A novel *A* allele with 664G>A mutation identified in a family with the A_m phenotype

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BACKGROUND: The A_m phenotype has been characterized as a weak expression of the A antigen on red blood cells but the presence of a normal quantity of the A antigen in saliva. This study describes a molecular genetic analysis of members of an A_m family.

STUDY DESIGN AND METHODS: The eight exon regions of the *ABO* genes of the A_m proposita were amplified by polymerase chain reaction and cloned, and their sequences were analyzed. The α-1,3-*N*-acetylgalactosaminyltransferase (A-transferase) activities of the A_m serum and the expressed A_m transferase were analyzed.

RESULTS: An *A* gene with a 664G>A mutation, which predicts an amino acid alteration of Val222Met, was identified in the A_m proposita. This *A^m664A* allele was demonstrated in other three family members with the A_m phenotype. The A-transferase activity was virtually undetectable in the A_m sera, and the expressed A_m transferase showed weak A-transferase activity, when compared with the expressed A_1 transferase, in assays that use acceptor substrates mimicking the Type 2 H structure and Type 1 H structure.

CONCLUSION: A novel *A* allele with 664G>A mutation was demonstrated in a pedigree with the A_m phenotype. The mechanism leading to the formation of the A_m phenotype still awaits elucidation.

The A and B antigenic epitopes of the human histo-blood group ABO system have been characterized as the defined carbohydrate determinants, GalNAcα1-3(Fucα1-2)Galβ1-R and Galα1-3(Fucα1-2)Galβ1-R, respectively, carried on glycolipids and glycoproteins (reviewed in Watkins[1] and Daniels[2]). They are synthesized by the respective glycosyltransferase products of the *A* and *B* alleles at the *ABO* locus. The *A* allele encodes an α-1,3-*N*-acetylgalactosaminyltransferase (A-transferase), which catalyzes the transfer of GalNAc from the donor substrate UDP-GalNAc to the H precursor structure to give the A determinant, and the *B* allele encodes an α-1,3-galactosyltransferase (B-transferase), which is responsible for the formation of the B determinant by the transfer of Gal from the UDP-Gal donor to the same H precursor. The A and B antigenic structures are not only present on the red blood cells (RBCs), but are also detected in various mucosa secretions including saliva.[2,3] It has been shown that the main H precursor structure on RBCs, onto which the A and B determinants are built, is different from that in secretions. The A and B determinants on RBCs are mostly built on the H structure with a Type 2 chain (Fucα1-2Galβ1-4GlcNAcβ1-R), whereas in saliva the H Type 1 chain (Fucα1-2Galβ1-

ABBREVIATIONS: 2′-FL = 2′-fucosyllactose; A-transferase = α-1,3-*N*-acetylgalactosaminyltransferase; B-transferase = α-1,3-galactosyltransferase; LNFP I = lacto-*N*-fucopentaose I.

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3GlcNAcβ1-R) is present dominantly and serves as the acceptor substrate for the synthesis of the A and B epitopes.

In 1990, Yamamoto and colleagues[4,5] first characterized the respective nucleotide sequences of the three major alleles, *A*[1], *B,* and *O,* of the *ABO* locus and elucidated the molecular genetic basis of the human ABO blood group system. The *A, B,* and *O* cDNA structures were found to be highly homologous. The primary sequences of the A- and B-transferases differ in only four amino acid residues at positions 176, 235, 266, and 268 (Arg, Gly, Leu, and Gly in A-transferase and Gly, Ser, Met, and Ala in B-transferase), and it has been shown that the differences in these four residues are responsible for the different specificities for the donor substrate between the two transferases.[6-9] Most of the *O* cDNAs possess a deletion at nucleotide 261, which leads to reading frameshift and thus this allele fails to encode a complete transferase product. In further investigations the *ABO* gene was shown to span approximately 20 kb of genomic DNA, and it was found recently that, in addition to the known complete *ABO* transcript structure comprising seven exon regions,[10,11] the *ABO* gene expresses another variant form, which contains a different starting exon 1 region through utilization of an alternative promoter.[12,13]

