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Multiple arrangements of the human embryonic zeta globin genes

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ABSTRACT

Rearrangements which are most readily explained by homologous crossover between misaligned segments of DNA in the region of the human embryonic zeta (ζ) globin genes have been identified in individuals of three different racial origins. These recombination events have resulted in a surprisingly high prevalence of chromosomes with single (0.4%) and triplicated (1.3%) ζ genes with apparently no significant effect on the phenotype.

INTRODUCTION

The human α-like globin genes occur in a multigene cluster on chromosome 16 arranged in the order 5'-ζ2-ζ1-ψα1-α2-α1-3' (1,2). This gene family probably evolved by duplication and subsequent divergence of pre-existing genes. However, the structures of the duplicated α genes within this cluster have remained remarkably similar despite the fact that they are thought to have arisen by a duplication event which occurred several million years ago (2,3,4,5). Likewise, the structures of the duplicated ζ genes are also similar, although it is clear that single base changes in the ζ1 sequence result in this being a pseudogene (N.J. Proudfoot, personal communication). It has been suggested that such gene pairs represent examples of concerted evolution, although the precise mechanism by which duplicated genes may evolve in unison is not yet clear (5,6). One hypothesis suggests that concerted evolution may have resulted from cycles of loss and duplication of similar genes by interchromosomal recombination (5). Unequal crossover events may occur between duplicated DNA sequences giving rise to reciprocal arrangements with either one or three genes on one chromosome. Specifically, it has been shown that both deletion

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and triplication of the α globin genes occur in vivo and in cloned sequences in vitro (2 and 7-12). If this is an example of a more general phenomenon it can be predicted that, since the two ζ globin genes are also similar in their structure, analogous rearrangements should also occur between them.

We have examined the arrangement of the ζ genes in a number of individuals to determine whether unequal crossover occurs between these genes. The results of this study show that such rearrangements do occur, confirming the notion that duplicated gene sequences give rise to the formation of single and triplicated gene arrangements. Evidence from this study shows that such rearrangements, like the α globin gene rearrangements, are surprisingly common, suggesting that either different genotypes afford a variation in selective advantage or that the DNA sequences within or around the ζ globin genes inherently predispose to a high rate of recombination.

MATERIALS AND METHODS

DNA from thalassaemic and non-thalassaemic individuals, was prepared from the buffy coats from 10-20 ml of heparinised blood by phenol-chloroform extraction. Approximately 10 μg of DNA obtained in this way was digested with 10-30 units of the restriction endonuclease Bam Hl (or its isoschizomer Bst I), Bgl II, EcoRl, Hind III, Hpa I, Kpn I, and Sac I (or Sst I) in single or double digests. Each reaction was carried out in the appropriate buffer at 37°C for 6-12 h. The restricted DNA was then electrophoresed in 0.8% agarose gel for 16, 40 or 72 h at 1.0-2.0 volts/cm (with recirculation of the buffer) and transferred to nitrocellulose filters (13) which were then hybridised with either α- or ζ-specific ^{32}P labelled probes as previously described (14).

A third probe was prepared from the plasmid pBR ζ (2) which includes DNA from between the two ζ genes. It has been shown that a hypervariable region, previously identified in larger Bgl II and Bam Hl fragments (15) is contained within a Sac I fragment of genomic DNA also present between the two ζ genes (unpublished). A Sac I/EcoRl digest of the recombinant plasmid provides a unique sequence probe (V-ζ probe) ∿ 0.3 kb

in length which enables the hypervariable region to be readily detected in a Sac I digest of genomic DNA by blot hybridisation.

RESULTS

Normal individuals have two α and two ζ globin genes per chromosome 16 (2). A variety of deletions of different sizes from within the α gene complex give rise to chromosomes with only one (-α) or no (--) α genes and this leads to the easily recognisable phenotype of α thalassaemia (16). These DNA rearrangements are readily detected by blot hybridisation and have been well characterised in terms of their structure and function (17).

