Determining the cause of patchwork *HBA1* and *HBA2* genes: recurrent gene conversion or crossing over fixation events

Hai-Yang Law*
Hong-Yuan Luo*
Wen Wang*
Julia F.V. Ho
Hossein Najmabadi
Ivy S.L. Ng
Martin H. Steinberg
David H.K. Chui
Samuel S. Chong

Background and Objectives. Recombinations are common between the two homologous α-globin genes. We report on the identification and characterization of two *patchwork* α-globin genes.

Design and Methods. Multiplex polymerase chain reaction assays were performed to rule out the presence of α-globin gene deletions and triplications. The *HBA1* (α1-globin) and *HBA2* (α2-globin) genes were individually amplified and sequenced.

Results. Two variants of the *HBA1* and *HBA2* genes were identified. One variant allele, *α121*, consists primarily of the *HBA1* gene sequence except for a small segment of IVSII in which an octanucleotide segment has been replaced by an *HBA2* -specific nucleotide. Conversely, the *α212* variant consists primarily of the *HBA2* gene sequence except for a segment of IVSII in which *HBA2* -specific nucleotides at two sites have been replaced by *HBA1*-specific sequences. Both variant alleles are found in individuals of different ethnicity, geographical origin, and haplotype backgrounds. The simplest model for the origins of these patchwork alleles is a single crossover between a normal allele and an existing recombinant allele such as the $-\alpha^{3.7}$ single gene deletion or the $\alpha\alpha\alpha^{anti3.7}$ triplicated allele, but we cannot exclude a reciprocal double crossover or a non-reciprocal gene conversion between misaligned *HBA1* and *HBA2* genes.

Interpretation and Conclusions. The α-globin patchwork alleles have arisen independently on several occasions, most likely through a single crossover between a normal and a recombinant allele. Further studies are necessary to evaluate the possible effect of these changes on α-globin gene expression.

Key words: patchwork, α-globin genes, gene conversion, crossover fixation.

Haematologica 2006; 91:297-302

**These three authors contributed equally to this work.*

Department of Pediatric Medicine, KK Women's & Children's Hospital, Singapore 229899, Singapore (HLaw, ISN); Departments of Medicine and Pathology, Boston University School of Medicine, Boston, MA 02118, USA (HLuo, MHS, DHC); Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119074, Singapore (WW, JFH, SSC); Kariminejad-Najmabadi Pathology & Genetics Center, 14665/154, Tehran, Iran (HN).

Correspondence:
Samuel S. Chong, PhD, FACMG, Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Level 4, NUH, 5 Lower Kent Ridge Road, Singapore 119074, Singapore.
E-mail: paecs@nus.edu.sg

The human α-globin gene cluster is located on chromosome 16 pter-p13.3, and is arranged in the order, 5'-ζ2-ψζ1-ψα2-ψα1-α2-α1-θ1-3'.[1] Recent re-examination of the ψα2 gene revealed that it is expressed at a very low level, and was renamed as mu-globin gene.[2] The cluster was thought to result from duplication events that occurred more than 300 million years ago.[3] Unequal homologous recombinations are common between the two ζ- and two α-globin genes of this cluster, due to the extensive sequence homology between the *HBZ* (ζ2-globin) and *HBZP* (ψζ1-globin) genes and between the *HBA1* (α1-globin) and *HBA2* (α2-globin) genes, respectively.

Despite their ancient origin, the *HBA1* and *HBA2* genes have remained very similar, comprising three segments of homology (the X, Y, and Z boxes) that are punctuated with non-homologous regions (I, II, and III).[4] They are identical throughout the 868 base pairs upstream of the cap site, except for two positions: nucleotide -634 is an A in *HBA1* and a G in *HBA2*, while nucleotide –733 is a C in *HBA1* and a T in *HBA2*. Both genes are also identical in their 5'untranslated regions, the first intron (IVSI) and all three coding exons. In the second intron (IVSII), only two sites of difference exist: a single nucleotide difference at IVSII,55 (G in *HBA1* and T in *HBA2*), and the substitution of an octanucleotide in *HBA1* (positions 119-126, 5'-CTCGGCCC-3') with a single G at position 119 in *HBA2*. The 3' untranslated regions of the two genes share 78% homology, preceding a short region of 100% identity adjacent to the polyadenylation site.[5] Several models have been suggested for this *concerted evolution* of the α-globin gene cluster, the most prominent being gene conversion and crossover fixation.

