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The Haptoglobin-Gene Deletion Responsible for Anhaptoglobinemia

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Summary

We have found an allelic deletion of the haptoglobin (Hp) gene from an individual with anhaptoglobinemia. The Hp gene cluster consists of coding regions of the α chain and β chain of the haptoglobin gene (*Hp*) and of the α chain and β chain of the haptoglobin-related gene (*Hpr*), in tandem from the 5′ side. Southern blot and PCR analyses have indicated that the individual with anhaptoglobinemia was homozygous for the gene deletion and that the gene deletion was included at least from the promoter region of *Hp* to *Hpr* α but not to *Hpr* β (Hp^{del}). In addition, we found seven individuals with hypohaptoglobinemia in three families, and the genotypes of six of the seven individuals were found to be Hp^2/Hp^{del}. The phenotypes and genotypes in one of these three families showed the father to be hypohaptoglobinemic (Hp2) and Hp^2/Hp^{del}, the mother to be Hp2-1 and Hp^1/Hp^2, one of the two children to be hypohaptoglobinemic (Hp2) and Hp^2/Hp^{del}, and the other child to be Hp1 and Hp^1/Hp^{del}, showing an anomalous inheritance of Hp phenotypes in the child with Hp1. The Hp^2/Hp^{del} individuals had an extremely low level of Hp (mean ± SD = 0.049 ± 0.043 mg/ml; $n = 6$), compared with the level (1.64 ± 1.07 mg/ml) obtained from 52 healthy volunteers having phenotype Hp2, whereas the serum Hp level of an individual with Hp^1/Hp^{del} was 0.50 mg/ml, which was approximately half the level of Hp in control sera from the Hp1 phenotype (1.26 ± 0.33 mg/ml; $n = 9$), showing a gene-dosage effect. The other allele (Hp^2) of individuals with Hp^2/Hp^{del} was found to have, in all exons, no mutation, by DNA sequencing. On the basis of the present study, the mechanism of anhaptoglobinemia and the mechanism of anomalous inheritance of Hp phenotypes were well explained. However, the mechanism of hypohaptoglobinemia remains unknown.

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Introduction

Haptoglobin (Hp), synthesized in the liver, is a hemoglobin-binding polymorphic plasma protein and is remarkable for being the first example in which both a polymorphism of human plasma proteins and a partial gene duplication have been substantiated by starch-gel electrophoresis, by amino acid analysis, and by DNA sequence analysis (Smithies and Walker 1955; Black and Dixon 1968; Maeda et al. 1984). In human populations, there are three common genetic Hp phenotypes—Hp1, Hp2, and Hp2-1—which are determined by a pair of codominant alleles— Hp^1 and Hp^2. The Hp protein is composed of two pairs of an α chain and a β chain, and the polymorphism of the protein reflects inherited variations in the Hp α-chain polypeptides (Bowman and Kurosky 1982). The α chain of the Hp2 protein is composed of 142 amino acid residues and is a product of a partial gene duplication of the Hp1 α chain, which has 83 residues. An inert allele, Hp^0, at the Hp locus has been postulated by studies on the anomalous inheritance of Hp phenotypes, and the homozygosity of Hp^0 has also been suggested as a cause for so-called anhaptoglobinemia (Harris et al. 1958; Matsunaga et al. 1970).

Maeda et al. (1984) demonstrated that the Hp2 protein bears a duplication of 59 α-chain amino acid residues corresponding to intragenic duplication of 1.7 kb occurring at precise intronic sites of Hp^1. In addition, an extra Hp gene, which shares a high degree of nucleotide-sequence homology with Hp^1, has been isolated (Maeda et al. 1984; Bensi et al. 1985; Maeda 1985). This gene has been designated as the "haptoglobin-related gene" ("*Hpr*"). *Hp* and *Hpr* are located within the 30-kb region in chromosome 16q22.3 (Maeda 1985), suggesting that *Hpr* has evolved by gene duplication and subsequent divergence.

