Wide Variety of Point Mutations in the H Gene of Bombay and Para-Bombay Individuals That Inactivate H Enzyme

By Mika Kaneko, Shoko Nishihara, Naoko Shinya, Takashi Kudo, Hiroko Iwasaki, Taiko Seno, Yasuto Okubo, and Hisashi Narimatsu

The H genes, encoding an α1,2fucosyltransferase, which defines blood groups with the H structure, of four Bombay and 13 para-Bombay Japanese individuals were analyzed for mutations. Four Bombay individuals were homologous for the same null H allele, which is inactivated by a single nonsense mutation at position 695 from G to A (G695A), resulting in termination of H gene translation. The allele inactivated by the G695A was designated h1. The other 13 para-Bombay individuals possessed a trace amount of H antigens on erythrocytes regardless of their secretor status. Sequence analysis of their H genes showed four additional inactivated H gene alleles, h2, h3, h4, and h5. The h2 allele possessed a single base deletion at position 990 G (990-del). The h3 and h4 alleles possessed a single missense mutation, T721C, which changes Tyr 241 to His, and G442T, which changes Asp148 to Tyr, respectively. The h5 allele possessed two missense mutations, T460C (Tyr154 to His) and G1042A (Glu348 to Lys). The h2, h3, h4, and h5 enzymes directed by these alleles were not fully inactivated by the deletion and the missense mutations expressing some residual enzyme activity resulting in synthesis of H antigen on erythrocytes. Thirteen para-Bombay individuals whose erythrocytes retained a trace amount of H antigen were determined to be heterozygous or homozygous for at least one of h2, h3, h4, or h5 alleles. This clarified that the levels (null to trace amount) of H antigen expression on erythrocytes of Bombay and para-Bombay individuals are determined solely by H enzyme activity. These mutations found in the Japanese H alleles differ from a nonsense mutation found in the Indonesian population. To determine the roles of the H, Se, and Le genes in the expression of H antigen in secretions and Lewis blood group antigen on erythrocytes, the Lewis and secretor genes were also examined in these Bombay and para-Bombay individuals. The Lewis blood group phenotype, Le(a⁻ b⁻), was determined by the combinatorial activity of two fucosyltransferases, the Lewis enzyme and the secretor enzyme, and the secretor status was solely determined by the secretor enzyme activity, not by H enzyme activity. Bombay individuals were confirmed to be homozygous for the inactivated H and Se genes. As expected from the very low frequency of Bombay and para-Bombay individuals in the population, ie, approximately one in two or 300,000, the H gene mutations were found to be very variable, unlike the cases of the point mutations in the other glycosyltransferase genes; the ABO genes, the Lewis gene, and the secretor gene.

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such as saliva and milk. Para-Bombay individuals possess similar phenotypes to Bombay individuals. There are two different definitions of para-Bombay individuals. One defines them as the individuals who lack ABH antigens on erythrocytes, but possess them in secretions. Another definition includes, in addition to the above individuals, people who possess very few ABH antigens on erythrocytes compared with ordinary O-type individuals regardless of the presence or the absence of ABH antigens in secretions. The H genes of one Bombay individual and one para-Bombay individual in an Indonesian sample were analyzed, and point mutations were found that terminate translation and result in complete inactivation of the H enzyme. Although the H gene was proven to be responsible for the H antigen expression on erythrocytes by this study, it is not known what determines various levels of H antigen expression on the erythrocytes of para-Bombay individuals who possess a trace amount of ABH antigens, how precisely the H and Se enzymes are involved in the antigen expression on erythrocytes and in saliva, and whether any correlation exists among H, Se, and Le genes.

To explore these problems and to identify H gene mutation(s) in the Japanese population, we analyzed the H, Se, and Le genes of four Bombay individuals and 13 para-Bombay individuals, who were classified by the second definition. In addition, we had data on the Se and Le genes from more than 200 Japanese individuals. We found one mutant allele with G695A (Trp232 to termination) in the H genes of one Bombay individual and four mutant alleles with G990del, T721C (Tyr241 to His), G442T (Asp148 to Tyr) and T460C (Tyr154 to His) plus G1042A (Glu348 to Lys), respectively, in the H genes of the para-Bombay individuals. These results are described together with results indicating linkage between the H and Se genes, and we discuss the phylogenetic aspects of H gene mutations.