Rearrangements involving the ζ globin genes are more difficult to interpret because these genes are separated by a hypervariable segment of DNA with the result that restriction maps may vary even between normal individuals (15). Nevertheless, in this study we have identified 18 individuals in whom Southern blot hybridisation studies indicate that in each case at least one chromosome contains a substantial DNA rearrangement in the region of the ζ genes (Table 1). In some cases, coexistent deletions of the α genes on one chromosome have facilitated the assignment of particular restriction fragments to one or other α gene complex.

(1) Evidence for chromosomes which have a single ζ gene

When DNA from normal individuals is digested with Bam Hl, two ζ-specific fragments are seen by blot hybridisation, one 5.9 kb long containing the ζ2 gene and one, containing the ζ1 gene, which is between 10.0 kb and 11.6 kb long, depending on the size of the hypervariable segment which is present within this fragment (15). However, in two individuals (A and B, Table 2) we observed an abnormal ζ-specific band corresponding to 6.5 kb in A, and 6.2 kb in B (Figure 1). Since the hypervariable fragment between the ζ genes normally only varies by up to ∿ 1.5 kb, it seems unlikely that these bands are due to variation in this region. Furthermore, the hypervariable segment is also contained in an overlapping Bgl II-ζ-specific fragment (10.5-12.0 kb) and since no corresponding fragment was seen in that digest, variation in this region is not the cause

TABLE 1

Summary of blot hybridisation results using ζ-specific and V-ζ probe

Subject	α-genotype	ζ-genotype	ζ								
			Bam H1					Bgl II			
A+	αα/αα	ζ/ζζ	5.9	6.5	10.8				11.3	12.6	
B+	-α/αα	ζ/ζζ	5.9	6.2	10.8				11.3	12.6	16.0
C	--/-α	ζ/ζζ	5.9	6.2		∿20	10.5				16.0
D	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.3						
E	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.3						
F	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.3						
G	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,10.8						
H	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.6			10.5,11.0,12.0		12.6	
I	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.6						
J	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.6						
K	αα/αα	ζζ/ζζζ	5.9		10.0‡10.4						
L	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,10.8						
M	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,11.6						
N	αα/αα	ζζ/ζζζ	5.9		10.0,10.8,11.3			10.5,11.3,12.0		12.6	
O*	αα/αα	ζζ/ζζζ	5.9		10.0,10.4,10.8						
P*	αα/αα	ζζ/ζζζ	5.9		10.0‡10.4						
Q	--/αα	ζζ/ζζζ	5.9		10.8,11.3,	∿20	10.5		11.3,12.0	12.6	
R	--/αα	ζζ/ζζζ	5.9		10.0,10.4	∿20	10.5		11.3,10.5	12.6	

TABLE 1 (continued).

	ζ																V-ζ		
	EcoRl				Kpn I			Hind III			Hpa I	Bam Hl/Hind III			Sac I				
A+	5.0		19.0		∿23				13.5	18.0	16.5						4.2		
B+	5.0		19.0	∿20					13.5	18.0	16.5						7.6		
C	5.0	17.2	15.3																
D																			
E	5.0		11.5		∿23	11.0	11.5	∿23.5	13.5	11.0	16.5,18.0		5.9	8.0,8.5,11.8			6.3	4.2	4.8
F	5.0		11.5		∿23	11.0	11.5	∿23.5	13.5	11.0	16.5,18.0						6.3	4.2	4.8
G																			
H	5.0		11.5		∿23				13.5	11.0	16.5,18.0						6.3	4.2	4.8
I																			
J																			
K																			
L																			
M	5.0		11.5		∿23				13.5	11.0	16.5,18.0						6.3	4.2	4.8
N	5.0		11.5		∿23	11.0	11.5	∿23.5											
O*																			
P*																			
Q	5.0	17.2	11.5		∿23	11.0	11.5	∿23.5				11.8,12.5	5.9	8.0,8.5		19.5			
R	5.0	17.2	11.5		∿23														

The subjects studied were of South East Asian, Vanuatuan* and Jamaican+ origins.

