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Characterization of genomic rearrangements of the α_1-acid glycoprotein/orosomucoid gene in Ghanaians

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Abstract In this study, the structure of the α_1-acid glycoprotein (*AGP*), or orosomucoid (*ORM*), gene was investigated in a Ghanaian mother and her child, who shared an unusual variant, *ORM1 S2(C)*, found by isoelectric focusing. Three remarkable changes of nucleotide sequence were observed: (1) The two ORM1 alleles, *ORM1*S* and *ORM1*S2(C)*, had the *AGP2* gene-specific sequence at one and three regions, respectively, in exon 5 to intron 5. The variant allele originating from *ORM1*S* was characterized by a G-to-A transition, resulting in an amino acid change from valine to methionine, which is also detected in *ORM1 F2*, a form that is common in Europeans. (2) The *AGP2* gene of the child, inherited from the father, was duplicated, as revealed by long-range polymerase chain reaction. (3) Three new mutations were observed in two exons of the *AGP2* genes of the mother and child. All of these novel genomic rearrangements, which were not observed in Japanese subjects, may have arisen through point mutation, gene conversion, and unequal crossover events. It is likely that the rearrangement of the *AGP* gene has often occurred in Africans.

Key words α_1-Acid glycoprotein · Gene conversion · Gene triplication · Orosomucoid · Single-nucleotide polymorphisms

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Introduction

The human α_1-acid glycoprotein (AGP), or orosomucoid (ORM), is a highly glycosylated plasma protein, consisting of a single polypeptide chain and five carbohydrate chains, giving an overall molecular weight of about 40,000. It is a typical acute phase protein and has important immunomodulatory functions. Its expression in hepatocytes is stimulated by glucocorticoids and induced by the key cytokines, interleukin (IL)-1β, tumor necrosis factor-α, and IL-6 (Bowman 1993; Baumann and Gauldie 1994; Fournier et al. 2000). Another interesting feature of this protein is that it binds and carries basic drugs. The ORM protein, as well as the α_1-microglobulin and retinol-binding protein, belongs to the lipocalin superfamily (Pervaiz and Brew 1987; Kremer et al. 1988; Xu and Venge 2000).

The ORM protein is basically controlled by two closely linked loci, ORM1 and ORM2 (MIM Entry: 138600 and 138610; this study follows conventional nomenclatures: ORM is used for protein loci and alleles, and AGP is used for genes and gene polymorphisms) and is present as a mixture of products of the two structural loci (Yuasa et al. 1986; Escallon et al. 1987). The ORM1 and ORM2 proteins are encoded by the *AGP1* and *AGP2* genes, respectively, tandemly arranged on chromosome 9q34.1–34.3. The *AGP2* gene is located about 3.3 kb downstream of the *AGP1* gene. When digested with *Hin*dIII, the *AGP1* gene is contained in a 4.6-kb-long fragment and the *AGP2* gene in a 6.5-kb-long fragment. The *AGP1* and *AGP2* genes are similarly organized with six exons and five introns. The *AGP1* and *AGP2* genes are highly homologous, with 94% sequence identity in the coding regions, and are derived by duplication from a common ancestral gene (Dente et al. 1985, 1987; Merritt and Board 1988; Merritt et al. 1990).

The occurrence of gene duplication provides a good opportunity for gene conversion and unequal crossover between two tandemly arranged genes. A tandemly triplicated *AGP* gene with two *AGP2* genes was cloned from a cosmid library (Dente et al. 1987). Recently we developed a polymerase chain reaction (PCR)-based technique to demon-

strate further duplication in the *AGP* gene. The duplication of the *AGP1* gene occurs at a frequency as high as 20% in the Japanese population (Nakamura et al. 2000b). The second *AGP1* (*AGP1B*) and *AGP2* (*AGP2B*) genes were shown to arise from unequal crossover events and to be contained in a 6.5-kb fragment after digestion with *Hin*dIII (Dente et al. 1987; Nakamura et al. 2000b).

The ORM loci are highly polymorphic and a number of common and variant alleles have been identified in various populations. The two common alleles, *ORM1*F1* and *ORM1*S*, are distributed worldwide, but *ORM1*F2* is limited to Europeans and West Asians (Yuasa et al. 1986, 1993; Umetsu et al. 1989). The molecular basis of these three alleles was determined by gene-specific amplification followed by sequencing. They arise from two point mutations occurring at the codons for amino acid positions 20 in exon 1 and 156 in exon 5 of the *AGP1* gene: *ORM1*F1* was characterized by CAG (Gln) and GTG (Val), *ORM1*F2* by CAG (Gln) and ATG (Met), and *ORM1*S* by CGG (Arg) and GTG (Val). *ORM1*F2* evolved from *ORM1*S* through two mutational events. *ORM2*M*, the most common allele at the ORM2 locus, has CGG (Arg) and ATG (Met) (Yuasa et al. 1997). With regard to African populations, only an isoelectric focusing study of African-Americans has been performed, and two ORM1 and four ORM2 alleles have been detected (Escallon et al. 1987). Recently we encountered an unusual ORM1 variant after isoelectric focusing was performed on serum samples from a Ghanaian mother and her child in a case of disputed paternity. In the present study, we investigated the structure of their *AGP* genes, in which novel genomic rearrangements arising through point mutation, gene conversion, and unequal crossover events were observed.