In addition to the common ABO phenotypes, A_1, A_2, B, A_1B, A_2B, and O, numerous phenotypes with a weak expression of the A or B antigens on RBCs, have been found (reviewed in Daniels[2] and Issitt and Anstee[14]). Each of these subgroups has their own defined serologic characteristics, and numerous genotypes of the *A* and *B* suballeles responsible for the formation of such subgroups have been identified.[15,16] Various suballeles, which possess missense mutations and consequently alter the primary structures of the encoded proteins, produce A- or B-transferase with weakened transferase activities, and additionally some suballeles, such as the *B(A)700G* and the *cis-AB700T* alleles, have been demonstrated to encode transferase products having dual or reversed specificities for donor substrates.[17-19]

In most of the subgroups, which have weakened expression of the A or B antigens on RBCs, lesser quantities of the A or B antigenic determinants in saliva are also detected. This is not the case for the A_m and B_m subgroups, however. Saliva of individuals with the A_m or B_m phenotypes contains normal quantities of the corresponding A or B antigens, although their RBCs have a weaker expression for the A or B antigens.[2,14]

The A_m phenotype is rare. One example of A_m was found in 150,000 French donors[20] and one example was identified in 400,000 Taiwanese donors.[21] This study carries out a molecular genetic analysis of the samples from a Taiwanese family with the A_m phenotype. The A^1 allele with G to A change at nucleotide position 664, which predicts the amino acid alteration of Val to Met at residues 222, was identified in the family members with the A_m phenotype. The transferase activities of the A_m serum and the expressed product from the A^m cDNA were examined, with Type 2 H and Type 1 H structures as acceptor substrates.

MATERIALS AND METHODS

Samples and serology

Our use of human subjects was conducted under the tenets of the Helsinki protocol and the program was approved by the Institutional Review Board at Mackay Memorial Hospital. Genomic DNA samples of the four family members in the A_m pedigree, and 40 randomly selected individuals with the common A_1 phenotype were prepared from their peripheral blood cells with a DNA blood mini kit (QIAamp, Qiagen GmbH, Hilden, Germany). All of the assessed individuals belong to the Taiwanese populations, and informed consent was obtained from all participants.

Blood grouping of the A_m proposita and her family members was performed by standard hemagglutination tests at room temperature. Monoclonal anti-A, anti-B, and anti-A,B reagents and anti-A_1 lectin (*Dolichos biflorus*) (Gamma Biologicals Inc., Houston, TX) and polyclonal anti-A, anti-B, and anti-A,B reagents (BCA, West Chester, PA) were used. The RBCs were also tested against anti-H lectin (*Ulex europaeus*) (Gamma Biologicals Inc.). Adsorption and elution studies of RBCs were performed with group B sera. The salivary ABH profile was determined by the salivary ABH inhibition test.[22]

Sequence analysis of the *ABO* gene

The eight exon regions of the *ABO* genes were divided into four segments and amplified by polymerase chain reaction (PCR) with specific primer sets as shown in Table 1. Fifty nanograms of genomic DNA and 5 pmol of each forward and reverse primers were combined in 12.5 µL of PCR buffer containing 0.2 mmol per L dNTP and 0.625 unit of hotstart DNA polymerase (*PfuUltra*, Stratagene, La Jolla, CA). The PCR products were cloned into the pCR4Blunt-TOPO vectors with a TOPO PCR cloning kit (Zero Blunt, Invitrogen, Groningen, the Netherlands). DNA sequences were determined with a cycle sequencing kit (BigDye Terminator, Applied Biosystems, Foster City, CA). Multiple clones from two batches of PCR products were sequenced to separate any PCR-induced errors from actual sequence polymorphisms.

PCR-restriction fragment length polymorphism analysis

As the 664G>A nucleotide substitution in the A^1 gene, identified in the A_m proposita, creates a *Nla*III recognition

