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Heterogeneity of the Human *H* Blood Group α (1,2) Fucosyltransferase Gene among Para-Bombay Individuals

Abstract

Background and objectives: The para-Bombay phenotype has a relatively high frequency of about 1 in 8,000 Taiwanese. Studies were carried out on eight healthy and unrelated Taiwanese with the para-Bombay phenotype to cast light on its immunogenetic basis. **Materials and methods:** Blood and saliva samples were tested with standard hemagglutination techniques. Salivary ABH substances were determined by hemagglutination inhibition. PCR techniques were used to amplify the coding region of the *H* genes. **Results:** Five different *h* alleles, designated as *h1, h2, h3, h4* and *h5,* were identified in the Taiwanese with the para-Bombay phenotype. The *h1* allele loses one of the three AG repeats located at the nucleotides 547–552 of the *H* gene, whereas two of the three T repeats located at the nucleotides 880–882 are deleted in the *h2* allele. The *h3* allele contains a C^{658} to T missense mutation, whereas two missense mutations, C^{35} to T and A^{980} to C were identified in the *h4* allele. A T^{460} to C missense is present in the *h5* allele. The *h5* allele was identified in an individual whose red blood cells contain blood group A antigen but not H antigen, and thus may be considered a weak variant of the *H* gene. **Conclusions:** So far no biologic relevance of the H antigen has been discovered, and its deficiency does not seem to produce any deleterious effects. There may be better understanding of the evolutionary basis for the polymorphisms at these loci after systematic study of different ethnic populations.

Introduction

The human ABO blood group antigens are oligosaccharide molecules constructed by the sequential action of specific glycosyltransferases [reviewed in 1]. The allelic glycosyltransferases encoded by the *ABO* blood group locus [2, 3] determine the final step in this pathway, using a common substrate, the H blood group antigen (Fucα1–2Galβ1-4G1cNAcβ1-R). The H blood group antigen on red blood cells (RBC) is synthesized by the transfer of fucose to the C-2 position of Gal on type II precursor substrate (Ga1β1–4G1cNAcβ1-R), through the action of α(1,2)-fucosyltransferase encoded by the *H (FUT1)* blood group locus [4]. Individuals of the rare Bombay [5–7] or para-Bombay

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[7–9] phenotypes lack the H antigen in the erythroid lineage, and thus the downstream products of A and B antigens are also absent (or markedly reduced) on RBC. The H blood group deficiency in these individuals is believed to result from a defect in the *H* gene.

In 1994, the molecular bases for the Bombay and para-Bombay phenotypes were first reported by Kelly et al. [10]. Three null *h* alleles, bearing the T^{491} to A, C^{826} to T and C^{948} to G point mutations, respectively, resulting in amino acid codon alterations of Leu^{164} to His, Gln^{276} and Tyr^{316} to stop codons, respectively, were demonstrated to be responsible for the H blood group deficiencies in the Bombay and para-Bombay phenotypes. In the same year, four other mutations identified in the *H* genes of H antigen-deficient individuals were reported by Johnson et al. [11]. These mutations comprised base substitutions at nucleotide positions 801 and 832, predicting amino acid substitutions of Trp^{267} to Cys and Asp^{278} to Asn, respectively, and two and one base deletions of $TTT^{880–882}$ to T and $CT\underline{G}^{990}$ to CT at amino acid codons 294 and 330, respectively.

H blood group-deficient phenotypes are very rare in most populations, but there is a relatively high frequency of about 1 in 8,000 among the Taiwanese [12]. All the H antigen-deficient individuals identified among the Taiwanese are classified as the para-Bombay phenotype, since the RBC A or B antigens in these individuals are usually detectable only by absorption and elution tests with anti-A or anti-B antibodies. In addition, all cases have been secretors with normal amounts of ABH substances present in the saliva [13]. In this paper, five different *h* alleles with deletions or missense mutations in the coding region of the *H* gene identified among the Taiwanese of the para-Bombay phenotype are reported.