Although certain pathological states, such as severe hemolysis and liver dysfunction, are known to lead to secondary anhaptoglobinemia (Rougemont et al. 1980), some previous reports have claimed that the hypo- or anhaptoglobinemia (Hp0 phenotype) observed in tropical countries has a genetic origin (Allison et al. 1958; Giblett and Steinberg 1960). However, it should be pointed out that, to date, a satisfactory complete genetic model has not yet been reported for the Hp0 phenotype.

Since no detectable change in the Hp gene clusters (including promoter region) of Hp0 individuals has been reported in previous studies (Hill et al. 1986; Maeda 1991), Maeda (1991) have speculated that the null expression of Hp is related to the *trans*-acting factors necessary for Hp expression, rather than to *cis*-acting promoter sequences.

In the present study, we found (1) an individual with the true anhaptoglobinemia phenotype and (2) that the homozygosity of a deletion in the Hp gene cluster (Hp^{del}) was responsible for the anhaptoglobinemia. The anomalous inheritance of Hp phenotypes was well explained by the presence of allele Hp^{del}, since, for a long time, hypohaptoglobinemia has been referred to simply as "anhaptoglobinemia."

Material and Methods

Determination of Hp Phenotype and Quantification of Serum Hp Level

Phenotypes were determined by mixing 20 μl serum with 10 μl hemolysate (~3% hemoglobin concentration), followed by electrophoresis in a 5% polyacrylamide gel (Linke 1984). The gel was soaked, for 15 min, in 40% acetic acid solution containing 0.4% leucomalachite green and then was stained with 3% hydrogen peroxide. Individuals with serum Hp below the limit of detection in this method were classified as an- or hypohaptoglobinemic (Hp0 phenotype). The serum Hp level was then measured by the enzyme-linked immunosorbent–assay (ELISA) method, by use of polyclonal anti-human Hp and alkaline phosphatase–conjugated anti-human IgG (EY Laboratories) and with purified Hp (Sigma) as the standard protein. An individual in whom ELISA showed complete absence of serum Hp was classified as anhaptoglobinemic, and individuals in whom ELISA showed a low but detectable level of serum Hp were classified as hypohaptoglobinemic. Since anhaptoglobinemia reported in previous studies may have included either or both anhaptoglobinemia and hypohaptoglobinemia, we have classified such phenotype as Hp0.

Southern Blot Analysis

The genotypes of *Hp* were determined as described by Maeda et al. (1984). Genomic DNA was isolated from peripheral leukocytes from an individual with true anhaptoglobinemia, from family members, including individuals with hypohaptoglobinemia, and from healthy volunteers, by the organic-solvent method (Sambrook et al. 1989). For Southern blot analysis, genomic DNA (2–5 μg) was separated in a 0.8% agarose gel after digestion with the appropriate restriction endonuclease, was transferred onto a nylon membrane, and was hybridized with the digoxigenin-labeled α chain–coding or β chain–coding region of the *Hp* cDNA probe (a kind gift from Université Libre de Bruxelles, Service de Génétique Appliquée) (Hyland 1988). To normalize the DNA quantity, the same membrane was rehybridized with the digoxigenin-labeled 0.5-kb catalytic domain of human H-type α(1,2)fucosyltransferase gene (*FUT1*) probe (Larsen et al. 1990; Koda et al. 1996; Wang et al. 1997). The digoxigenin-labeled DNA probe was prepared by use of a DIG DNA labeling kit (Boehringer Mannheim). Hybridization was performed overnight at 68°C with 500 mM phosphate buffer (pH 7.2) containing 7% SDS. The final wash was performed for 40 min at 68°C with 40 mM phosphate buffer (pH 7.2) and 1% SDS. The intensities of the hybridized bands of x-ray film were estimated by use of a densitometer (Shimadzu CS-930).

PCR Amplification of Hp *or* Hpr*, and DNA Sequencing*

Genomic DNA was subjected to PCR amplification (Saiki et al. 1988). Previously described primers (Maeda 1991) were used for amplification of the promoter region of *Hp* (30 cycles of 20 s at 94°C, 1 min at 55°C, and 1 min at 72°C).