MATERIALS AND METHODS

Peripheral blood samples and saliva samples. Peripheral blood samples that were donated by volunteers to Red Cross Blood Centers in Japan for use in blood transfusion were routinely subjected to ABO blood typing by hemagglutination tests. The donors whose erythrocytes showed the rare phenotypes having none or few ABH antigens were suspected to be Bombay or para-Bombay individuals. A total of 5 mL of peripheral blood was bled with heparin from the suspected Bombay and para-Bombay individuals who also donated saliva samples. ABH antigens were determined on erythrocytes and in saliva.

Detection of ABH antigens on erythrocytes. Two hemagglutination methods were employed to detect ABH antigens on erythrocytes. In the ordinary method, one drop of antibody was placed on a glass slide and mixed with washed red blood cells (RBCs). After a 1-hour incubation at room temperature, hemagglutination was carefully observed. A more sensitive method, the adsorption-elution test, was employed for detection of trace amounts of ABH antigens. For detection of H antigen, 100 µL of washed and packed RBCs was incubated with 100 µL of 10% Ulex lectin at 37°C for 7 minutes with continuous agitation. After centrifugation, the supernatant was discarded and the RBCs were washed five times with phosphate-buffered saline (PBS) (pH 7.2). The lectin bound to RBCs was eluted by 30 minutes incubation at 50°C. The amount of the lectin contained in the eluate was measured by the routine hemagglutination of O-type RBCs. The same adsorption-elution test was used to detect the trace amount of A and B antigens on the RBCs of Ah, Bh, and ABH individuals using anti-A or B antibody instead of Ulex lectin. The first incubation was done at 4°C and the elution of the antibody bound to RBCs was done at 50°C for 30 minutes. Antibody in the eluate was assayed by the hemagglutination of each type RBC.

Detection of ABH antigens in saliva. The presence or absence of ABH antigens in saliva was determined by hemagglutination inhibition using standard serological techniques.

Primers and the first polymerase chain reaction (PCR) amplification. PCR primers and PCR conditions used in this study are listed in Table 1. To amplify the full-length H, Se, and Le genes, the 5’- and 3’-flanking sequences of each gene were used as sense mk1, tk1, and sn1 primer sequences, and antisense mk2, tk2, and sn2 primer sequences, respectively. Each primer (1 µmol/L) was added to 1 µg of genomic DNA in a total volume of 50 µL containing 200 µmol/L of each deoxynucleoside triphosphate (dNTP), 10 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 2.5 mmol/L MgCl2, and 0.1 mg/mL gelatin. Thirty cycles in the conditions described in Table 1 were done at 4°C using anti-A or B antibody instead of Ulex lectin. The first incubation at 50°C was done at 4°C and the elution of the antibody bound to RBCs was done at 50°C for 30 minutes. Antibody in the eluate was assayed by the hemagglutination of each type RBC.

Sequence analysis. Sequencing of the 40 clones from five individuals in total were regarded as false mutations. They were confirmed afterward by the PCR-RFLP method for detection of this mutation was established using the mk1 and mk2 primers shown in Table 1. In the same manner, we amplified the ml allele by PCR-RFLP. After determination of H antigen, 100 µL of each of each deoxynucleoside triphosphate (dNTP), 10 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 2.5 mmol/L MgCl2, and 0.1 mg/mL gelatin. Thirty cycles in the conditions described in Table 1 were done at 4°C using anti-A or B antibody instead of Ulex lectin. The first incubation at 50°C was done at 4°C and the elution of the antibody bound to RBCs was done at 50°C for 30 minutes. Antibody in the eluate was assayed by the hemagglutination of each type RBC.

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Electrophoresis of the digested second PCR products for detection of the point mutations was performed on a 4% agarose gel (NuSieve GTG agarose, FMC Corp BioProducts, Rockland, ME), and DNA was visualized by ethidium bromide staining.

Determination of Se and Le genotypes. The PCR-RFLP method for the determination of Japanese Se and Le genotypes was described in detail in our previous reports. The principle of the PCR-RFLP method is the same as described in the previous section. In brief, PCR-RFLPs for the detection of the C357T and A385T mutations in the Se gene were performed using the tk5 and tk6 primer set and the tk7 and tk8 primer set, respectively. The PCR-RFLPs for detection of the C357T and A385T mutations in the Le gene were performed using the tk5 and tk6 primer set and the tk7 and tk8 primer set, respectively. These primers, conditions of PCR, and enzymes used for digestion of PCR products are shown in Table 1.