Evidence for mispairing and reciprocal crossing over between *HBA1* and *HBA2* can be inferred from the existence of the single α-globin gene deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$)[6-8] and their reciprocal triplicated alleles ($\alpha\alpha\alpha^{anti3.7}$ and $\alpha\alpha\alpha^{anti4.2}$).[9-13] The demonstration of α-globin gene deletions in transformed *E. coli* bacteria, identical to those found in humans, further reinforces the theory that sequence homology at the α-globin gene cluster promotes unequal crossing over.[14] Although

gene conversion has been well described in yeast,[15] and although it cannot be definitively distinguished from double crossovers events, in humans, gene conversion has been inferred from short DNA segments identical to one allele appearing in a different allele.

We now report two natural, complex hybrid variants of the *HBA1* and *HBA2* genes in man. In the *HBA1* gene variant *α121*, an octanucleotide within IVSII has been replaced by a single *HBA2*-specific nucleotide. In the *HBA2* gene variant *α212*, two sites within IVSII have been replaced by *HBA1*-specific sequences. We discuss the possible origin of these *patchwork* α-globin genes.

Design and Methods

Patients

Patient AI was a 14-year old African-American girl. She was referred for diagnosis because of severe anemia. Hemoglobin analysis revealed Hb S (74%) and Hb F (19%). DNA-based diagnostic results confirmed that she had sickle cell anemia (homozygous for the Hb S gene) and was heterozygous for the C/T polymorphism at nucleotide -158 upstream of the Gγ-globin gene.[16] Patient AP was a 2-year old Hispanic-American boy. He was referred for diagnosis because of microcytosis (mean corpuscular volume, MCV, 55 fL), and Hb 10.3 g/dL. DNA-based diagnostics showed that he was a heterozygous carrier of the IVSI,5 G→C β^+-thalassemia mutation.

Molecular analysis

Two multiplex polymerase chain reaction (PCR) assays were performed on both patients to screen for the presence of the seven most common α-globin gene deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{FIL}$, $--^{THAI}$, $-(\alpha)^{20.5}$, $--^{MED}$) and triplications.[17,18] This was followed by sequencing of the *HBA1* and *HBA2* genes, which were individually amplified using gene-specific forward and reverse primers as previously described.[19]

Southern blot analyses of *Bam*HI and *Bgl*II digested DNA fragments hybridized with α- and ζ-globin probes were performed to confirm the PCR-based results.

Results

Patients AI and AP were negative for α-globin gene deletions and/or triplications as determined by multiplex PCR analyses, and these results were confirmed by Southern blot analysis (*data not shown*).

For patient AI, PCR sequencing of her *HBA1* gene was negative for any mutations (*data not shown*). However, sequencing of her *HBA2* gene revealed the presence of a variant allele in addition to the wildtype one, differing only within a segment of IVSII (Figure 1A-C). This segment was heterozygous for a T→G substitution at IVSII,55 as well as for a G→CTCGGCCC insertion/substitution at IVSII,119. Closer scrutiny revealed that the IVSII sequence of this variant *HBA2* allele was a complete match with the wildtype *HBA1* IVSII sequence. We therefore conclude that we have identified a variant allele of *HBA2* in the heterozygous state in patient AI, whereby a segment of IVSII has been substituted with *HBA1*-specific IVSII sequences. We refer to this *HBA2* variant as an *α212* patchwork allele due to its alternating α2, α1, and α2 sequences (Figure 2). For patient AP, PCR sequencing of his *HBA1* gene revealed heterozygosity for a variant allele, which differs from the wildtype *HBA1* sequence only within a short segment of IVSII (Figure 1D-F). Nucleotides 119-126 of IVSII (CTCGGCCC) were replaced by a single G,