TABLE 1. PCR primers for amplification of the coding regions of the *ABO* gene

Encompassing region	Sequence	Location	Product size (bp)
Exon 1a-1			
Fa7	AGGGTCCTGTGAACGGGGTATCAGTGTAAA	113 bp upstream of exon 1a	
Rp*	GGTAGGTGCTGAAAATAGCAGCTCATGGAAGG	331 bp downstream of exon 1	1248
Exon 2-3			
Fb	GCAGGTGAGAGAAGGAGGGTGAGTGATGTG	23 bp upstream of exon 2	
Rx*	CAGCATGGATGCTCCACCTGCTCTTCCCTG	7 bp downstream of exon 3	941
Exon 4-6			
Fd	TCCTGCTCCTAGACTAAACTTCATCTCCTGTG	14 bp upstream of exon 4	
Rm*	TCACTCGCCACTGCCTGGGTCTCTACCCTC	19 bp downstream of exon 6	2554
Exon 6-7			
Fh3	GGGTGGTCAGAGGAGGCAGAAGCTGAGTGG	91 bp upstream of exon 6	
Rz*	GACGGGGCCTAGGCTTCAGTTACTCACAAC	99 bp downstream of stop codon	2119

* Antisense sequences.

sequence (CATG), a PCR-based restriction fragment length polymorphism (RFLP) test was developed to demonstrate the mutation in the *ABO* genes. Fifty nanograms of genomic DNA and 5 pmol of each forward (ABOF312, TACAAGCGCTGGCAGGACG, nucleotides 532-550 of *ABO* cDNA) and reverse (ABOR802, GGATGTAGGCCTGG GACTG, complement to nucleotides 751-769 of *ABO* cDNA) primer were combined in 12.5 µL of PCR buffer containing 0.2 mmol per L dNTP and 0.25 units of *Taq* polymerase (Promega, Madison, WI). The PCR program included 5 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 62°C, and 1 minute at 72°C. The 238-bp PCR products were subjected to digestion by *Nla*III restriction endonuclease and then analyzed by 3.0 percent agarose gel electrophoresis.

Serum A-transferase activity assay

A-transferase activity was analyzed by the transfer of radioactivity from [^{3}H]UDP-GalNAc to the acceptor substrates 2′-fucosyllactose (2′-FL; Fucα1-2Galβ1-4Glc) (Sigma Chemical Co., St Louis, MO) and lacto-*N*-fucopentaose I (LNFP I; Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc) (Glyco Inc., Novato, CA), which mimic the Type 2 H and Type 1 H structures, respectively. The reactions were carried out in a total volume of 100 µL with 50 mmol per L sodium cacodylate (pH 6.5), 20 mmol per L $MnCl_2$, 5 mmol per L ATP, 8 mmol per L NaN_3, 50 µmol per L of UDP-GalNAc, 0.2 µCi of UDP-[6-^{3}H]GalNAc (10 Ci/mmol) (American Radiolabeled Chemicals Inc., St Louis, MO), 5 mmol per L 2′-FL or LNFP I, and 25 µL of serum. After incubation at 37°C for 15 hours, the reaction solutions were extracted twice with 0.5 mL of Dowex 1X8-400 (chloride form, 1:2 in H_2O; Sigma Chemical Co.) slurry to separate the enzymatic reaction product from the donor substrate. Under this condition, no more than 5 percent of the donor substrate was transferred. Radioactivity of the supernatants after extraction was measured by scintillation counting.

Expression of the A^m*664A* cDNA and functional analysis

Total RNA samples were prepared from the peripheral blood cells of a group A_1 individual and the A_m proposita with a RNA blood mini kit (QIAamp, Qiagen, GmbH). The first-strand cDNAs were primed by oligo(dT) primer and synthesized by reverse transcriptase (SuperScript III, Invitrogen, Carlsbad, CA). The cDNA fragment encompassing the region from nucleotide 60 to nucleotide 1065, which encodes amino acid residues 54 to 354, of the A^1 gene, were PCR amplified with the primers ABOF4 (aattggcccagccgg ccGCTGTTAGGGAACCTGACCATCTGCAGC) and ABOR6 (ttaagggcccTCACGGGTTCCGGACCGCCTGGTGG, antisense sequence), which contained *Sfi*I and *Apa*I restriction endonuclease recognition sequences (underlined) at their 5′ ends, respectively. The amplified cDNA fragments were cloned into the *Sfi*I and *Apa*I sites of the mammalian expression vector pSecTaq2A (Invitrogen), which is designed to allow secretion of the expressed protein via the N-terminal secretion signal from the V-J2-C region of mouse Igκ chain. Plasmids containing the wild-type A^1 and A^m*664A* cDNAs were selected and their sequences were confirmed. The two constructed plasmids and mock pSecTaq2A plasmids were prepared for transfection with a plasmid kit (EndoFree, Qiagen GmbH).