H gene expression in COS-1 cells. Modification of the pCDM8 vector to introduce an EcoRI I site was done to facilitate subcloning. Inserts with real mutations were excised by EcoRI and Xho I digestion from pBS vector and subcloned into the EcoRI and Sal I sites of the expression vector pCDM8. COS-1 cells were transfected with 15 μg of each vector with insertion in combination with 1 μg of the β-actin promoter driven luciferase expression vector as an indicator of transfection efficiency.

Assays of αL2FT and luciferase activities. Luciferase activity was measured as previously described. The detailed method for measuring αL2FT activity was described in a previous report. In brief, phenyl-β-D-galactose (Phe-Gal) was used as an acceptor substrate, and assays were performed in a total volume of 100 μL containing 50 mM/ L Tris-HCl (pH 7.2), 0.3% Triton X-100, 10 mM/ L NaNO3, 10 mM/ L MnCl2, 5 mM/ L adenosine triphosphate (ATP), 25 μM/ L guanosine diphosphate (GDP)-fucose, 0.2 μM/ L B-H-GDP–fucose, 30 mM/ L Phe-Gal, and 10 μg of protein of soluble fraction of homogenate of the transfected COS-1 cells. After incubation at 37°C for 1 hour, the reaction mixture supernatant was diluted with 5 mL of water and applied to Sep-Pak plus C18 (Waters, MA) for binding of Phe-Gal. B-Gucose–incorporated Phe-Gal was eluted from the Sep-Pak plus C18 with 5 mL of methanol, and the radioactivity in the eluate was counted in a liquid scintillation counter. The actual radioactivity was divided by luciferase activity directed by the constant from transfection to transfection with errors within plus or minus 20%.

RESULTS

RBC- and saliva-phenotypes of Bombay and para-Bombay individuals. The RBC and saliva phenotypes of two control ordinary O-type individuals (YN and KN), four Bombay individuals (KK, KaT, JM, and GO) and 13 para-Bombay individuals (AI, MO, AM, HN, YH, MY, HF, KT, HiN, NK, HA, SK, and YT) were determined to confirm the alleged phenotypes, and results are summarized in Table 2. ABH antigens on erythrocytes of all Bombay and para-Bombay individuals were not detected by the ordinary method of hemagglutination. Using a more sensitive method, the adhesion-elution test described in Materials and Methods, 13 para-Bombay individuals, including two Omh (B)-type (MO and AI), two Ah-type (YT and AM), two Bh-type (NK and HiN), one ABh-type (HF), and six Omh-type (HN, YH, MY,
was ®rst determined. The PCR products of the full-length 

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Abbreviations: NT, not tested; +w, weakly positive.

Table 2. Genotypes and Phenotypes of Bombay and Para-Bombay Individuals

KT, HA, and SK) individuals, were determined to have a trace amount of ABH antigens on erythrocytes, whereas three Oh-type (KK, KaT, and JM) and one Oh (A)-type individual (GO) completely lacked erythrocyte expression of the ABH antigens. Among the 13 para-Bombay individuals, AI, MO, HA, SK, and YT possessed fewer H antigens than the others. Their secretor status was also determined by detection of ABH antigens in saliva, and six persons, KK, KaT, JM, GO, HA, and SK, were determined to be nonsecretors. Thus, three Oh-type and one Oh (A)-type individuals, KK, KaT, JM, and GO, were confirmed to be Bombay individuals who lack ABH antigens either on erythrocytes or in saliva. The other 13 were classified as para-Bombay individuals because they had different phenotypes from the Bombay individuals. They were determined to be para-Bombay individuals, as they had markedly decreased levels of ABH antigens on erythrocytes, regardless of their secretor status.

Point mutations in H genes of Bombay and para-Bombay individuals found by sequencing. The full-length sequences of the H genes of one Bombay, KK, in Table 2, and four para-Bombay individuals, MO, KN, KA, and NK, in Table 2, were determined to identify point mutations. Identification of point mutations in the H genes of these individuals was performed sequentially as described in Materials and Methods by combining full-length sequencing of the H gene for identification of mutations with PCR-RFLP assay for the detection of mutations. The sequence of the H gene of KK was first determined. The PCR products of the full-length H gene from KK amplified by mk1 and mk2 primers were cloned into a pBS vector and then eight clones were fully sequenced. The eight clones shared the same point mutation, G695A, which creates a termination codon, changing TGG (Trp232) to a termination codon TAG, and terminates the H gene translation. The allele having this mutation was named h1. As all eight clones of KK shared the G695A nonsense mutation, he was expected to be a homozygote for the h1 allele. PCR-RFLP assay was performed to detect the G695A mutation on the 17 Bombay and para-Bombay individuals. From the individuals who were determined not to have h1 allele by this assay, the H gene of MO was fully sequenced and was found to have a single base deletion, G990-del, which we named the h2 allele. The G990-del, located at the C-terminal, changes the reading frame and the original 35 amino acids are replaced by an additional five amino acids.