Materials and methods

Blood samples

Blood samples were obtained from a Ghanaian mother and her child living in Germany. A biological sample from the true father was unavailable, after an alleged father was excluded from paternity. Genomic DNA was extracted by a standard method. DNA samples from Japanese subjects were also obtained to compare nucleotide sequences.

Isoelectric focusing and immunodetection

ORM phenotyping of desialylated serum samples was performed by three isoelectic focusing techniques. Method A was performed in the pH gradient established with a 3:1 mixture of Pharmalytes pH 4.0–6.5 and pH 4.5–5.4 (Pharmacia, Uppsala, Sweden) in the absence of Triton X-100. Method B was performed in a gel prepared with Pharmalyte pH 4.5–5.4 in the presence of 0.2% Triton X-100. In Method C, desialylated samples were alkylated with iodoacetamide and subjected to isoelectric focusing in the presence of 8 M urea. The banding patterns were visualized by immunoprinting and immunoblotting (Yuasa et al. 1993; Rocha et al. 1993; Dülmer et al. 1998).

Long-range PCR

To amplify the intergenic regions, we designed two forward primers, AGP1-E4K and AGP2-E4K, on a divergent region at the 3′ side of exon 4 in the *AGP1* and *AGP2* genes, and two reverse primers, AGP1-L4R and AGP2-L4R, on another divergent region at the 5′ side of exon 4 in the two genes. Four pairs of primer sets were combined (Nakamura et al. 2000b). The long-range PCR was performed using TaKaRa LA Taq polymerase (Takara Shuzo, Otsu, Japan), instead of TaKaRa Ex Taq polymerase. The DNA fragment was amplified in a volume of 25 μl containing about 50 ng genomic DNA, 20 pmol of each primer, 400 μM of each dNTP, 2.5 mM $MgCl_2$, and 1.25 U TaKaRa LA Taq in 1× reaction buffer provided by the manufacturer. Cycle conditions were 94°C for 4 min, then 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 5 min with a final extension step of 7 min at 72°C, in a Robocycler Gradient 96 (Stratagene, La Jolla, CA, USA). The PCR products are referred to as the p11, p12, p21, and p22 products (Nakamura et al. 2000b). Subsequently, the products were digested with *Bam*HI, *Eco*RI, *Hin*dIII, and *Sph*I (New England Biolabs, Beverly, MA, USA) according to the supplier's instructions. To determine haplotypes, the p12 and p22 products from the child were digested with *Sph*I and then different fragment lengths were excised from 2% agarose gel followed by further purification using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Gemany).

Nested PCR of exons and introns

Gene-specific amplification of exons 1–3 and 4–6, and the nested PCR of six exons were performed as described previously (Yuasa et al. 1997; Nakamura et al. 2000a, 2000b). In this study, the *AGP1* and *AGP2* gene-specific products are referred to as the p1S and p2S products, respectively. A partial fragment of exon 5 (84 bp) was obtained using two primers: AGP1-G5F, 5′-GCTCTCGACTGCTTGCGC-3′ on exon 5 and AGP-G5R, 5′-GGGCAATGTCCAATC CCTT-3′ on intron 5. To amplify a complete fragment of intron 5 (about 1.14 kb), two primers were used: AGP1-G5F and AGP-Y5R, 5′-CTTCTCGTGCTGCTTCTCC-3′ on exon 6.

Analysis of nested PCR products

Restriction-fragment-length polymorphism analysis of nested PCR products was performed using *Hsp*92II, *Bsr*SI (Promega, Madison, WI, USA), and *Rsa*I (New England Biolabs) according to the instructions of the suppliers. Single-strand conformation polymorphism (SSCP) analysis and direct sequencing have also been described (Yuasa et al. 1997; Nakamura et al. 2000a, 2000b). The nucleotide

numbering was usually according to Merritt and Board (1988).