COS-7 cells (purchased from the American Type Culture Collection, Manassas, VA) were grown in 90 percent Dulbecco's modified eagle medium and 10 percent fetal bovine serum containing 50 units per mL penicillin and 50 µg per mL streptomycin. The day before transfection, cells were split into 60-mm culture dishes at a density of 5×10^4 per mL, and after culture for 24 hours, the cells were transfected with 1 µg of the expression vector. Transfection of the cells was performed with a transfection reagent (Effectene, Qiagen GmbH). After culturing for an additional 72 hours, the medium was harvested, concentrated 50-fold by a centrifugal filter unit (Centriplus YM-10, Millipore Intertech, Bedford, MA), and then used for the A-transferase activity assay.

The A-transferase activity assay for the expressed products was performed in a 100-µL reaction mixture containing 20 µL of concentrated medium. The reagents and procedures were similar to those in the serum A-transferase activity assay, except that 1 mmol per L acceptor substrate was used. Reactions without acceptor substrate were performed for each test to correct for background caused by endogenous transfer. Under these conditions, no more than 10 percent of the donor substrate was transferred. Because the optimal pH value for A_2-transferase activity has been shown to be between 7 and 8, the transferase activity assays, for the sera and for the expressed transferases, were also tested at pH 7.6.

RESULTS

The A_m family and serology

The pedigree of the family with the A_m phenotype is illustrated in Fig. 1. The A_m proposita, member II-1, was discovered as a result of discrepancies between forward and reverse typing in routine ABO grouping by the standard hemagglutination test. Further serologic study suggested the A_m phenotype for the individual. Samples from three other family members were shown to have similar serologic characteristics to the proposita.

The RBCs of the four family members were weakly agglutinated (+/–) with monoclonal anti-A, monoclonal anti-A,B, and polyclonal anti-A reagents, but not by monoclonal and polyclonal anti-B, polyclonal anti-A,B, and anti-A_1 lectin reagents. The cells reacted strongly (4+) with anti-H reagent; the cells were found to adsorb anti-A and the elutes reacted moderately (2+) with A cells. The sera samples of the four members contained strong anti-B activity and weak anti-A_1 activity. The saliva of the four members contained normal amounts of A and H substances compared to the saliva from common A_1 individuals in salivary ABH inhibition test.

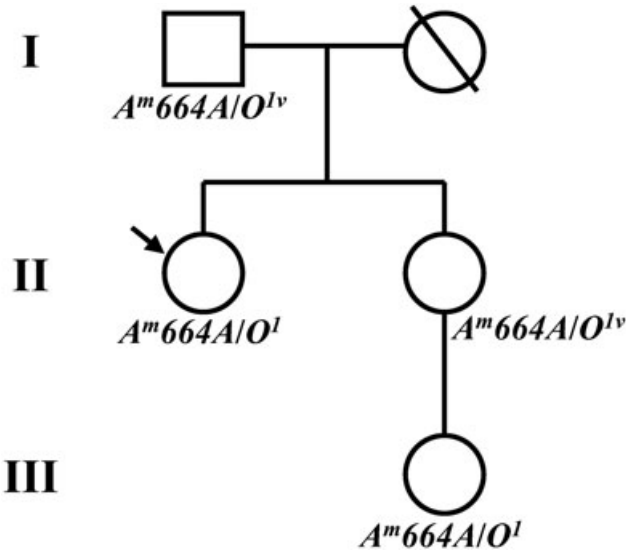

Fig. 1. The pedigree of family members with the A_m phenotype. The four family members, I-1, II-1, II-2, and III-1, were all typed as A_m phenotype. Each family member's *ABO* genotype is shown under each square (male) or circle (female) symbol. An arrow indicates the A_m proposita.