In this manner, we found three more mutant alleles in the H genes of para-Bombay individuals. The H genes of KN and HA were found to have a single missense mutation each for the catalytic region of the enzyme, T721C (Tyr241 to His) and G1042A (Glu348 to Lys), which we named the h3 and h4 alleles, respectively. The H gene of NK was found to have two missense point mutations, T460C (Tyr154 to His) and G442T (Asp148 to Tyr), which we named the h5 allele. The G990-del, located at the C-terminal, changes the reading frame and the original 35 amino acids are replaced by an additional five amino acids.
For detection of the G990-del in the \( h_2 \) allele, the mk6 primer, mismatched at position 992 to the original \( H \) gene sequence, was designed to create an \( Xho \) I site in the \( h_2 \) allele 116 bp PCR product. As seen in Fig 2B, both alleles of AI and MO were cleaved into two fragments, 93 and 23 bp, by \( Xho \) I digestion, indicating that these individuals were homozygotes with the \( h_2/h_2 \) genotype, whereas the other 15 individuals did not have the \( h_2 \) allele. Detection of the \( h_3 \) allele was performed with the mk7 and mk8 primer set, and the result is shown in Fig 2C. Five para-Bombay individuals, AM, HN, YH, MY, and HF, were determined to have an \( h_3/h_3 \) genotype because both their alleles were cleaved into two fragments, 77 and 24 bp, by \( Apa \) I digestion. The other three para-Bombay individuals, KT, HiN, and NK, were heterozygotes having a single \( h_3 \) allele because \( Apa \) I digestion of their PCR products resulted in three fragments of 101, 77, and 24 bp.

The original \( H \) gene sequence possesses an \( Eam \) 1105 I site (GACNNNNNGTC) at position 442. The G442T missense mutation in the \( h_4 \) allele removes this \( Eam \) 1105 I site. The full-length \( H \) genes of Bombay and para-Bombay individuals amplified with the mk1 and mk2 primer set were subjected to \( Eam \) 1105 I digestion for detection of the G442T mutation in the \( h_4 \) allele. As seen in Fig 2D, the 1.2-kb full-length \( H \) genes of all 15 individuals, except for HA and SK, were cleaved into two fragments, 0.7 and 0.5 kb, while the \( H \) gene of HA and SK showed an undigested 1.2 kb band. This indicated that HA and SK are homozygotes for the G442T mutation and that the others were negative for the \( h_4 \) allele. Determination of the two \( h_5 \) allele mutations, T460C and G1042A, by PCR-RFLP was performed using the mk9 and 10 primer set and the mk11 and 12 primer set, for detection of each mutation, respectively. PCR with mk9 and mk10 creates an \( Apa \) I site on the \( h_5 \) allele PCR product, and the product is separated into 106 and 20 bp fragments by \( Apa \) I digestion. The G1042A mutation creates a \( Bst\) XI site (CCANNNNNNTGG) in the \( H \) gene sequence, so the 125-bp PCR product of the mutated gene with the mk11 and mk12 primer set, which amplify the fragment encompassing the position 1042, was cleaved by \( Bst\) XI. As seen in Fig 2E and F, YT was homozygous for the \( h_5 \) allele, possessing both the mutations in both alleles and NK was heterozygous for the \( h_5 \) allele. The \( H \) genotypes of all 17 individuals examined are summarized in Table 2.