‡ Double intensity

Table 2.

EXAMPLES OF ζ/ζζ ARRANGEMENT IN INDIVIDUALS WITH αα/αα, -α/αα and --/-α GENOTYPES

		Genotype	Bam H1*	Bgl II*	EcoR1*	Sac I‡
A	(i)	ζ2-ζ1-ψα1-α2-α1	5.9, 10.8	11.3, 12.6	5.0, ∿23	4.2
	(ii)	ζ-ψα1-α2-α1	6.5	12.6	19.0	
B	(i)	ζ2-ζ1-ψα1-α	5.9, 10.8	11.3, 16.0	5.0, ∿20	7.6
	(ii)	ζ-ψα1-α2-α1	6.2	12.6	19.0	
C	(i)	ζ2-ζ1—	5.9, ∿20	10.5	5.0, 17.2	—
	(ii)	ζ-ψα1-α	6.2	16.0	15.3	—

Three examples explaining the assignment of various fragments to particular haplotypes in the ζ/ζζ genotypes. Results of blot hybridisation using ζ-probe* and V-ξ‡ probe; the sizes of fragments are given in kilobases (kb). In addition to containing the hypervariable region the upstream Sac I site of this fragment also appears to be polymorphic and therefore more than one size of Sac I fragment may contain the same length of the hypervariable DNA segment.

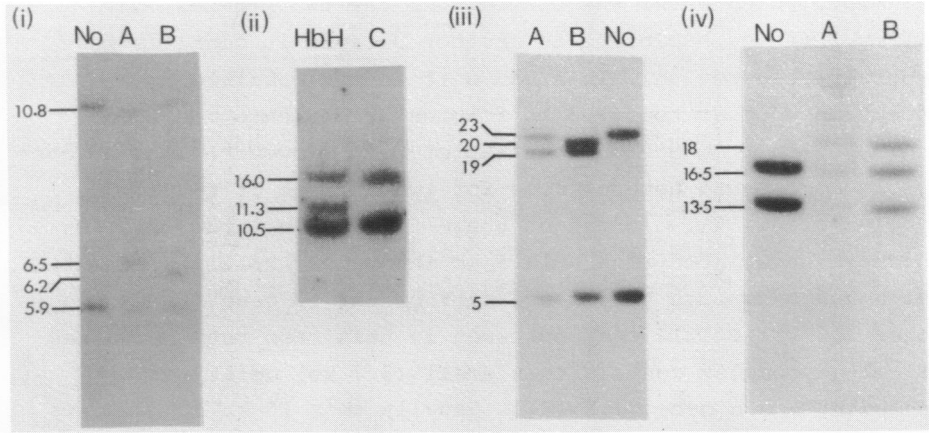

Figure 1 Blot hybridisation results using ζ-specific probe in subjects with ζ/ζζ genotypes (i) Bam Hl (ii) Bgl II (iii) EcoRl and (iv) Hind III digested genomic DNA. The subjects studied, denoted A, B and C correspond to those shown in Table 1. No denotes normal DNA sample, Hb H represents DNA from an individual with the --/-α genotype. Sizes of DNA fragments are in kilobases (kb).

of these abnormal fragments. If they were due to restriction site polymorphisms rather than DNA rearrangements there should be no other abnormal ζ-specific restriction fragments: the presence of abnormal EcoRl fragments (∿19 kb) and Hind III fragments (∿18 kb) confirms that there are DNA rearrangements in these haplotypes.

The results of blot hybridisation studies in the two haplotypes (A ii, and B ii, Table 2) are best explained by deletions of at least two different types which remove between 10 kb and 11 kb of DNA and include the two Bam Hl and single EcoRl sites between the ζ genes giving rise to 6.5 {5.9 + (10.0-11.6)* minus ∿ 10 kb} (Case A) or 6.2 {5.9 + (10.0-11.6)* minus ∿ 10 kb} (Case B), Bam Hl fragments, a ∿ 19 {5.0 + (∿23)* minus ∿ 10 kb} EcoRl fragment and a ∿ 18 {13.5 + (16.5)* minus ∿ 10 kb} Hind III fragment. (*Since these fragments all contain the hypervariable segment they may differ in size from one chromosome to another).