To analyze Hp^2 of individuals ST and TN (of families T and N, respectively) with the Hp0 phenotype, all exons of Hp^2 were amplified by PCR and then were sequenced. Exon 1U (5′-GCA GTG TGA AAA TCC TCC AAG ATA A-3′; 683–707 bp) and exon 1L (5′-AAT TTA GCC CAT TTG CCC GTT TCT T-3′; 1,135–1,159 bp) primers or exon 5U (5′-ATT CTC AGA ACC AGA GGC AAA GAC C-3′; 4,286–4,310 bp) and exon 6L (5′-AGT GCC CTT CAG GCC CTA ATG AAC A-3′; 5,761–5,785 bp) primers were used for amplification of exon 1 or exons 5 and 6 (30 cycles of 10 s at 98°C, 1 min at 60°C, and 1 min 30 s at 72°C). To avoid the amplification of *Hpr,* whole Hp^2 was amplified by use of exon 1U and exon 7L (5′-CTG GAC CAT AAA GGA GCA AAT AAA A-3′; 7,473–7,497 bp) primers and a LA PCR kit (Takara) (30 cycles of 10 s at 98°C and 10 min at 68°C). Nested or seminested PCRs were then performed by use of exon 2U (5′-TAG CTT TCC ACT CCT CCT TGT CTT C-3′; 2,515–2,539 bp) and exon 4L (5′-CAG TGC CCT TCA GGC CCC AAT GAA C-3′; 4,047–4,071 bp) primers, for amplification of exons 2–4, or by exon 7U (5′-AGA TGG AAA GGC TCT TGC ACA TTT C-3′; 6,445–6,469 bp) and exon 7L primers, for amplification of exon 7, by use of 10,000-fold–diluted first PCR product as a template (30 cycles of 10 s at 98°C, 1 min at 60°C, and 1 min 30 s at 72°C). For amplification of exons 2–4 of *Hpr,* PCR was performed by use of *Hpr* exon 2U (5′-CAG CTT TCC GTT CCT CCT TGT TTT C-3′; 14,226–14,250 bp) and exon 4L (see above; 15,780–15,840 bp) (30 cycles of 10 s at 98°C, 1 min at

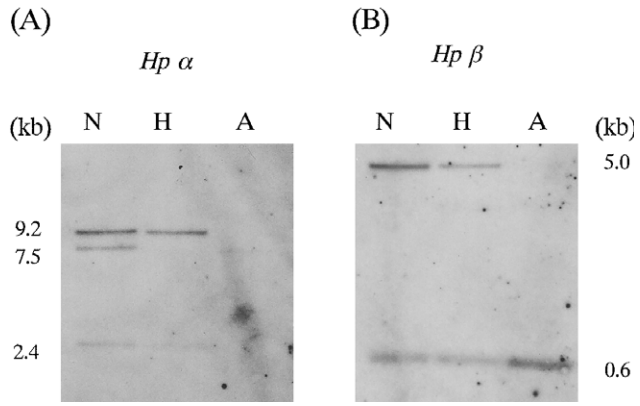

Figure 1 Southern blot analysis of genomic DNAs, using *Hp α* (*A*) or *Hp β* (*B*) cDNA probes. Genomic DNAs (2 μg) from an individual with anhaptoglobinemia (lanes A), an individual with hypohaptoglobinemia (lanes H), and a normal control individual (lanes N) were digested by *Bam*HI. The sizes (in kb) of hybridized fragments are indicated.

60°C, and 1 min 30 s at 72°C). For coamplification of exon 7 of *Hp* and of exon 5 of *Hpr,* PCR was performed by use of exon 7U and exon 7L primers. Numbering of the nucleotide sequence followed the notation of Maeda (1985). The resulting PCR products were subcloned into plasmid pGEM by use of a pGEM-T vector system I (Promega), and then the DNA sequence was determined by use of an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 373 sequencer (Applied Biosystems) (Koda et al. 1996, 1997).

Results

An Individual with Complete Anhaptoglobinemia

In the initial stage of this study, we investigated the Hp phenotypes of 9,711 unrelated individuals and found, by ELISA analysis, an individual with the complete absence of serum Hp (Yoshioka et al. 1991).