The A^1 gene with the 664G>A missense mutation was identified in the A_m pedigree

The eight exon regions of the *ABO* genes of the A_m proposita, exon 1a and exons 1 to 7, were PCR amplified and cloned as described under Materials and Methods, and the sequences of the exons and the adjacent splice acceptor and splice donor sites were inspected. Recombinant plasmids containing PCR fragments encompassing exon 4 through exon 6 and exon 6 through exon 7 regions were first distinguished into *O* and non-*O* allele origin by the PCR-RFLP analysis that detects the 261G deletion of the *O* allele. The exon 6 and exon 7 region of the *O* allele clone was demonstrated to have the wild-type sequence associated with the O^1 allele. The exon 6 and exon 7 region of the non-*O* allele clones from two batches of PCR products were sequenced, and sequences of the two clones demonstrated the A^1 gene backbone aside from a substitution of G to A at nucleotide 664. One non-*O* allele clone containing the exon 4 through exon 6 region was sequenced and was shown to have wild-type A^1 exon sequences. Eight clones for exon 2 to exon 3 region and eight clones for exon 1a to exon 1 region were analyzed, and all demonstrated the wild-type A^1/O^1 exon sequences. These results suggest that the A_m proposita is a heterozygote possessing the wild-type O^1 gene and a mutant A^1 gene with a 664G>A mutation. Direct sequencing of the PCR product also showed the heterozygous state for the G and A nucleotides at that position (Fig. 2, right panel). Direct sequencing of the PCR product amplified from an individual with the common A_1 phenotype did not show the G to A change (Fig. 2, left panel). The 664G>A nucleotide substitution in the A^1 gene predicts an amino acid alteration from Val to Met at residue 222.

The DNA fragments encompassing the exon 6 to 7 region of the *ABO* genes of the other three family members, member I-1, II-2, and III-1, were PCR amplified and directly sequenced, and the results showed that the three A_m members all carried the same 664G>A mutation. Their *O* allele genotypes were also determined, as shown in Fig. 1.

The 664G>A mutation is virtually absent in the general A^1 gene pool

The 664G>A mutation identified in the A^1 gene of the A_m proposita creates a *Nla*III recognition sequence, and thus

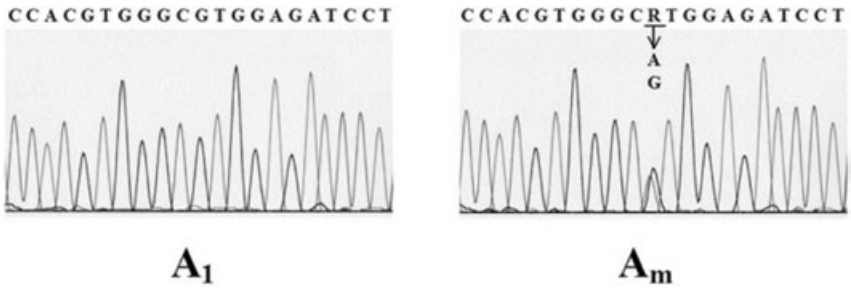

Fig. 2. Sequencing results of the exon 7 regions of the *ABO* genes of a common group A_1 individual (left panel) and the A_m proposita (right panel). Genomic DNA samples were purified from an A_1 individual and the A_m proposita. The exon 7 regions of the *ABO* genes were amplified by PCR, and the sequences were analyzed by direct sequencing. In the A_m sample, the heterozygous state of the G and A nucleotides (underlined) at the 664 position is demonstrated.

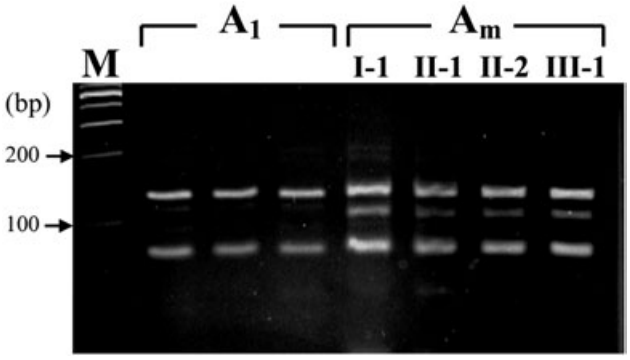

Fig. 3. PCR-RFLP analysis to demonstrate the *A^m664A* allele. The 664G>A mutation identified in the *A^1* gene of the A_m proposita creates a *Nla*III site. The 238-bp PCR product amplified from the wild-type *A^1* allele was cleaved into 128-, 75-, 26-, and 9-bp fragments by *Nla*III digestion, whereas that amplified from the mutant *A^m664A* allele was cleaved into 104-, 75-, 26-, 24-, and 9-bp fragments. The *Nla*III-cleaved products were analyzed by 3.0 percent agarose gel electrophoresis. Samples from the A_m family members, I-1, II-1, II-2, and III-3, together with samples from 40 randomly selected group A_1 individuals were subjected to the PCR-RFLP analysis. Three of the results obtained from the group A_1 individuals are shown in Lanes A_1. (Lane M) Molecular mass standards of the 100-bp ladder.