To examine the extent of this deletion, DNA was cut with Bgl II which produces two ζ-specific fragments of 11.3 and 12.6 kb in normal individuals. Again the size of the usual 11.3 kb

fragment, which spans the DNA between the two ζ-genes, may differ from one haplotype to another because it contains the hypervariable region. In A and B it is not possible to ascertain whether the 11.3 kb fragment represents the intergenic region from one or both haplotypes. However, a third case (C) is a compound heterozygote for two defects of the α gene complex (Table 2). In one of these there is a large deletion which is associated with a 10.5 kb intergenic Bgl II fragment which contains both the ζ1 and part of the ζ2 gene (14). The other (16 kb) Bgl II fragment seen in this case represents the ζ1-ψα1-α- complex containing a small (3.7 kb) deletion including one α gene (7,8,18). Usually this 16 kb fragment is linked to a second Bgl II fragment containing the DNA which spans the hypervariable region and part of the ζ2 gene. No second band is seen in DNA from this individual (Figure 1). Although the data could be explained by a shorter intergenic fragment, also 10.5 kb long, associated with the novel 6.2 kb Bam Hl fragment, it is more likely that in this and in fact all of these cases, the intergenic fragment has been deleted together with one ζ gene. This conclusion was strengthened by blot hybridisation of Sac I digested DNA using the V-ζ probe which hybridises to the intergenic region. Whereas most commonly two Sac I fragments are seen, each derived from the hypervariable region of one chromosome, in A and B only one fragment was seen indicating that in these cases the intergenic region from one chromosome was deleted (Table 1 and 2 and Figure 2) and that they have a genotype ζ/ζζ. The removal of this large fragment of DNA including the intergenic region is most simply explained by a crossover event between homologous but misaligned regions in or around the ζ genes (Figure 3). Similar rearrangements in this complex between the highly homologous regions which include the α genes are well documented and they result in chromosomes with one (-α) or three (ααα) genes (2,7,12).

(2) Evidence for chromosomes with three ζ genes

The reciprocal nature of the previously reported α globin rearrangements supports the hypothesis that they arose by crossover events and therefore if the single ζ gene arose in

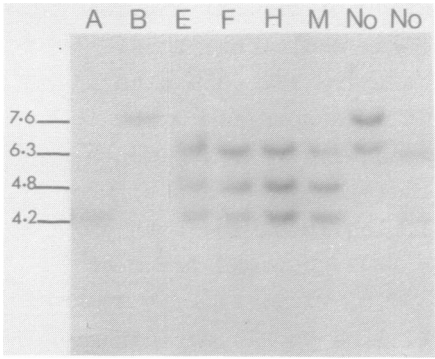

Figure 2 Blot hybridisation results using V-ζ probe in DNA from subjects A,B,E,F,H,M digested with Sac I. The size of the two bands seen in normal individuals varies from 3.0-7.6 kb. No denotes DNA from normal individuals. Sizes of fragments are in kilobases (kb).

this way it would predict the existence of a ζζζ gene arrangement (Figure 3). To examine this possibility we studied DNA from thalassaemic and non-thalassaemic individuals using several enzymes which cut outside and between the ζ-genes (Table 1). Normal DNA produces two ζ-specific fragments on digestion with EcoRl, one 5.0 kb long containing the ζ2