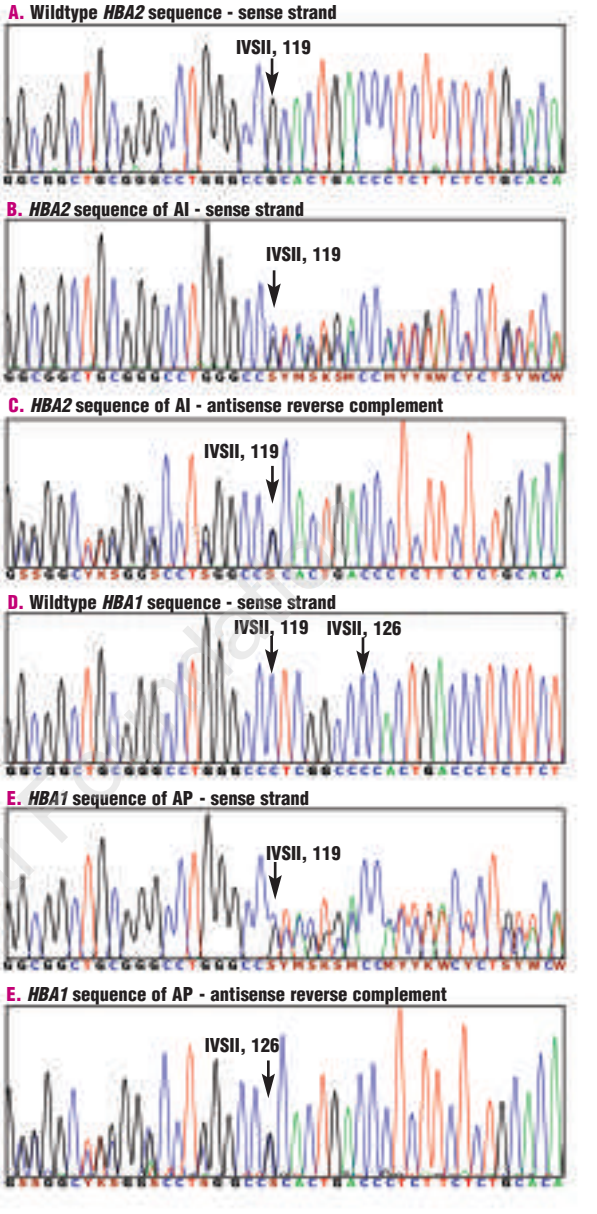

Figure 1. Partial *HBA1* and *HBA2* gene sequence chromatograms of wildtype (panels A & D), patient AI (panels B & C) and patient AP (panels E & F). **A.** Wildtype *HBA2* gene sequence around IVSII,119. **B-C.** Corresponding gene sequencing result from patient AI showing overlapping wildtype (*HBA2*-specific) and variant (*HBA1*-specific) IVSII sequences. **D.** Wildtype *HBA1* gene sequence around IVSII,119. **E-F.** Corresponding gene sequencing result from patient AP showing overlapping wildtype (*HBA1*-specific) and variant (*HBA2*-specific) IVSII sequences.

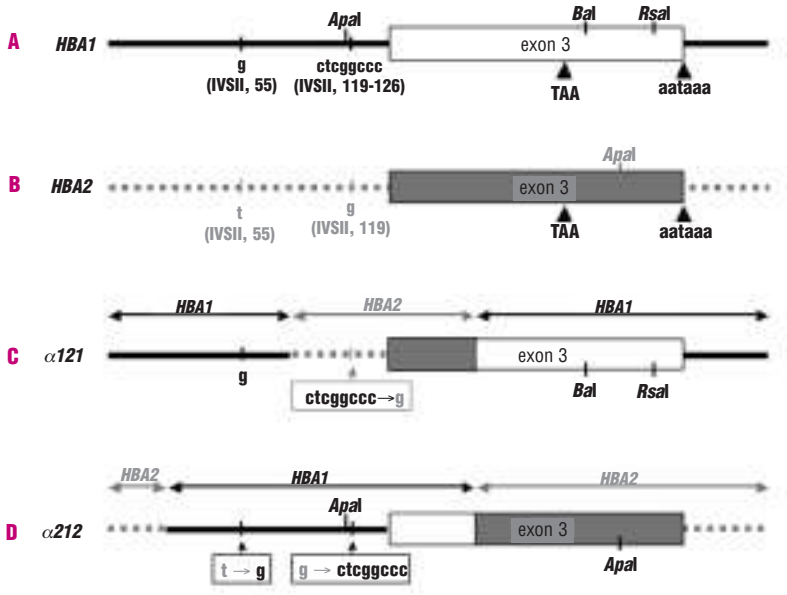

Figure 2. Schematic illustration of the 3' end of wildtype *HBA1* and *HBA2* genes and their patchwork variants. A-B. Wildtype *HBA1* and *HBA2* genes, respectively, highlighting the nucleotide differences within *IVSII*, as well as restriction site differences. C-D. *α121* and *α212* patchwork genes, respectively, and their IVSII sequence compositions. TAA, translation termination codon; aataaa, polyadenylation signal.