Results

Characterization of a Ghanaian variant by isoelectric focusing

Figure 1 depicts the human ORM patterns after separation of the same set of desialylated serum samples under the conditions described earlier. A new variant shared by the Ghanaian mother and child had a relatively high intensity and was suggested to be a product of the ORM1 locus. The phenotypes of the mother and child were ORM1 S-Variant/ORM2 M and ORM1 F1-Variant/ORM2 M, respectively. In Fig. 1a, the Ghanaian variant was located at the same position as the ORM2 M band. The distance between the variant and the ORM1 S bands was similar to that between the ORM1 F1 and ORM1 F2 bands. In Fig. 1b, the variant band was slightly anodal to the ORM1 S band, as was the ORM1 F2 band to the ORM1 F1 band. In addition, the ORM2 M band from the mother appeared slightly broad, although the band was never divided into two bands. In Fig. 1c, the variant was shifted cathodally, like the ORM1 F2 and ORM2 M bands. Thus, the Ghanaian variant behaved similarly to ORM1 F2 under the three different conditions of isoelectric focusing.

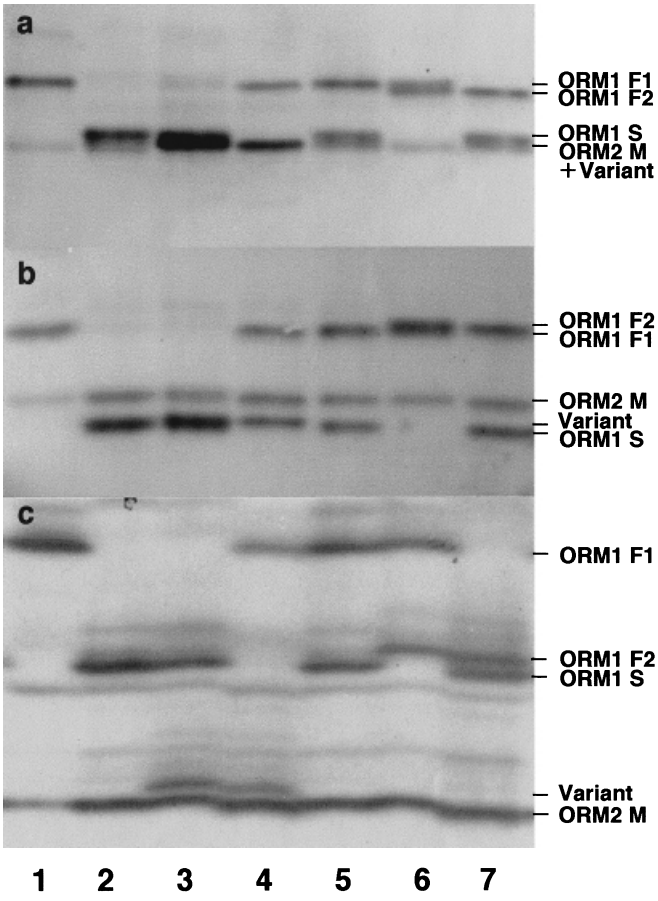

Fig. 1a–c. Banding patterns of desialylated orosomucoid (ORM) proteins after isoelectric focusing under three different conditions: **a** pH 4.0–6.5 in the absence of detergent, **b** pH 4.5–5.4 in the presence of 0.2% Triton X-100, and **c** pH 3.5–6.0 in the presence of 8 M urea after alkylation. Anode at *top*. *Lane 1*, ORM1 F1 (control); *lane 2*, S (control); *lane 3*, S-Variant (mother); *lane 4*, F1-Variant (child); *lane 5*, ORM1 F1-S (control); *lane 6*, F1-F2 (control); *lane 7*, F2-S (control). All of the samples are ORM2 M

Amplification of the intergenic regions

Figure 2 shows the electrophoretic patterns of products obtained by the long-range PCR with the four primer sets. When a primer set of AGP1-E4K and AGP2-L4R was used, a PCR product (p12) was obtained in samples from both the mother and child. These results were consistent with the tandem arrangement of the *AGP1* and *AGP2* genes in order from 5′ to 3′. With the primer set AGP2-E4K and AGP2-L4R, a band (p22) was observed only in the child. The positive reactions suggested duplications of the *AGP2* gene, *AGP2A* and *AGP2B* (Fig. 3). The p12 and p22 products were about the same as the 6.5-kb-long p11 and p12 products observed in Japanese subjects with duplicated *AGP1* genes (Nakamura et al. 2000b). When the other sets of primers were used, no products were detected. Neither the tandem duplications of the two *AGP1* genes nor those of the *AGP2* and *AGP1* genes occurred in the mother or child.

