rubin for providing genomic DNA and guidance of this project. Also, lots of thank to Leleesha, Joe, Liu Bo, and Jinsong Qiu for helping me to complete this project.

References

1. Leung NT, Lau TK, Chung TKH. 2005. Thalassemia screening in pregnancy. *Curr Opinion in Ob Gyn*. 17: 129–34.
2. Chong SS, Boehm CD., Higgs DR, and Cutting GR. 2000. Single-tube multiplex-PCR screen for common deletional determ-inants of α-thalassemia *Blood*. 95: 360-362.
3. Chui DHK, Waye JS. 1998. Hydrops fetalis cause by alpha-thalassemia: an emerging health care problem. *J Am Soc Hematol*. 91 (7): 2213–2222.
4. Ko TM, Chen TA, Hsieh MI, Tseng LH, Hsieh FJ, Chuang SM, Lee TY. 1993. α-thalassemia in the four major aboriginal groups in Taiwan. *Hum Gene*. 92: 79-80.
5. Ko TM, Tseng LH, Kao CH, Lin YW, Hwa HL, Hsu PM, Li SF, Chuang SM. 1998. Molecular characterization and PCR diagnosis of Thailand deletion of α-globin gene cluster. *Am J Hematol*. 57: 124-130.
6. Higgs DR, Ayyub H, Chong SS. 1999. The —^{THAI} and —^{FIL} determinants of α-thalassemia in Taiwan. *Am J Hematol*. 60: 80-81.
7. Higgs DR, Vickers MA, Wilkie AOM, Pretorius IM, Jarman AP, Weatherall DJ. 1989. A review of the molecular genetics of the human α-globin gene cluster. *Blood*. 73: 1081-1104.
8. Kazazian Jr H. The thalassemia syndromes: molecular basis and prenatal diagnosis in 1990. 1990. *Semin Hematol*. 27: 209-228.
9. Harteveld KL, Losekoot M, Heister AJ, van der Wielen M, Giordano PC, Bernini LF. 1997. alpha-Thalassemia in The Netherlands: a heterogeneous spectrum of both deletions and point mutations. *Hum Genet*. 7: 114-122.

10. Hardison R, Riemer C, Chui DHK, Huisman THJ, Miller W. 1998. Electronic access to sequence alignments, experimental results and human mutations as an aid to studying globin gene regulation. *Genomics* 47: 429-437.