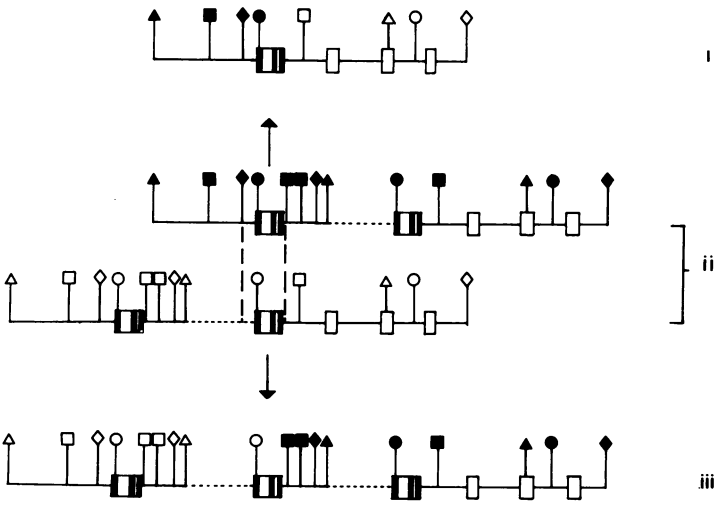

Figure 3 Proposed model for crossover events between two normal chromosomes (ii) in which the genes are arranged 5'-ζ2-ζ1-ψα1-α2-α1-3'. This results in (i) one chromosome with a single ζ-globin gene and (iii) a chromosome with triplicated ζ genes. Restriction sites shown are △ ▲ Hind III, □ ■ Bam Hl, ○ ● Bgl II and ◊ ♦ EcoRl. (---) denotes hypervariable regions of DNA.

gene and another ∿ 23 kb long containing the ζ1, ψα1 and two α genes. In 7 individuals we detected an additional EcoR1 fragment which was 11.5 kb in length (Figure 4). Since no abnormal fragments were detected using an α-specific probe it appeared that this new fragment represented a rearrangement in the region of the ζ-genes. In 2 individuals (Q and R) who were compound heterozygotes for a deletion involving both α genes (--) which is associated with a 17.2 kb EcoR1 fragment (14) four ζ-specific bands were seen (5.0, 11.5, 17.2, ∿ 23 kb). The enzymes Kpn I, Hind III and Hpa I, like EcoR1, cut either side and once between the two ζ genes. Digestion of DNA from several individuals with these enzymes similarly gave additional ζ-specific fragments (Table 1 and 3, and Figure 4). This suggests that the abnormal fragments represent an insert of ∿ 11 kb of DNA which also contains an additional ζ globin gene.

Since Bam H1 and Bgl II ζ-specific fragments overlap each other and both span the hypervariable region between the ζ genes, a single digest of genomic DNA with either enzyme may produce two fragments representing the two different

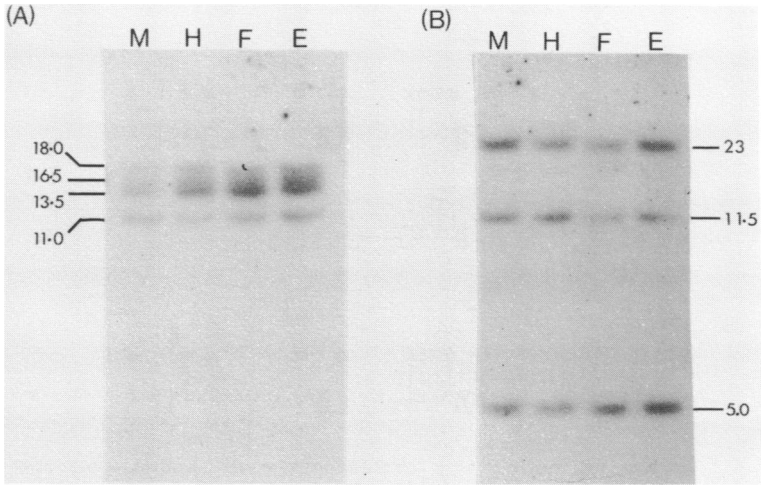

Figure 4 Results of blot hybridisation studies, using ζ-specific probe, of (A) Hind III digested DNA and (B) EcoR1 digested DNA from the subjects M,H,F and E. Sizes of DNA fragments are in kilobases (kb).