the latter interestingly being a characteristic of the wildtype *HBA2* IVSII sequence. The nucleotide at IVSII,55, however, remained wildtype *HBA1*. We therefore conclude that we have identified a variant allele of *HBA1* in the heterozygous state in patient AP, whereby a short stretch of IVSII has been substituted with an *HBA2*-specific IVSII sequence. We refer to this *HBA1* variant as a *α121* patchwork allele due to its alternating α1, α2, and α1 sequences (Figure 2). PCR sequencing of the *HBA2* gene of patient AP was negative for any mutations (*data not shown*). Separately, in the course of routine *HBA1* and *HBA2* gene screening of asymptomatic individuals with borderline low normal MCV of ~85 fL or less, we identified one additional individual of Asian Indian ethnicity who was heterozygous for the *α121* patchwork allele, and a further eight unrelated individuals of different ethnicity who were heterozygous for *α212* (Table 1). The single *α121*-positive individual was identified from a total of 348 unrelated Singaporean samples (176 Chinese, 106 Malay, 37 Indian, and 29 of other ethnicities) that underwent *HBA1* gene screening, suggesting an *α121* allele frequency of 1.35% in the Asian Indian population. In marked contrast, out of 416 unrelated Singaporeans (208 Chinese, 128 Malay, 47 Indian, and 33 of other ethnicities) underwent *HBA2* gene screening, we identified four Indian and two Malay individuals who were heterozygous for the *α212* patchwork allele, suggesting *α212* allele frequencies of 4.25% and 0.78% in the Asian Indian and Malay populations, respectively. A further two out of 120 Iranian samples screened were positive for the *α212* allele (0.83% allele frequency). It should be noted, however, that these data may not be representative of the true population allele frequencies, due to the relatively small sample sizes involved and the fact that the samples were selected for analysis based on certain MCV criteria. Interestingly through sequencing and/or pedigree analysis, the Indian and Malay *α212* alleles were discovered to be linked in *cis* to a variant nucleotide (G) at position -4 relative to the cap site, while the *α212* allele in the Iranian samples

Table 1. Additional individuals heterozygous for the *α212* or *α121* patchwork allele.

S/No.	Ethnicity	MCV	Patchwork allele	Linked nucleotide at position -4 on *α212*	Other mutations
1	Indian	82.9	α121	n.a.	
1	Indian	77.3	α212	G	
2	Indian	81.1	α212	G	
3	Indian	84.8	α212	G	
4	Indian	73.1	α212	G	
5	Malay	76.4	α212	G	
6	Malay	73.8	α212	G	Concurrent heterozygous Hb E
7	Iranian	74.5	α212	C	
8	Iranian	81.0	α212	C	

n.a.: not applicable.

is linked to wildtype C at position -4. The predicted 5' and 3' crossover sites differ between the two patchwork alleles described. In *α212*, both IVSII,55 and IVSII,119-126 are *HBA1*-specific, implying a 5' crossover occurring upstream of IVSII,55 and a 3' crossover occurring downstream of IVSII,119-126 but not much beyond the TAA translation stop (Figure 2). In *α121*, only the IVSII,119-126 site is *HBA2*-specific. This observation indicates that these patchwork alleles do not represent reciprocal derivatives of an intragenic double crossover between the *HBA1* and *HBA2* genes.