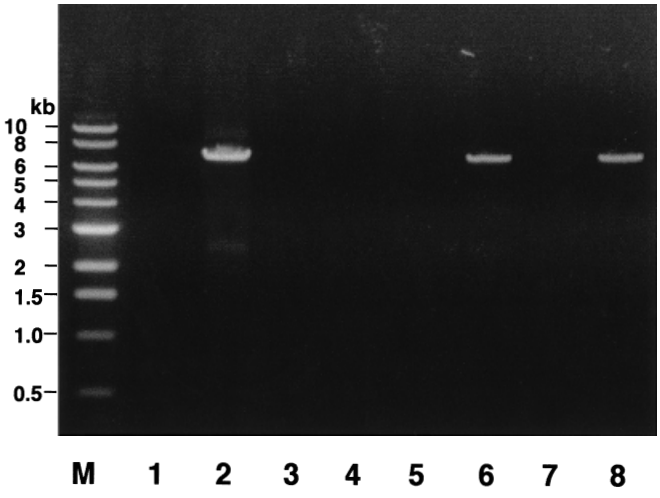

Fig. 2. Long-range polymerase chain reaction (PCR) of DNA samples from the mother (*lanes 1–4*) and child (*lanes 5–8*). Four primer sets of α_1-acid glycoprotein (AGP)1-E4K/AGP1-L4R (*lanes 1* and *5*), AGP1-E4K/AGP2-L4R (*lanes 2* and *6*), AGP2-E4K/AGP1-L4R (*lanes 3* and *7*), and AGP2-E4K/AGP2-L4R (*lanes 4* and *8*) were used for amplification. *M* indicates a 1000-bp ladder marker (New England Biolabs, Beverly, MA, USA). Ten microliters of each PCR product was separated by electrophoresis on a 0.8% agarose gel. The PCR products are located between 6.0- and 8.0-kb markers

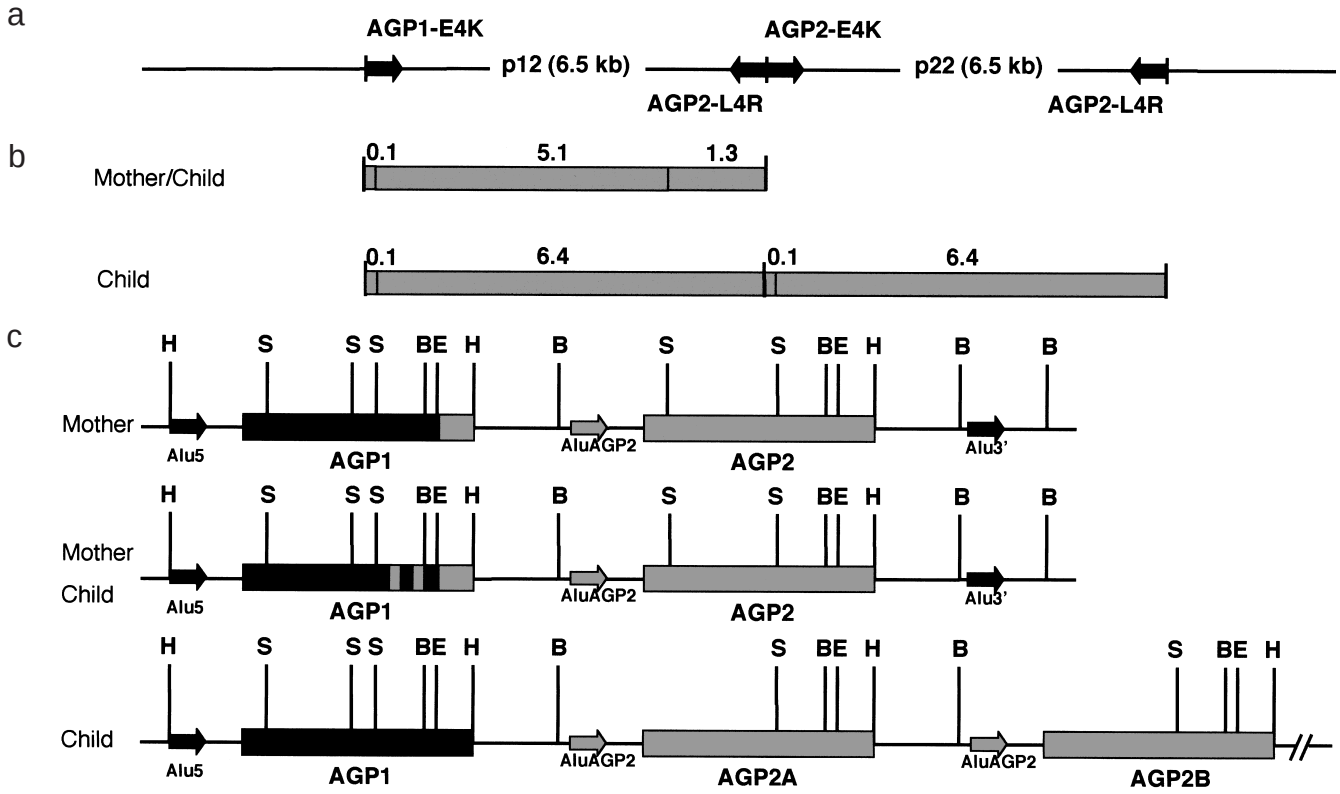

Fig. 3. **a** Location of primers for detection of triplicated genes and resultant PCR products. **b** Fragments obtained by *Sph*I digestion of the p12 and p22 products from the mother and child. **c** Restriction maps of *AGP* genes from the mother and child. *B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII; *S*, *Sph*I. The *AGP1* gene shared by the mother and child showed the *AGP2* gene-specific sequences at three regions from exon 5 to exon 6