Characterization of the Hp *Gene Deletion*

We investigated the Hp gene cluster in an individual with anhaptoglobinemia, using Southern blot analysis with *Bam*HI-digested genomic DNA. As shown in lane A of figure 1*A,* no hybridized signal was detected in an individual who was supposed to be truly anhaptoglobinemic, when the *Hp α* cDNA probe was used, whereas only a 0.6-kb band corresponding to *Hpr*—but not a 5.0-kb band corresponding to *Hp*—was identified, when the *Hp β* cDNA probe was used (fig. 1*B,* lane A). This gene deletion was also identified by a failure in PCR amplification of *Hpr α,* whereas *Hpr β* was amplified in both normal control and anhaptoglobinemic individuals (data not shown). In addition, the promoter region of *Hp* was also not amplified in an anhaptoglobinemic individual (fig. 2, lane A). These results suggested that a homozygous gene deletion of the promoter region of *Hp, Hp α, Hp β,* and *Hpr α*—but not *Hpr β* (Hp^{del})—was responsible for the anhaptoglobinemia phenotype.

To identify the 3′ end of the gene deletion, Southern blot analysis was performed after digestion of genomic DNA from the individual with anhaptoglobinemia, by *Aat*I and the *Hp β* cDNA probe, since *Hpr β* appeared to be not deleted. As shown in figure 3, a >19.3-kb band, corresponding to *Hp β,* and a 4.5-kb band, corresponding to *Hpr β,* were found in a control individual (lane N), whereas only an unexpected band of 16.0 kb was observed in the individual with anhaptoglobinemia (lane A). Maeda (1985) reported that the endonuclease site for *Aat*I was present in the 5′ flanking region of *Hpr β* (intron 4). Although Hp^{del} appeared to be digested at a different site for *Aat*I, compared with *Hpr β* from the normal allele (figs. 3 and 4), the size of a *Bam*HI fragment of *Hpr β* was the same in both the normal and the Hp^{del} alleles. These results suggested that the 3′ end of the gene deletion occurred within the 284-bp stretch between the *Aat*I site within intron 4 and the *Bam*HI site in exon 5, of *Hpr* (fig. 4*A*). Furthermore, *Hpr β* was amplified by PCR in the anhaptoglobinemic individual (data not shown). Since the 5′ PCR primer was ~200 bp downstream from the *Aat*I site, the 3′ end of the gene deletion was within this region.

Screening and Identification of Families with Hypohaptoglobinemia

We investigated the Hp phenotypes of 300 undergraduate students in Kurume University and found three hypohaptoglobinemic individuals (ST, TN, and HH) (fig.

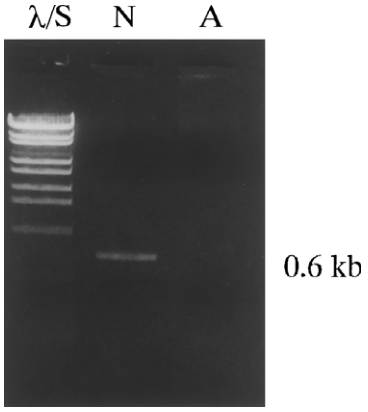

Figure 2 PCR amplification of promoter region of *Hp.* PCR products underwent electrophoresis in a 1.2% agarose gel and were stained by ethidium bromide. The size of the PCR product is indicated. *Sty*I-digested lambda DNA (lane λ/S) was used as a molecular-size marker.

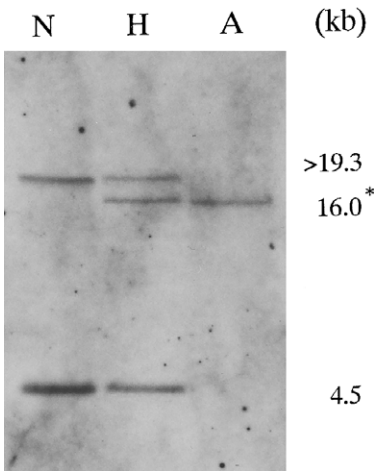

Figure 3 Southern blot analysis of genomic DNAs, using *Hp* β cDNA probe. Genomic DNAs (2 μg) from an individual with anhaptoglobinemia (lane A), an individual with hypohaptoglobinemia (lane H), and a normal control individual (lane N) were digested by *Aat*I. The band indicated by an asterisk (*) is unexpected. The sizes (in kb) of the hybridized fragments are indicated.