Discussion

Duplication of the ancestral α-globin gene is thought to have occurred millions of years ago. Yet, the resultant *HBA1* and *HBA2* genes have maintained a remarkable sequence homology to each other over time. Segmental

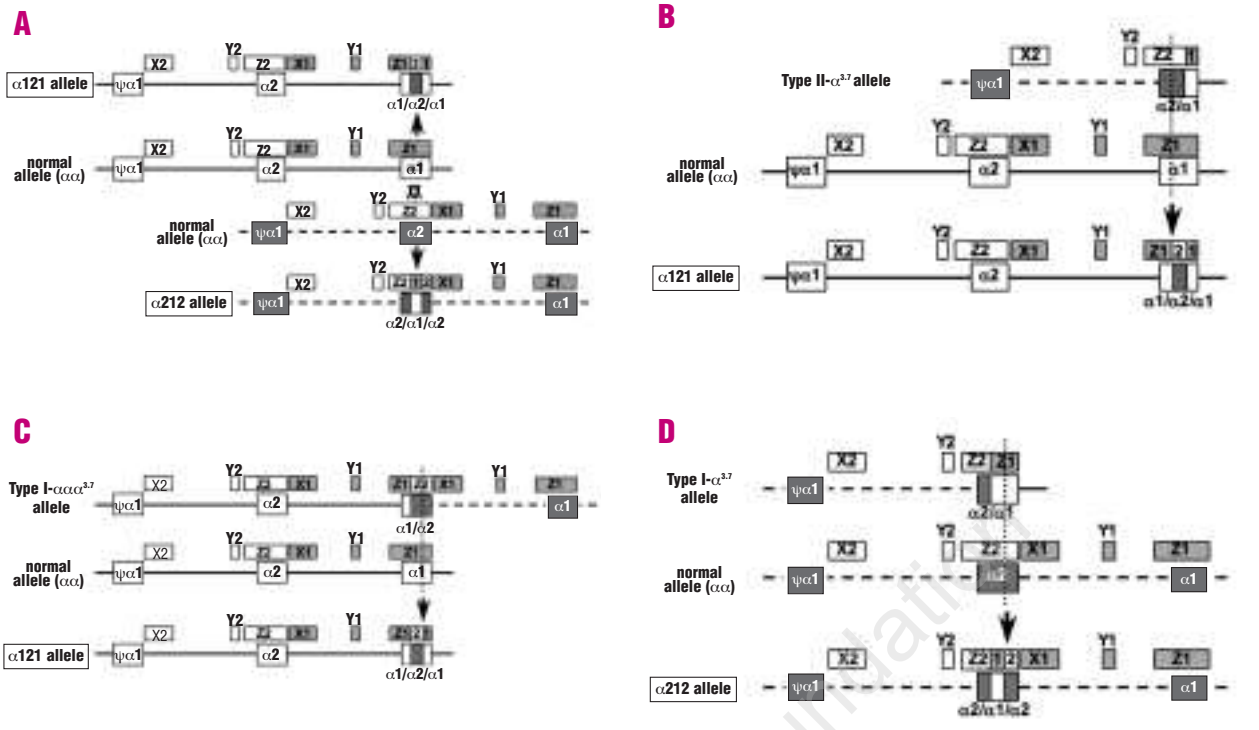

Figure 3. Derivation of patchwork genes via double crossover, gene conversion or unequal crossing over between a normal allele and a single gene deletion or triplicated allele. A. Double crossover or gene conversion mechanism for the origin of the patchwork genes. Misalignment between two normal alleles and an intragenic double crossover between the *HBA1* and *HBA2* genes will produce an *α121* derivative and its reciprocal *α212* derivative. Alternatively, an intragenic gene conversion event between *HBA1* and *HBA2* could produce an *α121* or *α212* patchwork gene. B-E. Unequal crossing over between a normal allele and a type II -$\alpha^{3.7}$ allele (B) or a type I anti-3.7 allele (C) can give rise to an *α121* allele. Similarly, unequal crossing over between a normal allele and a type I -$\alpha^{3.7}$ allele (D) or a type II anti-3.7 allele (E) can give rise to an *α212* allele. The X, Y, and Z boxes are the homologous segments in the α-globin gene cluster.

gene conversion and/or unequal crossing over between these two genes has been invoked to account for this *concerted evolution*.[4,5,20] The two natural hybrid patchwork α-globin genes described in this study may help further our understanding of these genetic recombination mechanisms during evolution.