To explore the role of \( H \) and \( Se \) genes on the expression of \( H \) antigens on erythrocytes and in saliva and to examine the relationship between \( H \), \( Se \), and \( Le \) genes, the \( Se \) and \( Le \)
genes of the four Bombay and 13 para-Bombay individuals was determined. In our previous studies on more than 200 Japanese individuals randomly sampled,\textsuperscript{7,11} Japanese Se genes were classified into two active alleles, Se\textsubscript{1} with no mutation and Se\textsubscript{2} with the C357T silent mutation, and one inactivated allele, se\textsubscript{j} having the C357T and A385T missense mutations. Le genes were classified into one active allele, Le with no mutation, and two inactivated alleles, le\textsubscript{1} with the T59G and G508A mutations and le\textsubscript{2} with the T59G and T1067A mutations. The Se genes of the 17 Bombay and para-Bombay individuals were examined by PCR-RFLP as described previously.\textsuperscript{7,11} As shown in Fig 2G, 13 of the 17 individuals were homozygous for the C357T mutation, as both Se alleles amplified by PCR were cleaved into a 70- and a 28-bp fragment by Ase I digestion. The other individual (NK) was determined to be heterozygous for this mutation. AI, MO, and YT were determined to be homozygotes with the Se\textsubscript{1}/Se\textsubscript{1} genotype because they did not have the C357T

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**Fig 2.** PCR-RFLPs for detection of each of mutant H and Se alleles. Profiles of PCR-RFLPs for detection of h\textsubscript{1} (A), h\textsubscript{2} (B), h\textsubscript{3} (C), h\textsubscript{4} (D), and h\textsubscript{5} (E and F) alleles in H gene, and profiles of PCR-RFLPs for genotyping of Se gene (G and H) are presented. Initials of each individual are at the top. M, marker.
mutation. Results shown in Fig 2H indicate that KK, KaT, JM, GO, HA, and SK were A385T homozygotes, as both alleles of their amplified Se genes were cut by Alu I. From the results in Fig 2G and H, both Se gene alleles of KK, KaT, JM, GO, HA, and SK had the C357T and A385T mutations. Their Se genotypes were assigned to be sej/sej.

Family studies of the mutant h genes. Family studies both the H and Se genes (h1/h3 and Se2/sej). These results indicate that in relation to the Se gene, the h1 and h3 alleles form h1-sej and h3-Se2 haplotypes, respectively. The G695A point mutation in the h1 allele and T721C in the h3 allele must have occurred on the chromosome with the sej allele or the Se2 allele, respectively. The Se genotype of the remaining one h3 allele heterozygote, HN (h1/h3), was determined to be Se2/Se2. To explain HN’s H and Se genotypes, we assume that a recombinatorial event between the H gene and the Se gene must have previously given rise to the chromosome possessing the h1 allele.

In the present study, we could find only two h2/h2 (AI and MO), two h4/h4 (HA and SK), one h5/h5 (YT) homozygotes, and one h3/h5 heterozygote (NK). Although numbers of individuals with h2, h4, or h5 gene were limited, determination of Se genotypes of these individuals seemed to indi-
COS-1 cells mock-transfected with pCDM8 vector; pCDM8/H, activity of H-transfected COS-1 cell lysate; pCDM8/h1, h2, h3, h4, or h5- transfectected COS-1 cell lysate, respectively. The activity in each lysate was normalized by transfection efficiency, which was determined by expression of cotransfected luciferase expression vector. Data represent means and standard deviation (SD) of three independent assays.

Fig 4. α1,2FT activity directed by each of the wild-type H and five mutant H alleles. From left to right, pCDM8, activity of lysate of COS-1 cells mock-transfected with pCDM8 vector; pCDM8/H, activity of H-transfected COS-1 cell lysate; pCDM8/h1, h2, h3, h4, or h5- transfectected COS-1 cell lysate, respectively. The activity in each lysate was normalized by transfection efficiency, which was determined by expression of cotransfected luciferase expression vector. Data represent means and standard deviation (SD) of three independent assays.

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From the results in Table 2, no haplotype linkage was observed between the H gene and the Le gene or between the Se gene and the Le gene.

α1,2FT activity directed by each of the mutant h alleles. We first measured the luciferase activity directed by the cotransfected luciferase cDNA for normalization of transfection efficiency and the amount of H gene transcripts by Northern blot analysis in the COS cell transfection assays (data not shown). It was confirmed that the mRNA expression of the H genes was almost at the same level between the wild type gene and the five mutant genes. Translation must occur at the same level between the wild type and the mutant enzymes because it is very hard to consider that only a few amino acid substitutions in h3, h4, and h5 enzymes and a frameshift in h2 enzyme affects the translational efficiency.