Mapping of the PCR products with restriction endonucleases

The three products (p12 and p22) amplified by the long-range PCR of DNA from mother and child were mapped with four enzymes, including *Bam*HI, *Eco*RI, *Hin*dIII, and *Sph*I (Fig. 4). After digestion with *Bam*HI, *Eco*RI, and *Hin*dIII, the fragments of the p12 and p22 products were the same size. When cleaved with *Sph*I, the digested fragments of the p12 product from the mother were identical to those from Japanese subjects (Nakamura et al. 2000b). However, in the digested fragments from the p12 and p22 products of the child, an approximate 6.4-kb-long fragment was observed, which must have been inherited from the true father. The difference in size between the *Sph*I fragments arose from the presence or absence of an *Sph*I site in intron 1, which is polymorphic at nucleotide (nt) 2006, as described previously (Nakamura et al. 2000b). The mother was homozygous for the *Sph*I restriction site in the p12 products, i.e. in intron 1 of the *AGP2* gene, whereas the child was heterozygous in the p12 products and hemizygous in the p22 product (Fig. 3). The mapping results were consistent with the structure of the *AGP* genes described previously (Dente et al. 1987; Merritt and Board 1988; Nakamura et al. 2000b). The p12 products contained exons 5–6 of the *AGP1* gene, a spacer region, and exons 1–3 of the *AGP2* or *AGP2A* genes, and the p22 products contained exons 5–6 of the *AGP2A* gene, a spacer region, and exons 1–3 of the *AGP2B* gene. The *AGP2A* and *AGP2B* genes, like the *AGP1B* gene, were deduced to span about 6.5kb between the two *Hin*dIII sites (Fig. 3).

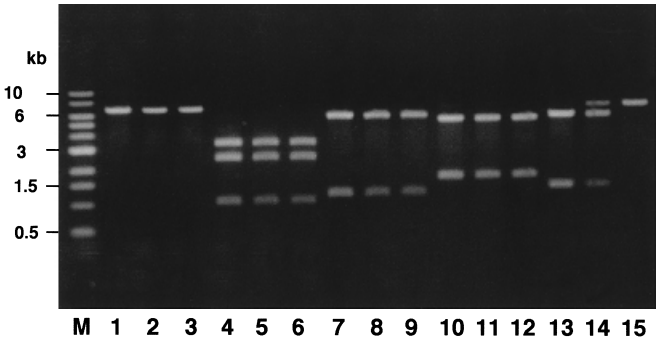

Fig. 4. Digestion of the PCR products with four restriction endonucleases. *Lanes 1*, *4*, *7*, *10*, and *13* were from the p12 products obtained using the primer set of AGP1-E4K/AGP2-L4R (corresponding to *lane 2* of Fig. 2). *Lanes 2*, *5*, *8*, *11*, and *14* were from the p12 products of AGP1-E4K/AGP2-L4R (corresponding to *lane 6* of Fig. 2). *Lanes 3*, *6*, *9*, *12*, and *15* were from the p22 products of AGP2-E4K/AGP2-L4R (corresponding to *lane 8* of Fig. 2). *Lanes 1–3*, undigested controls; *lanes 4–6*, *Bam*HI; *lanes 7–9*, *Eco*RI; *lanes 10–12*, *Hin*dIII; *lanes 13–15*, *Sph*I. *M* indicates a 1000-bp ladder marker. Five microliters of each PCR product was digested with enzymes and thereafter separated by electrophoresis on a 0.8% agarose gel

Table 1. Comparison of nucleotide sequences in exon 5 to intron 5 of the human *AGP* genes