Given the high sequence identity between the *HBA1* and *HBA2* genes and their surrounding sequences, the patchwork α-globin genes could have arisen from a double crossover or a gene conversion between misaligned *HBA1* and *HBA2* genes of normal alleles (Figure 3A). In a double crossover, two breakpoints occur within the misaligned α-globin genes followed by exchange of genetic material between the breakpoints, resulting in an *α121*-like patchwork gene and its reciprocal *α212*-like derivative. Because the region of DNA exchange involved in these patchwork alleles is in the sub-kilobase range, a simultaneous double crossover with 5' and 3' crossing over sites within a few hundred nucleotides of each other is expected to be extremely rare. Gene conversion involves the non-reciprocal transfer of information from a donor sequence to an acceptor sequence. There is strand invasion of a part of the *HBA1* or *HBA2* gene to its misaligned *HBA2* or *HBA1* counterpart, respectively, followed by heteroduplex formation, mismatch repair, and finally synthesis of the complementary strand of the recipient gene directed by the donor strand as template. This results in the formation of either an *α212*-like or *α121*-like patchwork gene, but not both. Although it has been suggested that sequence gaps such as the IVSII 7 bp insertion/deletion difference between *HBA1* and *HBA2* act as a barrier to gene conversion,[4,5,20] observations at the ζ-globin locus suggest that small deletions or insertions do not always act as barriers to genetic recombination.[20,21] The existence of *α121* and *α212* patchwork genes may also be construed as further evidence that gene conversion can occur in regions of insertion/deletion difference. A simpler

model to account for the origin of the patchwork genes, however, is through unequal crossing over between a common recombinant allele such as the -$\alpha^{3.7}$ single gene deletion (or the $\alpha\alpha\alpha^{anti3.7}$ triplication) and a normal allele. Thus, an $\alpha 121$ allele could be derived from an unequal crossover between a wildtype *HBA1* gene and a type II -$\alpha^{3.7}$ allele, with the crossing over occurring between IVSII,55 and IVSII,119 of *HBA1* (Figure 3B). It is also possible to derive an $\alpha 121$ allele through unequal crossover between a wildtype *HBA1* gene and a type I anti-3.7 triplicated allele, with crossing over occurring between the *Apa*I site in IVSII and the *Bal*I site in exon 3 of *HBA1* (Figure 3C). The reciprocal derivatives of these two recombinations are a type I -$\alpha^{3.7}$ allele and a type II anti-3.7 triplicated allele, respectively (*not shown*). Similarly, an $\alpha 212$ allele could be generated through a single unequal crossover between a wildtype *HBA2* gene and a type I -$\alpha^{3.7}$ allele, with crossing over occurring between the *Apa*I site in IVSII and the *Bal*I site in exon 3 (Figure 3D). It could also be derived from an unequal crossover between a wildtype *HBA2* gene and a type II anti-3.7 triplicated allele, with crossing over occurring upstream of IVSII,55 (Figure 3E). The reciprocal derivatives of these two recombinations are a type II –$\alpha^{3.7}$ allele and a type I anti-3.7 triplicated allele, respectively (*not shown*). We have now detected the $\alpha 121$ allele in 2 unrelated individuals of different ethnicity (Latino and Asian Indian), and the $\alpha 212$ allele in a total of nine unrelated individuals from four different ethnic backgrounds (African American, Asian Indian, Malay and Iranian). Sequencing and/or pedigree analyses also showed that the $\alpha 212$ allele in the Iranians was linked to the wildtype C nucleotide at position -4 relative to the cap site of the *HBA2* gene, while the same allele in the Indians and Malays was linked to the variant G nucleotide at position -4 (Table 1). The different ethnic, geographical, and haplotype backgrounds of both the $\alpha 121$ and $\alpha 212$ alleles strongly suggest that they have arisen independently on at least two different occasions. In fact, we predict that in regions with a high frequency of the type II -$\alpha^{3.7}$ allele or type I anti-3.7 allele, the presence of the $\alpha 121$ allele is highly probable. Similarly, in regions with a high frequency of the type I -$\alpha^{3.7}$ allele or type II anti-3.7 allele, the probability of the $\alpha 212$ allele being present may be high.