Table 3.
EXAMPLES OF ζζ/ζζζ ARRANGEMENT IN INDIVIDUALS
WITH αα/αα and αα/-- GENOTYPES

		Bam H1*	Bgl II*	EcoR1*	KpnI*	Sac I‡
H (i)	ζ2-ζ1-ψα1-α2-α1	5.9, 11.6	12.0, 12.6	5.0, ∿23	——	4.8
H (ii)	ζ2-ζ-ζ1-ψα1-α2-α1	5.9, 10.0, 10.4	10.5, 11.0, 12.6	5.0, 11.5, ∿23	——	4.2, 6.3
Q (i)	ζ2-ζ1——	5.9, ∿20	10.5	5.0, 17.2	11.0	——
Q (ii)	ζ2-ζ-ζ1-ψα1-α2-α1	5.9, 10.8, 11.3	11.3, 12.0, 12.6	5.0, 11.5, ∿23	11.0, 11.5, ∿23.5	

Two examples explaining the assignment of various fragments to particular haplotypes in the ζζζ/ζζ gene arrangement. Results of blot hybridization using ζ-probe* and V-ζ probe‡; the sizes of fragments are given in kilobases (kb).

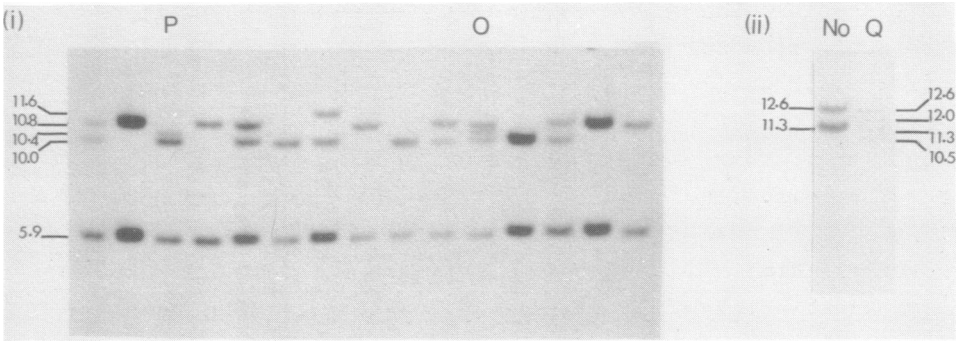

Figure 5 Results of blot hybridisation using ζ-specific probe with (i) Bam Hl digested DNA from 13 normal individuals and 2 individuals (O and P) with the ζζζ/ζζ genotype and (ii) DNA from subject Q with Bgl II.

haplotypes (Figure 5). The difference in size between such haplotypes is represented in both Bgl II and Bam Hl digests (Tables 1 and 3). In those cases described above where an additional ζ-specific fragment was detected, either three, rather than two, Bam Hl and Bgl II fragments were seen; or where there were two, one was twice as intense as the other (Figure 5). Since these three fragments were difficult to resolve by agarose gel electrophoresis a double digest, which produced smaller fragments representing the same areas, was used to confirm these observations (Table 1). These data are consistent with the presence of three different variable regions in these subjects and taken together with the results of the EcoRl, Hind III, Hpa I and Kpn I digests suggest that on one chromosome there is a complete repeat of a ζ gene and the intergenic region (Table 1; and Table 3, haplotypes H ii and Q ii).

As before, this was confirmed by blot hybridisation of Sac I digested DNA and with the V-ζ intergene specific probe. In 4 individuals (E,F,H, and M), with the proposed ζζ/ζζζ gene arrangements, three rather than the normal two fragments were seen (Table 1 and Figure 2).

DISCUSSION

Restriction maps of both the single and triplicated ζ gene arrangements are consistent with the model set out in Figure 3.