	Exon 5 (E5)				Intron 5 (I5)																															
Genes	3594	3604	3615	3626	3657	3711	3826	3858	3870	3927	3976	3988	3989	4102	4153	4174	4281	4298	4300	4320	4324	4325	4329	4359	4381	4401	4472	4515	4517	4524	4528	4533	4549	4558	4573	4708
AGP1 (p1S)																																				
Japanese	C	A	G	T	g	g	a/g	a/g	a/g	g	-	c	a/g	t	c	-	t/c	t	g	cc	t/c	g	t	a	c	c	g	t	g	c	a	a	a	a	t	t
Mother	C	A	G/*A*	T/*C*	g	g	g	g	g	c	-	c	a	t	c	-	t	t/*g*	g/*a*	cc/*c-*	c	g/*c*	t/*c*	a	c	c	g	*c*	*t*	*a*	*t*	*t*	*g*	*g*	*g*	*c*
Child	C	A	G/*A*	T/*C*	g	g	g	g	g	g/c	-	c	a/g	t	c	-	t	t/*g*	g/*a*	cc/*c-*	c	g/*c*	t/*c*	a	c	c	g	t/*c*	g/*t*	c/*a*	a/*t*	a/*t*	a/*g*	a/*g*	t/*g*	t/*c*
p12(5.1 kb)	C	A	*A*	*C*	g	g	g	g	g	c	-	c	a	t	c	-	t	*g*	*a*	*c-*	c	*c*	*c*	a	c	c	g	*c*	*t*	*a*	*t*	*t*	*g*	*g*	*g*	*c*
p12(6.4 kb)	C	A	G	T	g	g	g	g	g	g	-	c	g	t	c	-	t	t	g	cc	c	g	t	a	c	c	g	t	g	c	a	a	a	a	t	t
AGP2 (p2S)																																				
Mother	*T*	*G*	*A*	T	g	g	g	g	g	g/c	-	c/t	g	*g*	*t*	*t*	c/t	*g*	*a*	*c-*	c	*c*	*c*	*g*	*t*	*t*/c	g	*c*	*t*	*a*	*t*	*t*	*g*	*g*	*g*	*c*
Child	*T*	*G*	*A*	*C*/T	g	*a*/g	g	g	g	g	-	c/t	g	*g*	*t*	*t*	c/t	*g*	*a*	*c-*	c	*c*	*c*	*g*	*t*	*t*	g/a	*c*	*t*	*a*	*t*	*t*	*g*	*g*	*g*	*c*
p22(6.4 kb)	*T*	*G*	*A*	*C*	g	*a*	g	g	g	g	-	c	g	*g*	*t*	*t*	c	*g*	*a*	*c-*	c	*c*	*c*	*g*	*t*	*t*	a	*c*	*t*	*a*	*t*	*t*	*g*	*g*	*g*	*c*
Japanese	T	G	A	C/T	g	a	g	g	g	g	-	c	g	g	t	t	c	g	a	c-	c	c	c	g	t	t	g	c	t	a	t	t	g	g	t/g	c
Merritt and Board[a]	T	G	A	C	t	a	g	g	g	g	a	c	g	g	t	t	c	g	a	c-	c	c	c	g	t	t	g	c	t	a	t	t	g	g	t	c

The *AGP1*- and *AGP2*-specific sequences in the *AGP2*, *AGP2A*, and *AGP2B* genes of the mother and child are underlined and italicized, respectively

-, gap

[a] Merritt and Board (1988)

SSCP analysis of each exon

To elucidate the molecular basis of the Ghanaian variant, we obtained the products for each exon by nested PCR from the p1S and p2S products and subjected them to SSCP analysis under various conditions. The mobility shifts were observed in exons 1 and 5 of the *AGP1* gene and in exons 2, 3, and 5 of the *AGP2* gene. The SSCP patterns of the fragment for exon 1, where a nucleotide substitution distinguishing *ORM1*F1* and *ORM1*S* resides (Yuasa et al. 1997), revealed that the mother and child had patterns identical to ORM1 S and ORM1 F1-S, respectively. The Ghanaian variant was shown to originate from *ORM1*S*.

Molecular analysis of the Ghanaian variant

The nucleotide sequencing of the products for exon 1 obtained from the p1S products confirmed the results of the SSCP analysis. In exon 5 of the *AGP1* and *AGP2* genes, the consensus sequences differ by four nucleotides at positions 3594, 3604, 3615, and 3626 (Dente et al. 1987; Merritt and Board 1988). The nucleotide sequence of the products for exon 5 obtained from the p1S and p2S products is shown in Table 1. In the p1S products, both mother and child were homozygous for the *AGP1* gene-specific C and A at nt 3594 and 3604, respectively. In contrast, they had both *AGP1* and *AGP2* gene-specific sequences at nt 3615 and 3626. The A and C at nt 3615 and 3626 were the same as the consensus sequence of the *AGP2* gene. The 84-bp-long fragments obtained with the primer set of AGP1-G5F and AGP-G5R were digested with *Hsp*92II and *Bsr*SI, which are able to cut the *AGP2* gene-specific sequence. It was shown that the *AGP2* gene-specific sequence of the Ghanaian variant was located at the same gene (data not shown). A G-to-A transition at nt 3615 resulted in the substitution of methionine (ATG) for valine (GTG) at amino acid position 156. This mutation corresponded to that of *ORM1*F2* arising from *ORM1*F1*. A T-to-C transition at nt 3626 was silent, and aspartic acid (GAT-GAC) at position 159 remained unchanged. This mutation has not been detected in *ORM1*F2*, which has a T at the same position. The Ghanaian variant was tentatively designated *ORM1*S2(C)*.