Interestingly, a hybrid *HBA2* gene has previously been reported among African Americans.[22] This hybrid *HBA2* gene is most similar to the $\alpha 212$ as it also displays *HBA1*-specific sequences at two sites within IVSII. Unlike $\alpha 212$, however, a CC dinucleotide normally present in wildtype *HBA1* (IVSII,126-127) is deleted in the hybrid gene. The authors postulated that the hybrid gene probably originated through a double crossover. A hybrid δ-globin (*HBD*) gene containing internal *HBB* (β-globin) gene sequences has also been described.[23,24] In this $\delta\beta\delta$ hybrid gene, the IVSI and small portions of exons 1 and 2 of the *HBD* gene are *HBB*-specific. The authors also suggest that this patchwork gene probably arose through a reciprocal non-homologous double crossover between the *HBB* and *HBD* genes.

The possible effect of the IVSII changes on α-globin gene expression from the $\alpha 121$ and $\alpha 212$ alleles is undefined at present. Although the majority of individuals who were positive for the $\alpha 121$ or $\alpha 212$ alleles were asymptomatic and had borderline low MCV (Table 1), this could simply be due to the fact that only asymptomatic individuals with borderline low MCV were screened, to investigate whether the low MCV could be explained by mild mutations within the *HBA1* or *HBA2* genes. Alternatively, the $\alpha 121$ and $\alpha 212$ alleles might truly represent mildly hypomorphic alleles of their respective normal α-globin genes. However, a preliminary analysis of MCV values in an Indian family segregating with the $\alpha 212$ allele suggests otherwise (Table 2). In this family, low MCV values of less than 80 fL were observed only in those members co-segregating with the -$\alpha^{3.7}$ allele alone or in combination with the $\alpha 212$ allele, while those members who were heterozygous for the $\alpha 212$ allele alone (but not the -$\alpha^{3.7}$ allele) had MCV values greater than 80 fL. These observations suggest that the $\alpha 212$ allele is unlikely to be associated with microcytosis. Systematic screening for these patchwork alleles in a larger cohort of unselected individuals, followed by hematologic investigations of those positive for either allele alone, is needed in order to dissect out their possible effect.

Table 2. MCV values in an Indian family segregating with $\alpha 212$ and -$\alpha^{3.7}$ alleles.

S/No.	Relationship	MCV (fL)	Genotype
1	Father	78.9	-$\alpha^{3.7}$/α212
2	Mother	82.9	$\alpha\alpha/\alpha\alpha$
3	Son	75.9	-$\alpha^{3.7}/\alpha\alpha$
4	Son	80.1	-$\alpha^{3.7}/\alpha\alpha$
5	Daughter	84.8	$\alpha\alpha/\alpha$212
6	Daughter	80.4	-$\alpha^{3.7}/\alpha\alpha$
7	Son	81.5	$\alpha\alpha/\alpha$212
8	Daughter	80.8	$\alpha\alpha/\alpha$212
9	Daughter	87.0	$\alpha\alpha/\alpha$212
10	Daughter	86.2	$\alpha\alpha/\alpha$212
11	Son	82.3	$\alpha\alpha/\alpha$212
12	Son	79.2	-$\alpha^{3.7}/\alpha\alpha$

HLaw, MHS, DHC, and SSC conceived and directed the study, and revised and finalized the manuscript. HLaw, HN, ISN, and DHC provided the patients' samples. HLuo and WW performed the bench work experiments, analyzed the data together with the other co-authors, and generated the Figures and Tables. JFH analyzed the consolidated data, and wrote the manuscript.

We sincerely thank Ms. Amy Y.Y. Chan and Dr. Edmond S.K. Ma, Department of Pathology, the University of Hong Kong, for carrying out the Southern blot analyses on patients AI and AP, and Professor Ross C. Hardison, Department of Biochemistry and Molecular Biology, Pennsylvania State University, USA, for critical review and valuable suggestions on the manuscript. The work carried out in Boston and Singapore was supported in part by NHLBI grant 1U54 HL 0708819 (MHS) and BMRC grant 03/1/21/ 18/222 (SSC), respectively.

Manuscript received September 14, 2005. Accepted January 2, 2006. Prepublished online on February 17, 2006. PII: 03906078_9221.