5). We then investigated the phenotypes of Hp and measured the serum Hp level in all the members of these three families, after obtaining fully informed consent. In two families (T and N), other individuals with the hypohaptoglobinemia phenotype were detected, whereas, in the third family (H), both the father and the mother had two normal alleles with the phenotype Hp2-1. The phenotypes in families T, N, and H were as follows: in family T, the father had the hypohaptoglobinemia phenotype (Hp2), the mother had Hp2-1, one of the two children (ST) had hypohaptoglobinemia (Hp2), and the other child had Hp1; in family N, the father had hypohaptoglobinemia (Hp2), the mother had Hp2, and the three children all had hypohaptoglobinemia (Hp2); and, in family H, both the father and the mother were Hp2-1, one of the two children (HH) had hypohaptoglobinemia (Hp2), and the other child had Hp2 (fig. 5). All the other genetic markers that were investigated, such as multilocus DNA polymorphism (with oligonucleotide $[CAC]_5$ used as a probe) (Pena et al. 1991) and the D1S80 (pMCT118) locus (Kasai et al. 1990), demonstrated that within each family the paternity and maternity matched (data not shown). Thus, one child with phenotype Hp1, in family T, showed an anomalous inheritance of the Hp phenotype.

The Hemizygous Genotype Hp^2/Hp^{del}*—but Not* Hp^1/Hp^{del}*—as a Possible Cause of Familial Hypohaptoglobinemia*

Since an anomalous inheritance of Hp phenotype was observed in family T, semiquantitative Southern blot