a PCR-RFLP analysis was developed and used to detect the mutation in 40 randomly selected individuals with common A_1 phenotype. The 238-bp PCR product amplified from the wild-type *A^1* allele was cleaved into 128-, 75-, 26-, and 9-bp fragments by *Nla*III digestion, whereas that amplified from the mutant *A^m664A* allele was cleaved into 104-, 75-, 26-, 24-, and 9-bp fragments. As shown in Fig. 3, the A_m proposita and the other three A_m family members (Lanes A_m) had one allele with the 664G>A mutation at their *ABO* loci, whereas none of the *A^1* or *O* alleles of the 40 random group A_1 individuals demonstrated the mutation (three of the results are shown in Lanes A_1 of Fig. 3), suggesting that the *A^1* allele with the 664G>A mutation is virtually absent in the general A_1 population.

The A-transferase activities of the A_m sera and the expressed A_m transferase

The A-transferase activities of the A_m sera and the expressed A_m transferase were analyzed and compared with those of the common A_1 serum and the expressed A_1 transferase. The A-transferase activity was measured as the amount of GalNAc incorporated onto 2′-FL or LNFP I substrates, which served as Type 2 H and Type 1 H acceptor substrates, respectively, from the donor substrate UDP-GalNAc.

Table 2 shows the results obtained from the A-transferase activity assay of the sera from the A_m proposita and a normal group A_1 individual with *A^1/O* genotype. The A_1 serum sample demonstrated significant A-transferase activities for both the Type 2 H and the Type 1 H acceptor substrates. Nevertheless, A-transferase activity in the A_m serum was virtually undetectable with both the Type 2 H and the Type 1 H substrates. The A-transferase activity was also not detected in the sera from the other three family members, I-1, II-2, and III-1, when LNFP I was used as acceptor substrate (data not shown).

The expressed A_m transferase, possessing the Val to Met alteration on residue 222, showed A-transferase activity (Table 3); however, the activities were weak on both Type 2 H and Type 1 H acceptor substrates when compared with those of the expressed normal A_1 transferase.

The A-transferase activity assays for the A_m serum and the expressed A_m transferase, shown in Tables 2 and 3, were performed in cacodylate buffer with a pH value of 6.5. The assays were also performed in pH 7.6 buffer, and the results were similar to those for the pH 6.5 buffer (data not shown).

DISCUSSION

In this study, an *A* gene with a 664G>A missense mutation was demonstrated in the A_m proposita, after the coding sequences and the splice donor and acceptor sites of the eight exon regions of the *ABO* genes were analyzed. The four A_m members from the same family were all demonstrated to possess the 664G>A mutation, whereas the nucleotide change was not detected in the general A_1 population.

TABLE 2. Comparison of the A-transferase activities of the A_1 and A_m sera*

	Acceptor substrate		
	—	2′-FL	LNFP I
A_1	1.65 ± 0.14	68.80 ± 3.48	49.10 ± 0.93
A_m	2.45 ± 0.46	2.40 ± 0.51	2.20 ± 0.12

* The amounts of transferred GalNAc (pmole) to acceptor substrates, 2′-FL and LNFP I, and the amounts of transferred GalNAc in the absence of acceptor substrate are shown. The results of the mean and standard deviation of three tests are shown. The assay was performed in cacodylate buffer with a pH value of 6.5, and similar results were obtained when the assay was performed at pH 7.6.

TABLE 3. The A-transferase activities of the enzymes encoded from the *A^1* and *A^m* cDNAs*

	Acceptor substrate	
	2′-FL	LNFP I
Vector only	26.63 ± 7.11	14.75 ± 2.04
A^1	231.03 ± 44.87	121.38 ± 26.82
A^m	41.86 ± 10.07	22.73 ± 3.90

* The amounts of transferred GalNAc (pmole) to 2′-FL and LNFP I acceptor substrates by the medium concentrates harvested from the cells transfected with respective expression vectors were measured. The results of the mean and standard deviation of four tests are shown. Endogenous transfer of GalNAc in the absence of acceptor substrate was corrected for each test. The assay was performed in cacodylate buffer with a pH value of 6.5, and similar results were obtained when the assay was performed at pH 7.6.