As shown in Fig 4, lysates of the COS-1 cells transfected with the wild type H allele showed full α1,2FT activity. The COS-1 cell lysates transfected with the mutant allele, h1, did not exert any activity at all, whereas those transfected with the h2, h3, h4, or h5 allele showed quite low, but positive activity. The h2, h3, h4, and h5 enzymes showed 3.3%, 11.8%, 3.5%, and 1.6% of the activity of the wild type H-transfected cell lysates, respectively. The single base deletion, G990-del in the h2 allele, and the missense mutations, T721C (Tyr241 to His) in the h3 allele, G442T (Asp148 to Tyr) in the h4 allele and T460C (Tyr154 to His) and G1042A (Glu348 to Lys) in the h5 allele did not cause complete inactivation of the encoded enzymes, and exerted residual activity.

H antigen levels on erythrocytes are determined solely by H enzyme activity, while expression of Lewis b (Leb) antigen on erythrocytes is determined by Le and Se enzyme activities and secretor status is determined solely by Se enzyme activity. It is now clear that the H gene and the Se gene encode different α1,2FTs. The results summarized in Table 2 indicate clearly that the H enzyme determines the expression of H antigens on erythrocytes, while the Se enzyme determines the secretor status and, in cooperation with the Le enzyme, the expression of Leb antigen on erythrocytes.

The residual H enzyme activity encoded by the h2, h3, h4, or the h5 allele was in good agreement with the level of ABH antigen expression on erythrocytes shown in Table 2. The 13 para-Bombay individuals, eight of whom having the Omh phenotype, two of whom having the Ah phenotype, two of whom having the Bh phenotype and one of whom having the ABh phenotype, possessed a trace amount of H antigen on their erythrocytes, which could be detected by the adsorption-elution test as shown in Table 2. All of the 13 para-Bombay individuals possessed at least one of the weakened H alleles, h2, h3, h4, or h5. AI, MO, HA, SK, and YT having fewer RBC ABH antigens than the other eight individuals as determined by the adsorption-elution test, were homozygotes with the h2, h4, or h5 allele, respectively. This agrees with the finding that the H enzyme activity directed by h2, h4, and h5 was weaker than that directed by h3. All of the individuals who completely lack H antigens on erythrocytes, KK, KaT, JM, and GO (Table 2) were determined to be completely inactivated h1 allele homozygotes (Table 2 and Fig 4).

All 11 secretors in Table 2 who secreted H antigen into saliva possessed at least one of the active Se genes, Se1 or Se2, and six nonsecretors possessed homozygous alleles of the inactivated Se gene, sej (Table 2). Nine individuals, AI, AM, YH, MY, HF, KT, HiN, NK, and YT, having the Le(a - b - ) phenotype, possessed at least one of the active Le and Se genes, homozygously or heterozygously. Six individuals, KK, KaT, JM, GO, HA, and SK, having the Le(a - b - ) phenotype, possessed at least one of the active Le genes, but their Se genes were inactive with the homozygous sej/sej genotype. Two Le(a - b - ) individuals, MO and HN, were confirmed to lack the active Le gene, regardless of the Se genotype.

DISCUSSION

Both the H and Se enzymes are α1,2FTs with subtle differences in substrate specificity. It was clearly shown in this study that the H enzyme activity determines the amount of ABH antigens on erythrocytes, while the Se enzyme activity determines the secretor status and, in cooperation with Le enzyme, Leb expression on erythrocytes. Both the H and Se genes of Bombay individuals were confirmed to be inactivated by point mutations. The absence of ABH antigens on erythrocytes of four Bombay individuals was explained by complete inactivation of their H genes due to the nonsense mutation. A variety of phenotypes of the 13 para-Bombay
individuals in this study were clearly explained by independent expression of H and Se enzymes. The weak expression of ABH antigen on erythrocytes of para-Bombay individuals was explained by residual h2, h3, h4, and h5 enzyme activity and the presence of ABH antigens in the saliva of some para-Bombay individuals was explained by the Se enzyme activity.

It is of interest to know where the active sites of the enzyme lie within the known primary structure. One study analyzed the donor substrate binding site of α1,3fucosyltransferases using sulfhydryl-group reagents, but the enzyme active sites of many fucosyltransferases have not been analyzed. The enzymes retaining residual activity like the h3, h4, and h5 enzymes might have a folded structure, and the positions of mutations might exist at active sites of the enzyme, such as the acceptor- or the donor-binding site. Together with missense mutations in h3, h4, and h5 alleles, further study of missense mutations differing from those in h3, h4, and h5 alleles in the other para-Bombay individuals would clarify the active sites of the H enzyme.