Analysis of intron 5

The two nucleotide substitutions found in *ORM1*S2(C)* were located at the 3′ end of exon 5 of the *AGP1* gene. To localize precisely the 3′ boundary of the rearrangement, we performed the sequencing of fragments for intron 5 (nt 3636–4775) from the p1S and p2S products. As shown in Table 1, the sequence was heterozygous in many nucleotide positions and the *AGP2*-specific sequence invaded at least two regions of intron 5 of the *AGP1* gene (nt 4298–4329 and nt 4515–4708). Interestingly, the nucleotide sequence at nt 4515–4708 was homozygous in the mother. The 5.1- and 6.4-kb-long fragments obtained by *Sph*I digestion of the p12 and p22 products from the child were subjected to haplotyping. Sequencing analysis of the 5.1-kb-long fragment of the p12 product inherited from the mother confirmed that *ORM1*S2(C)* was a mosaic with the *AGP2* gene-specific sequence at the three regions in exon 5 and intron 5. *ORM1*S* in the mother had the *AGP2* gene-specific sequence at nt 4515–4708, indicating that it was a hybrid gene. The exchange of nucleotide sequence at nt 4515–4708 in the two alleles of the mother may extend into exon 6 or the 3′-flanking region of the *AGP1* gene, because the *AGP1* and *AGP2* genes were identical in sequence in these regions (Dente et al. 1987; Merritt and Board 1988). In contrast, the nucleotide sequence in the 6.4-kb-long fragment of the p12 product that originated from *ORM1*F1* of

the true father was almost identical to that in *ORM1*F1* and *ORM1*S* in the Japanese subjects. A C-to-A transversion at nt 4524 resulted in the gain of an *Rsa*I restriction site. The screening by digestion with *Rsa*I revealed that intron 5 of the *AGP1* gene from 39 Japanese people (Nakamura et al. 2000b) was unlikely to be invaded by the *AGP2* sequence. In addition, several single-nucleotide polymorphisms were found in exon 5 and intron 5 of the *AGP2* gene. Some were the same nucleotides found in the consensus sequence of the *AGP1* gene, and others were new and not observed in both genes.

Analysis of recombination breakpoint in the *AGP2B* gene

An *AGP* gene consisting of one *AGP1* and two *AGP2* was observed in the child. The triplicated *AGP* genes must have arisen through a homologous unequal crossover event from two tandemly duplicated *AGP* genes. An interval between the 5′-flanking region and exon 2 of the *AGP2B* gene contained in the p22 product was sequenced. Its sequence was very similar to that of the *AGP2* genes in Japanese subjects (data not shown). Possible recombination breakpoints were suggested to exist between a downstream region of the *AGP2A* gene and *AluAGP2* of the *AGP2B* gene.

Heterogeneity of *ORM2*M*

As described earlier, mutations were suggested to be present in the PCR products for exons 2, 3, and 5 of the *AGP2* gene by SSCP analysis. As shown in Table 2, nucleotide substitutions were observed at a total of five sites, one of which was observed in the sequence of intron 2 contained in the PCR products for exon 3. Two of them were nonsynonymous: T2537C (Val[GTC]81Ala[GCC]) and G2558A (Val[GTC]88Ile[ATC]). These mutations are neutral and unlikely to bring about a marked shift in the isoelectric point of the ORM2 protein, as shown by isoelectric focusing. The others were silent: T2230C (Phe[TTT]51Phe[TTC]) and C3626T (Asp[GAC]159Asp[GAT]). Three of these four substituted nucleotides (nt 2230, 2558, and 3626) were the same as the nucleotides of the *AGP1* gene. Haplotypes in *AGP2*, *AGP2A*, and *AGP2B* of the child were also determined using purified *Sph*I-digested fragments of the p12 (1.3 kb), p12 (6.4 kb), and p22 (6.4 kb) products, as shown in Table 2. Three different haplotypes were seen in the mother and child. The *AGP2A* and *AGP2B* genes in the child are likely to have the same sequence as each other and as the common *ORM2*M*. In contrast, the two *ORM2*M* in the mother apparently differed from the common *ORM2*M*. Thus, *ORM2*M* was heterogenous at the DNA level, although its products were not successfully separated by isoelectric focusing.

Table 2. Comparison of nucleotide sequences in intron 1 to exon 5 of the human *AGP* genes

	I1		E2	I2	E3		E5
	2	2	2	2	2	2	3
	0	1	2	4	5	5	6
	0	2	3	5	3	5	2
Genes	6	5	0	6	7	8	6
AGP1							
Dente et al.[a]	a	ND	C	ND	T	A	T
Japanese	a	a	C/T	-	T	A	T
AGP2							
Dente et al.[a]	a	ND	T	ND	T	G	C
Merritt and Board[b]	a	c	T	c	T	G	C
Japanese	a/c	c/a	T	c/t	T	G	C/T
Mother (p2S)	a	a	*T*/C	*c*	T/C	*G*/A	T
Child (p2S)	a/c	a	*T*	*c*/*t*	T/C	*G*	*C*/T
AGP2	a	a	*T*	*c*	C	*G*	ND
AGP2A	*c*	a	*T*	*t*	T	*G*	*C*
AGP2B	*c*	a	*T*	*t*	T	*G*	ND