Among various A or B subgroups, the A_m and B_m have attracted considerable attention, as the expression of the A or B antigenic determinants becomes weak on RBCs but remains normal in saliva secretion. The molecular background underlying the A_m or B_m phenotypes, however, has not been defined. Previously the *A^1* gene in a Norwegian pedigree with the A_m phenotype was shown to have wild-type coding exon 6 and exon 7 sequences,[23] and thus it has been speculated that a mutation in the promoter region of the *A* gene, which leads to a blocking of the *A* gene expression in marrow cells but not in mucus-secreting cells, might be a possible molecular mechanism responsible for the formation of A_m phenotype.[2]

Differential expression of the *A* gene in blood cell lineage and in salivary mucus-secreting cells, because of a change in the regulatory region, could be an explanation for the A_m phenotype. A previous article, however, reported the identification of an *A^1* allele with a 761C>T mutation, which predicts the Ala254Val alteration, in a pedigree with the A_m phenotype.[24] In this study, the 664G>A missense mutation in the coding region of the *A^1* gene was identified in the family members with the A_m phenotype, and the *A^m664A* allele was found to be virtually absent in the general A_1 population. These two examples suggest that the A_m phenotype might also be caused by a molecular change in the coding region of the *A* gene.

Missense mutation in the *A* or *B* genes might weaken the enzyme activity of the encoded transferase. In our A_m case, the A-transferase activity was virtually undetected in the A_m serum, and the A-transferase expressed from the *A^m664A* cDNA only showed weak A-transferase activity when compared with the common A-transferase expressed from the wild-type *A^1* cDNA. These results support the possibility that the Val222Met alteration has led to decreased activity of the A_m transferase. A missense mutation in the *A* or *B* genes, however, might also shift donor substrate specificity of the encoded transferase. Hybrid A- or B-transferases encoded from variant *A* or *B* alleles, such as the *A*803C*,[25] *B*796C*,[26] and *B*703G*[27] alleles (designated as *cis-AB01*, *cis-AB02*, and *B(A)01* alleles, respectively, according to the Blood Group Antigen Gene Mutation Database,[28] http://www.bioc.aecom.yu.edu/bgmut/index.htm) have been suggested as possessing dual specificities for both UDP-GalNAc and UDP-Gal donor substrates.[6,7] Furthermore, it has been shown that the B-transferase encoded from the *B*700G* allele (*B(A)02*) possesses decreased B-transferase activity but increased A-transferase activity[17] and the B-transferase encoded from the *B*700T* allele (*cis-AB03*) has a reversed donor substrate specificity in enzyme kinetic analysis.[19]

Thus, we speculate that certain missense mutation in *A* or *B* genes might be able to produce variant A- or B-transferases with changed specificities for acceptor substrates. These variant A- or B-transferases may have different preferences when using the Type 2 H or Type 1 H precursor structures for the synthesis of the A or B epitopes. This might also explain the formation of the A_m or B_m phenotypes. Nevertheless, the results represented in Tables 2 and 3 show that the A-transferase activity in the A_m serum and the A-transferase activity of the expressed transferase from *A^m* cDNA does not have a different preference for the Type 2 H or Type 1 H acceptor substrates in our assays, suggesting that the Val222Met change does not alter the acceptor substrate specificity of the A-transferase. These results appear to eliminate the possibility that preference for the Type 1 H acceptor substrate by the A_m transferase is the mechanism for the A_m phenotype in this family; however, more detailed analysis is required to totally exclude the mechanism. It should be noted that whether the mutant *A^m664A* gene is expressed as an adequate amount of the A-transferase, in the sera of the A_m members and in the COS-7 cell-expression experiment, was not determined.

In this study, a novel *A* allele with the 664G>A mutation was identified in the A_m family; however, more detailed investigation and more A_m samples are required to elucidate the molecular mechanism responsible for the formation of the A_m phenotype.

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