The H and Se genes form a gene cluster on 19q13.3 and are located only 35 kb apart. The Le gene also maps to 19p13.3 within the gene cluster of the α1,3fucosyltransferase family, but is distant from the H and Se genes. In this study, determination of the H, Se, and Le genotypes of the four Bombay and 13 para-Bombay individuals showed interesting results that each of the mutant h alleles was closely associated with specific haplotype of Se gene, ie, h1-sej, h2- Se1, h3-Se2, h4-sej, and h5-Se1 haplotypes, in the Japanese population, although the number of individuals with h2, h4, or h5 gene was limited.

The sej allele inactivated by the single A385T missense mutation is known to be widely distributed in the Japanese, the Chinese in Taiwan, and Polynesians, although its distribution in other ethnic groups has not been analyzed. In our previous study, all 35 Japanese nonsecretors examined were homozygotes with the inactive allele, sej/sej. The Se enzyme-inactivating G428A mutation found in Caucasians has never been found in Japanese samples. From the observation of ethnic group specific point mutations, which inactivate Se gene, we assumed that Se gene inactivation must have occurred after these ethnic groups diverged. As for further study of missense mutations differing from those in h3, h4, and h5 alleles in the other para-Bombay individuals, we conclude that mutations that inactivate the H gene occurred after Se gene inactivation, as would clarify the active sites of the H enzyme.

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as the le1 allele inactivated by the G508A missense mutation, which is rather widely distributed in the Japanese population,\textsuperscript{3,11} is uncommon among Caucasians.\textsuperscript{9}

In the present study, we collected the 17 Bombay or para-Bombay individuals through routine determination of ABO blood types on a large number of randomly sampled volunteers who donated their blood to Red Cross Blood Centers. Thus, the incidence of Bombay and para-Bombay individuals was determined to be very rare and a doctor in the Red Cross Blood Center conjectured that it would be approximately one in two or 300,000 in the whole Japanese population. In contrast to the wide distribution of the single mutant alleles of Se and Le genes, it was found that the point mutations inactivating the H gene are very variable and are specific to the individuals and their family. This is consistent with the very rare frequency of inactivated H alleles in the whole human population. The birthplaces of the 17 individuals examined in this study are indicated in Table 2 and in the map of Japan of Fig 5, in which we notice that the birthplaces of three Bombay individuals with the h1/h1 and se1/se1 genotypes, KK, KaT, and GO, are localized in a restricted area, the Nobi plane. Although they are not aware of any blood relations, they must have common ancestors from the near past. Two h2/h2 homozygotes, AI and MO, who are also not aware of blood relations, were found in Osaka. The G990 deletion in the h2 allele may have occurred in the H gene of their common ancestor from the near past. Fukui, the birthplace of MY and YH with the h3/h3 genotype, has been culturally and geographically isolated by mountains. MY and YH also do not know their blood relations, but we assume that the h3 allele must have appeared in a common ancestor of MY and YH in the Fukui area. The h3 mutant allele seemed to be rather widely distributed in the Japanese population than the other mutant h alleles. The h4 allele found in HA’s family may be specific to the Tokushima area in Shikoku island, which is separated by the sea from the other places or may be specific to HA’s family because we have not found the h4 allele in anyone except them. As for the h5 allele, we found only one h5/h5 homozygote in Okayama, so it is impossible to make any conjecture on the original place of emergence of the h5 mutant allele. Kanagawa, the birthplace of KF (h2/h2), and Gunma, those of NK (h3/h5) and Hn (h1/h3), are relatively new areas of settlement within Japan’s history. Therefore, we conjecture that their ancestors moved from Fukui or the Nobi plane to Kanagawa or Gunma.

The above results suggested that consanguineous marriages in a closed society seemed to give rise to rare homozygous individuals with inactive H genes. H gene inactivation by point mutations must have occurred in the nearer past than that of Le and Se genes.

In a previous report,\textsuperscript{11} we raised an interesting question as to why a sole inactivated Se allele or only two inactivated Le alleles spread in each ethnic group. In the case of the H gene, which is highly homologous to the Se gene, the mutant H alleles do not seem to have spread in the population. There might be some specific selective advantage on the individuals with the mutant Se and Le alleles, but some selective disadvantage on the individuals with the mutant H alleles.

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REFERENCES


Wide Variety of Point Mutations in the H Gene of Bombay and Para-Bombay Individuals That Inactivate H Enzyme

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