The *AGP1* and *AGP2* gene-specific sequences in the *AGP2* genes of the mother and child are underlined and italicized, respectively
ND, no data; -, gap
[a] Dente et al. (1987)
[b] Merritt and Board (1988)

Discussion

In this study we have identified and characterized three remarkable nucleotide changes occurring in the *AGP* genes of a Ghanaian mother and child: (1) interlocus sequence exchanges in the *AGP1* gene, (2) a duplication of the *AGP2* gene, and (3) diversity of a common allele, *ORM2*M*.

The two ORM1 alleles, *ORM1*S* and *ORM1*S2(C)*, had the *AGP2* gene-specific sequence at one and three regions, respectively, in exon 5 to intron 5 (Fig. 3c). The interlocus sequence exchange at nt 4515–4708 in both alleles may have been generated through an unequal crossover event rather than a gene conversion event, because the *AGP2* gene-specific sequence was observed in a relatively large region (nt 4515–4708 at minimum). If crossover is a cause for generation of such a hybrid gene, the *AGP* gene with two tandem *AGP2* genes is necessary. The duplication of the *AGP2* gene may occur frequently in Ghanaian and other African populations, because such a duplicated *AGP2* gene has been detected in the child as described earlier. Two mutational steps are needed to generate *ORM1*S2(C)* from *ORM1*S*. Because the two sequence exchanges have occurred in relatively small intervals (nt 3615–3626 or nt 3605–3710 at maximum and nt 4298–4329 or nt 4282–4358 at maximum), these must have arisen through two gene conversion events but not through two double recombination events and multiple independent point mutations (Cooper 1999; Strachan and Read 1999).

The two nucleotide substitutions responsible for the *ORM1* variant allele designated *ORM1*S2(C)* were 3615G→A and 3626T→C in exon 5 of the *AGP1* gene. The former mutation yields a V156M substitution, which is the same as an amino acid substitution found in *ORM1 F2* (Yuasa et al. 1997). These results were consistent with a similarity in behavior between the variant and *ORM1 F2* bands by three different techniques of isoelectric focusing. The shift in isoelectric point of the *ORM1 S2(C)* band was

observed after treatment with iodoacetamide, just like *ORM1 F2* and *ORM2 M*, because the alkylation of a methionine residue produces a positively charged derivative (Rocha et al. 1993).

This study also presents evidence for further duplication of the *AGP2* gene, which must have arisen through homologous unequal crossover. Each *AGP2* gene in the duplicated gene was likely to be the same sequence in coding exons as each other and as a duplicated *AGP2* gene cloned from a cosmid library, the ethnic origin of which was not described (Dente et al. 1987). In the previous study of 39 Japanese individuals (Nakamura et al. 2000b), no duplication of the *AGP2* gene was observed, whereas duplications of the *AGP1* gene were detected.

The *ORM2*M* is the most predominant allele in all populations investigated thus far. It has been observed at more than 95% frequency in African-Americans and Asians (Escallon et al. 1987; Yuasa et al. 1993), and at a monomorphic frequency of more than 99% in Europeans (Yuasa et al. 1986, 1993; Umetsu et al. 1989). In a previous study (Nakamura et al. 2000b), we found a 3626C→T transition in exon 5 of the *AGP2* gene. In addition to this mutation, three new mutations were detected in exons 2 and 3 in the Ghanaian mother and child. No variation was observed at the three nucleotide positions in the 39 Japanese subjects (Nakamura et al. 2000b). These results suggest that African populations could be more diverse than the Japanese population. Three of the four nucleotides in the three coding exons (nt 2230, 2558, and 3626) were replaced by a copy of the *AGP1* gene. These single-base changes could have resulted from point mutations. However, the acquisition of sequences from the other genes may represent gene conversion-like events (Cooper 1999; Strachan and Read 1999).

Thus, it is likely that the rearrangement of the *AGP* gene has often occurred in Africans and is different from that in the Japanese population. Further studies on the structure of the *AGP* gene in Africans and other populations are needed to elucidate the molecular evolution and diversity of the human *AGP* gene. The new polymorphisms of the *AGP* genes found in this study may play an important role in drug transport. The AGP, or ORM, in plasma is a main carrier protein of basic drugs and other ligands, for which the binding is different among polymorphisms (Tinguely et al. 1985; Kremer et al. 1988; Hervé et al. 1993, 1996).

Finally, we propose that *ORM1*S* corresponding to *ORM1*F1* be renamed *ORM1*S1* from now on, because the variant corresponding to *ORM1*F2* was designated *ORM1*S2* in this study.

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