In each case, the crossover event has occurred to the right of the EcoRl site upstream from the ζ gene and the first Bam Hl site downstream from this gene and the corresponding region including the ζ1 gene on the other chromosome. This suggests that for misalignment and crossover to occur, considerable homology must exist between the ζ1 and ζ2 genes contained within this area, indeed DNA sequencing has shown that the two zeta genes are highly homologous (N.J. Proudfoot, personal communication). It is not clear why at least two different sized deletions giving rise to the single ζ gene arrangement exist unless there are also areas of non-homology, which differ in size between the ζ1 and ζ2 genes, separating homologous segments of DNA. In this case, the size of deletion would depend on the site of the crossover point with respect to the non-homologous areas: such a mechanism is known to occur in the α gene region (2). The differing lengths of the first intron in the ζ1 and ζ2 genes (N.J. Proudfoot; personal communication) may provide such a basis for these different sized deletions.

Crossovers giving rise to two different sized deletions involving the α genes also produce the corresponding types of reciprocal arrangements with three α genes (9-12). Therefore, different triplicated ζ gene arrangements should exist in populations where the two single ζ gene arrangements are found. However, the reciprocal arrangement in this case is complicated by the fact that since the crossover includes two hypervariable regions, each of which may vary by up to ∿ 1.5 kb, the patterns obtained by restriction mapping may vary considerably. Therefore it is not possible using blot hybridisation to determine whether the precise reciprocal arrangement exists or not.

Since all mammals have embryonic haemoglobins (16), it is probable that they are necessary for normal development. In man ζ globin chains are present in Hb Gower 1 ($\zeta_2\varepsilon_2$) and in Hb Portland ($\zeta_2\gamma_2$) which are two of the three types of embryonic haemoglobins, the third being Hb Gower 2 ($\alpha_2\varepsilon_2$) (16). A combination of protein sequencing, blot hybridisation and DNA sequencing has shown that whereas the ζ2 gene is functional the ζ1 gene can not produce normal ζ globin (14,19 and

N.J. Proudfoot, personal communication). This raises the point of whether a single gene resulting from these crossover events is functional or not. It would appear that the phenotype of adults with the ζζ/ζ genotype is normal. Similarly, those with the ζζζ/ζζ genotype appear normal although detailed clinical, haematological and biochemical observations will be needed to clarify this point. Furthermore, it may be possible to predict the expression of the remaining ζ gene when the DNA sequence of the two genes and the precise points of crossover in these rearrangements have been established. We have not identified individuals with the ζ/ζ gene arrangement probably because of the relatively low prevalence of chromosomes with a single ζ gene. However, it is also possible that this genotype is not compatible with normal development. Regardless of whether or not these arrangements cause an alteration in phenotype, they are found linked to both normal and α thalassaemia haplotypes.

Recombination events between various structural globin genes within both the α and non-α globin gene complexes have been recognised by genetic studies and haemoglobin analysis and more recently by the application of recombinant DNA technology (17). In general, unequal crossing over takes place between highly homologous regions of DNA that have originated from a duplication event. Such regions are usually in close proximity to each other; 3-4 kb in the case of the α genes, 5 kb for the γ globin genes and 7 kb for the δ and β globin genes (2,7,20 and 24). Recombination between these regions may result in reciprocal arrangements, as described here, or in a gene conversion in which there is correction of duplicated sequences against each other without an alteration of the number of genes on either chromosome. It is thought that these mechanisms provide a way in which duplicated sequences may remain conserved during evolution (5,23). Crossover events between genes that are further apart in the gene cluster have also been characterised but appear to be less common. For example, one fusion haemoglobin (Hb Kenya) is the result of a crossover between the γ and β globin genes which are 21 kb apart: in this case the reciprocal -βγ- arrangement has not been identified (25). The two ζ globin genes are ∿ 11 kb apart and the finding

of 3 haplotypes, from a total of 710 studied, with a single ζ gene and 10, from 790, with the ζζζ arrangement is surprisingly high. It is not clear whether this is due to differing selective advantages of the various gene arrangements or if it reflects a high rate of DNA rearrangements in this region of the α complex. However, it does appear that these cases represent more than one type of recombination event. Therefore, it may be that, as in the case of the α genes, there is a relatively high rate of recombination between these genes which in turn may serve to maintain considerable homology between them. There are insufficient data at present to determine when the ζ gene duplicated and therefore it is not known to what extent these sequences have been conserved by this or any other mechanism.

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