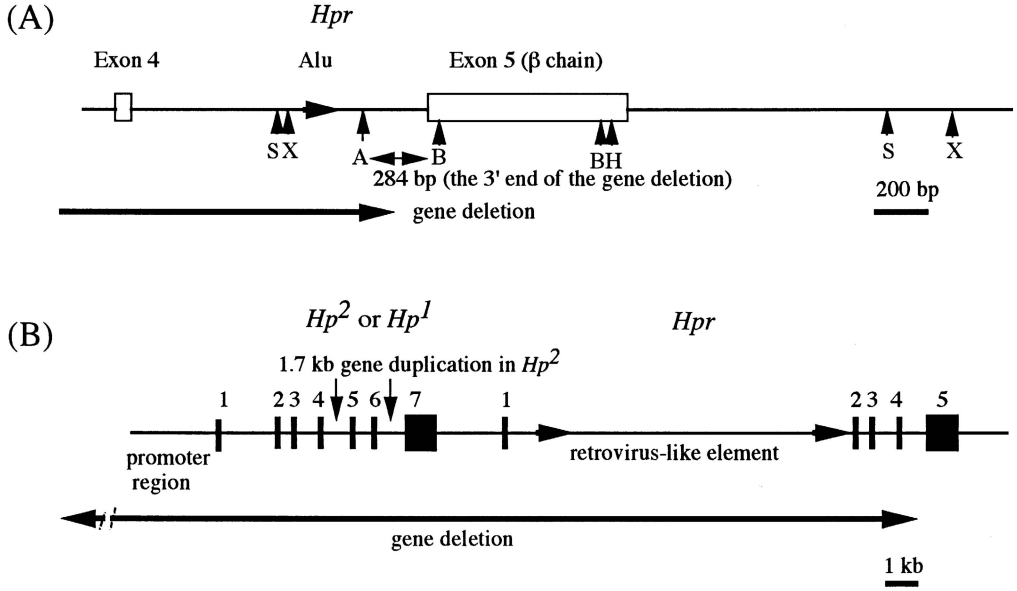

Figure 4 Restriction-endonuclease maps of *Hpr* gene (panel *A*) and of *Hp* gene cluster (panel *B*). In panel *A*, sites for restriction endonucleases (*vertical arrowheads*), coding regions of exons 4 and 5 of *Hpr* (*rectangles*), and an *Alu* sequence in intron 4 of *Hpr* (*horizontal arrow*) are indicated . A = *Aat*I; B = *Bam*HI; H = *Hin*dIII; S = *Sac*I; and X = *Xba*I. The expected position of the 3′ end of the gene deletion in *Hpr* in Hp^{del} is located within the 284 bp between the *Aat*I site in intron 4 and the *Bam*HI site in exon 5 of the *Hpr* gene. In panel *B*, the expected gene deletion in the *Hp* gene cluster is shown (*double-headed horizontal arrow*). The numbers in the *Hp* gene cluster denote the exons of *Hp* and *Hpr*. Scale bars are given in the lower-right corners of panels *A* and *B*.

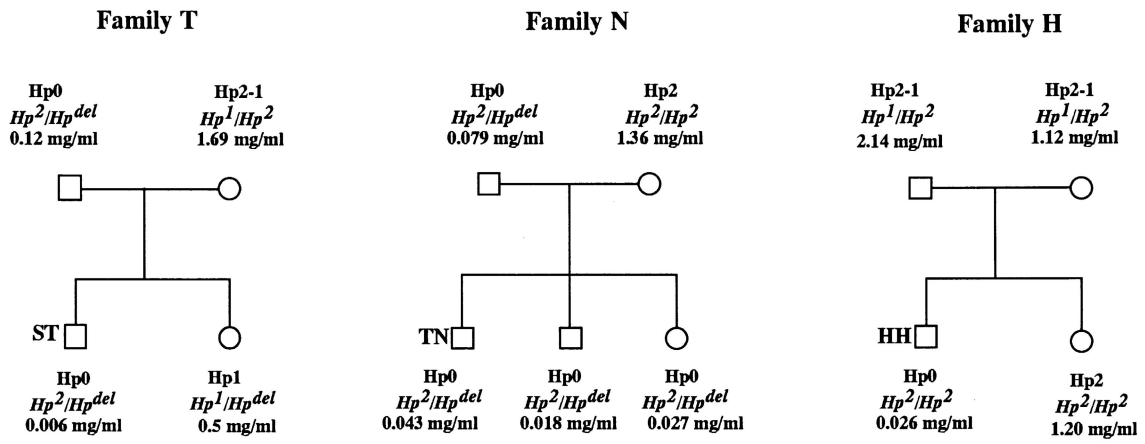

Figure 5 Summary of three families with hypohaptoglobinemic individuals. The phenotypes and genotypes of Hp, as well as the plasma Hp levels, of all family members are indicated. The propositi—ST, TN, and HH—in the three families are indicated.

analysis was performed to investigate the possibility of an *Hp* deletion. Genomic DNA from each individual in family T and from a normal control individual was digested with restriction enzyme *Bam*HI. After separation by electrophoresis in a 0.8% agarose gel and blotting onto a nylon membrane, the membrane was hybridized with *Hp* α cDNA probe and then was rehybridized with *Hp* β cDNA probe and *FUT1* probe, sequentially. As shown in figure 6, *Hp* α of the father (F) and of one of the children (C1) showed a single band indicating Hp^2, the other child (C2) showed a single band demonstrating Hp^1, and the mother (M) and a control individual (N) showed double bands of Hp^1/Hp^2. The ratios of *Hp* α, *Hpr* α, or *Hp* β to *FUT1* of the father and two children were approximately half those of the control individual, whereas those of the mother were similar to those of the control individual. However, the ratio of *Hpr* β to *FUT1* in all members of family T was similar to that of the control individual. The same results were obtained for family N. These results suggested that a hemizygous gene deletion was present in the father and two children in family T and in the father and all three children in family N. However, the deletion of the Hp gene cluster was not found in the one child in family H who had hypohaptoglobinemia (HH). When Southern blot analysis was performed after digestion with endonuclease *Sac*I of genomic DNA from each individual in family T, a single band of *Hp* α was obtained, irrespective of the Hp phenotype, and the ratios of *Hp* α to *FUT1*, of the father, the two children, and the mother, compared with those in the control individual, were 0.6, 0.6 and 0.4, and 1, respectively (data not shown), indicating a hemizygous gene deletion also in the Hp gene cluster of the father and two children. As shown in figure 3, the two bands corresponding to *Hp* β and *Hpr* β, as well as the 16.0-kb unexpected band, were found in an individual with hypohaptoglobinemia (lane H), indicating that this individual was heterozygous for Hp^2 and Hp^{del}. In addition, when genomic DNA was digested by *Sac*I, by *Xba*I, and by *Hin*dIII, only an unexpected band, at 7.5 kb, 6.5 kb, and 4.4 kb, respectively, was found in the individual with anhaptoglobinemia, and an unexpected band of similar size, plus bands corresponding to *Hp* β and *Hpr* β, were detected in individuals with hypohaptoglobinemia, by use of the *Hp* β cDNA probe (data not shown).