References

1. Higgs DR. Molecular Mechanisms of α Thalassemia. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Vol. 17, 1st ed. Cambridge: Cambridge University Press; 2001. p. 405-30.
2. Goh SH, Lee YT, Bhanu NV, Cam MC, Desper R, Martin BM, et al. A newly discovered human {α}-globin gene. Blood 2005;106:1466-72.
3. Zimmer EA, Martin SL, Beverley SM, Kan YW, Wilson AC. Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. Proc Natl Acad Sci USA 1980;77:2158-62.
4. Michelson AM, Orkin SH. Boundaries of gene conversion within the duplicated human α-globin genes. Concerted evolution by segmental recombination. J Biol Chem 1983;258:15245-54.
5. Higgs DR, Hill AV, Bowden DK, Weatherall DJ, Clegg JB. Independent recombination events between the duplicated human α globin genes; implications for their concerted evolution. Nucleic Acids Res 1984;12:6965-77.
6. Orkin SH, Old J, Lazarus H, Gurgey A, Weatherall DJ, Nathan DG. The molecular basis of α-thalassemias: frequent occurrence of dysfunctional α loci among non-Asians with Hb H disease. Cell 1979;17:33-42.
7. Embury SH, Lebo RV, Dozy AM, Kan YW. Organization of the α-globin genes in the Chinese α-thalassemia syndromes. J Clin Invest 1979; 63: 1307-10.
8. Embury SH, Miller JA, Dozy AM, Kan YW, Chan V, Todd D. Two different molecular organizations account for the single α-globin gene of the α-thalassemia-2 genotype. J Clin Invest 1980;66:1319-25.
9. Goossens M, Dozy AM, Embury SH, Zachariades Z, Hadjiminas MG, Stamatoyannopoulos G, et al. Triplicated α-globin loci in humans. Proc Natl Acad Sci USA 1980;77:518-21.
10. Higgs DR, Old JM, Pressley L, Clegg JB, Weatherall DJ. A novel α-globin gene arrangement in man. Nature 1980;284:632-5.
11. Lie-Injo LE, Herrera AR, Kan YW. Two types of triplicated α-globin loci in humans. Nucleic Acids Res 1981; 9: 3707-17.
12. Trent RJ, Higgs DR, Clegg JB, Weatherall DJ. A new triplicated α-globin gene arrangement in man. Br J Haematol 1981;49:149-52.
13. Traeger-Synodinos J, Kanavakis E, Vrettou C, Maragoudaki E, Michael T, Metaxotou-Mavromati A, et al. The triplicated α-globin gene locus in β-thalassaemia heterozygotes: clinical, haematological, biosynthetic and molecular studies. Br J Haematol 1996; 95:467-71.
14. Lauer J, Shen CK, Maniatis T. The chromosomal arrangement of human α-like globin genes: sequence homology and α-globin gene deletions. Cell 1980;20:119-30.
15. Fogel S, Hurst DD. Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics 1967; 57: 455-81.
16. Gilman JG, Huisman TH. DNA sequence variation associated with elevated fetal G γ globin production. Blood 1985;66:783-7.
17. Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for α-thalassemia. Blood 2001;98:250-1.
18. Wang W, Ma ES, Chan AY, Prior J, Erber WN, Chan LC, et al. Single-tube multiplex-PCR screen for anti-3.7 and anti-4.2 α-globin gene triplications. Clin Chem 2003;49:1679-82.
19. Sutcharitchan P, Wang W, Settapiboon R, Amornsiriwat S, Tan AS, Chong SS. Hemoglobin H disease classification by isoelectric focusing: molecular verification of 110 cases from Thailand. Clin Chem 2005;51:641-4.
20. Papadakis MN, Patrinos GP. Contribution of gene conversion in the evolution of the human β-like globin gene family. Hum Genet 1999;104:117-25.
21. Hill AV, Nicholls RD, Thein SL, Higgs DR. Recombination within the human embryonic ζ-globin locus: a common ζζ-chromosome produced by gene conversion of the ψζ gene. Cell 1985; 42:809-19.
22. Gu YC, Nakatsuji T, Huisman TH. Detection of a new hybrid α-2 globin gene among American blacks. Hum Genet 1988;79:68-72.
23. Adams JG 3rd, Morrison WT, Steinberg MH. Hemoglobin Parchman: double crossover within a single human gene. Science 1982;218:291-3.
24. Metzenberg AB, Wurzer G, Huisman TH, Smithies O. Homology requirements for unequal crossing over in humans. Genetics 1991;128:143-61.