Materials and Methods

Sample Preparation

Fresh blood and saliva samples of 8 healthy and unrelated Taiwanese with the para-Bombay phenotype, denoted TP1, YHK, HYTP, HuSC, JYT, HST, WRT and SCR, respectively, were collected and tested in this study. Their RBC ABH antigens and serum antibodies were detected by standard hemagglutination and absorption and elution tests, and salivary ABH substances were determined by a salivary inhibition study [14]. Samples of 7 of these 8 individuals demonstrated the typical serological reactions of other para-Bombay Taiwanese identified previously [13]. However, the ABH phenotype on RBC of HYTP was different to those of the other seven samples. A fairly large amount of A antigen (3+ agglutination with anti-A), but no H and B antigens, was detected on the RBC of HYTP and the serum of HYTP contained a very low titer of anti-A but a normal anti-B and a relatively high titer of anti-H. Genomic DNAs of these individuals were prepared from leukocytes by a proteinase K/SDS method [15].

Molecular Cloning of the H Allele

The polymerase chain reaction (PCR) and a pair of specific primers for the H α(1,2)fucosyltransferase gene [4] were used to amplify the coding region of the *H* genes. The sense primer locates at nucleotides –29 through –3 of the *H* gene (TGCTAATTCGCCTTTCCTCCCCTGCAG), 5' to the initiation codon, and the antisense primer (CTGAAGCCACGTACTGCTGGCTCTAG) is complementary to nucleotides 1134 through 1159 within the 3' untranslated region. 150 ng of genomic DNA and 0.6 μ*M* of each primer were combined in 50 μl of PCR buffer containing low concentrations of dNTP (25 μ*M* each) and Taq polymerase (0.5 unit; Promega, Madison, Wisc., USA) to minimize the PCR-mediated DNA sequence alteration. The PCR program consisted of 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 1 min at 65°C and 1.5 min at 72°C. The PCR products of 1,188 bp were cloned into pGEM-T vectors (Promega). DNA sequences were determined by the dideoxy chain termination method, using an AmpliCycle Sequencing Kit (Perkin Elmer, Foster City, Calif., USA). Multiple clones from each individual were analyzed to demonstrate the genotype of the *H* locus of each individual and to distinguish PCR errors from actual sequence polymorphisms.

Results

The coding sequences of the *H* genes in these para-Bombay individuals were cloned and analyzed. Five different *h* alleles, designated as *h1, h2, h3, h4* and *h5*, respectively, were identified among these 8 individuals (fig. 1). The *h1* allele loses one of the three AG repeats located at nucleotides 547–552 of the *H* gene, whereas two of the three T repeats at nucleotides 880–882 are deleted in the *h2* allele. The *h3* allele has a C^{658} to T point mutation, predicting an amino acid substitution of Arg^{220} to Cys. Two missense mutations, C^{35} to T and A^{980} to C, predicting amino acid substitutions of Ala^{12} to Val and Asn^{327} to Thr, respectively, are present in the *h4* allele. A T^{460} to C mutation was identified in the *h5* allele, which predicts a Tyr^{154} to His substitution. Apart from the same deletion in the *h2* allele as reported previously by Johnson et al. [11], the other four *h* alleles are newly identified.

The coding region of the *H* gene from a Taiwanese individual of normal ABH phenotype was also cloned and analyzed, and demonstrated a wild type *H* allele with a coding sequence identical to that reported by Larsen et al. [4].

Through analyzing multiple clones from each individual, the genotype at the *H* locus of each individual was determined and revealed different combinations of the five *h* alleles. YHK and HuSC were both *h1/h2* heterozygotes. TP1 was an *h1/h3* heterozygote, and the combinations *h2/h3* and *h2/h4* were identified in HST and JYT, respectively. HYTP was an *h1/h5* heterozygote. WRT and SCR both appeared to be homozygous for the *h1* allele, since all the thirteen clones from each individual demonstrated deletion of the *h1* allele.