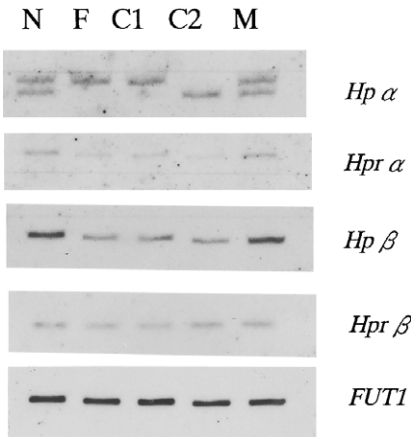

Figure 6 Semiquantitative Southern blot analysis of genomic DNAs, using *Hp* α cDNA, *Hp* β cDNA, or *FUT1* as probe. Genomic DNAs (5 μg) from individuals of family T (F, the father with hypohaptoglobinemia [Hp2]; C1, the elder child with hypohaptoglobinemia [Hp2]; C2, the other child, with Hp1; and M, the mother, with Hp2-1) and from a normal control individual (N) with Hp2-1 were digested by *Bam*HI. Coding regions of the α chains of both *Hp* and *Hpr* were visualized by use of the *Hp* α cDNA probe, and those of the β chains of both *Hp* and *Hpr* were visualized by use of the *Hp* β cDNA probe. The band *FUT1* was visualized by use of the *FUT1* probe. The intensity of the hybridized bands was determined by use of a densitometer.

We also obtained three bands from the genomic DNA of the one child (C2) with Hp1, in family T, and from that of each individual with hypohaptoglobinemia, except for child HH, whose gene deletion was not detected. In addition, we found no unexpected 4.4-kb *Hin*dIII band by use of Southern blot analysis in 95 healthy individuals. These findings clearly demonstrated that the same gene deletion found in the individual with anhaptoglobinemia was present in all members of families T and N who had hypohaptoglobinemia and in the one member of family T who had Hp1. Although we found the same deletion of *Hp* in two families, relatedness between the fathers of families T and N was not established.

To examine the other allele (i.e., Hp^2) of individuals with hypohaptoglobinemia, we isolated all the exons and exon/intron junctions of Hp^2 from individuals ST and TN (of families T and N, respectively), using PCR amplification. We found no mutation in these regions, using DNA sequence analysis.

The six individuals with Hp^2/Hp^{del} had an extremely low level of Hp (0.049 ± 0.043 mg/ml), compared with the level (1.64 ± 1.07 mg/ml) obtained from 52 healthy volunteers having phenotype Hp2 and genotype Hp^2, whereas the serum Hp level of the child (C2) with Hp^1/Hp^{del} in family T was 0.50 mg/ml, which was approximately half the level of Hp in the control sera from the Hp1 phenotype (1.26 ± 0.33 mg/ml; $n = 9$), showing a gene-dosage effect (fig. 5).

Discussion

We have described here an allelic deletion in *Hp* (Hp^{del}), and the homozygous gene deletion has been shown to be associated with the complete absence of serum Hp, whereas Hp^2/Hp^{del}—but not Hp^1/Hp^{del}—has been shown to be associated with hypohaptoglobinemia. Since the complete absence of the Hp gene and protein was identified in the individual in this study who had true anhaptoglobinemia, it seems that the Hp protein is not essential for human survival. The gene deletion occurred from at least the promoter region of *Hp* to the 5′ flanking region of *Hpr β*. The 3′ end of the gene deletion was present within a region ~200 bp downstream from the *Aat*I site of intron 4 in *Hpr*. However, we could not determine the position of the 5′ end of the gene deletion. On the basis of a map of the Hp gene cluster (Maeda 1985), the size of the gene deletion was calculated to be >20 kb (fig. 4*B*).

The anomalous inheritance of Hp phenotypes in families having one or more members with Hp0 has been known for a long time (Harris et al. 1958; Matsunaga et al. 1970), and a silent allele, Hp^0, has been thought to be responsible for the Hp0 phenotype and for the mode of inheritance of Hp in these families. It has been reported that individuals in previous studies who had Hp0—and who were probably hypohaptoglobinemic—were always associated with Hp2 phenotype, and anomalous inheritance has been demonstrated in individuals with the Hp1 phenotype (Harris et al. 1958; Matsunaga et al. 1970). We also found a similar family—that is, family T—in which both the father (F) and the elder child (C1) were hypohaptoglobinemic (Hp2) and in which the other child (C2), who had Hp1, showed an anomalous inheritance of the Hp phenotype, since the mother (M) had Hp1 and Hp2. The allelic deletion of *Hp* (Hp^{del}) found in the present study can explain the anomalous inheritance observed in the second child, C2. Maeda (1991), however, reported the existence of Hp0 in three African American individuals—one having Hp^2 and the other two having Hp^1—and in two individuals with Hp^2 who were not African American, with no change in the Hp gene clusters of any of these five Hp0 individuals, using Southern blotting. Since Hp0 in African American individuals is known to be frequently due to secondary anhaptoglobinemia (Allison et al. 1958; Giblett and Steinberg 1960; Rougemont et al. 1980), further study is required in two African American individuals with Hp0 associated with Hp^1. Azevedo et al. (1969) reported no Hp1 × Hp1 matings in 29 families, in northeastern Brazil, having one or more individuals with Hp0.

The Hp gene cluster is located in chromosome 16q22.3, which is well known to contain inheritable fragile sites (Shabtai et al. 1983; Sutherland et al. 1984). The loss of the heterozygosity of *Hp* has been observed frequently during carcinoma progression (Tsuda et al. 1990). A crossover event has produced a gene duplication, resulting in the generation of *Hp* and *Hpr* in tandem (Maeda et al. 1984; Maeda 1985). Moreover, Hp^2 has been generated by a nonhomologous crossover within different introns of two Hp^1 alleles (Black and Dixon 1968; Maeda et al. 1984; Maeda 1985). Furthermore, Maeda et al. (1986) found polymorphisms for many tandemly arranged *Hpr*'s in the Hp gene cluster, formed by gene duplication by homologous but unequal crossover in some African American individuals, and suggested that the tandemly arranged *Hpr* was linked to Hp^2. In addition, a rare phenotype, Hp Johnson, has been demonstrated to be a threefold tandem repeat of the same 1.7-kb DNA segment implicated in Hp^2 duplication (Oliviero et al. 1985). In the present study, we have found that the phenotype hypohaptoglobinemia is associated with Hp^2, in the presence of Hp^{del}. Therefore, these other findings concerning Hp^2, together with our findings, suggest that the chromosome region containing the Hp gene cluster is especially prone to genomic DNA rearrangement (Shabtai et al. 1983; Sutherland et al. 1984).

In the present study, we have detected both the ho-

mozygous gene deletion of the Hp gene cluster, in the individual with anhaptoglobinemia, and the heterozygous gene deletion, in individuals with hypohaptoglobinemia. Since no detectable change in the Hp gene clusters (including the promoter region) of Hp0 individuals in previous studies (Hill et al. 1986; Maeda 1991) and of one hypohaptoglobinemia individual (HH) in the present study has been identified by use of Southern blot analysis, the cause of hypohaptoglobinemia is not simple. However, this is the first report to propose a genetic model for anhaptoglobinemia and the anomalous inheritance of Hp phenotypes, although the precise mechanism of hypohaptoglobinemia associated with Hp^{2}/Hp^{del} remains unknown.

Acknowledgments

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