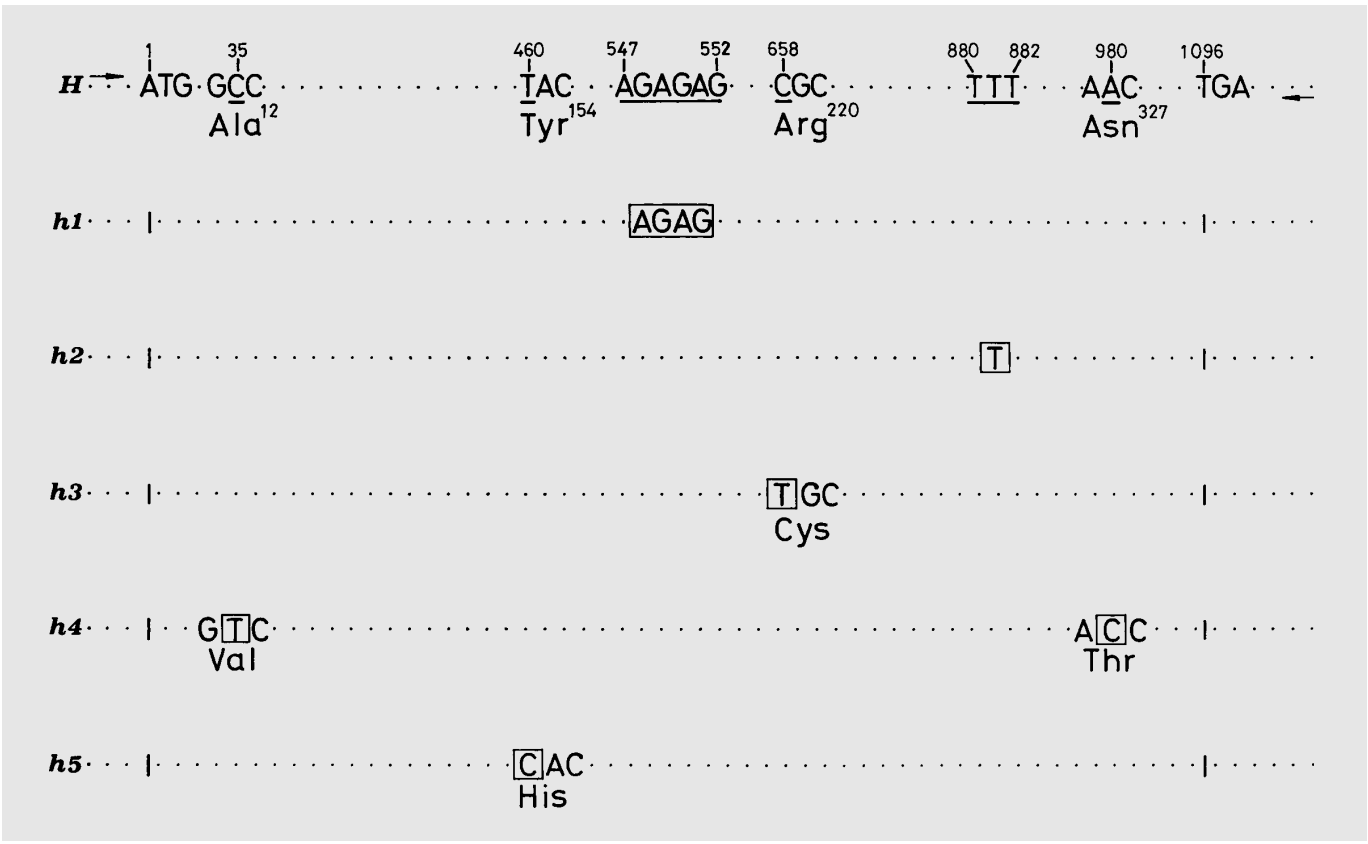

Fig. 1. *H* indicates the coding region of the *H* α(1,2)fucosyltransferase gene reported by Larsen et al. [4]. The ATG and the TGA codons correspond to the initiation and termination codons of the enzyme, respectively. PCR primers used to amplify the coding region are indicated by arrows. *h1, h2, h3, h4* and *h5* represent the variants of the *H* allele. Positions of deletions and point mutations found in these variants are boxed and the corresponding wild type nucleotides of the *H* allele are underlined.

Discussion

It appears that the deletions and missense mutations demonstrated in the *H* alleles in the above cases abolish, or at least greatly reduce, the activity of the corresponding α(1,2)fucosyltransferase, which results in the H blood group antigen deficiency observed in these para-Bombay individuals. The two alleles with deletions, *h1* and *h2*, are believed to express nonfunctional α(1,2)fucosyltransferase enzymes, since these deletions change the reading frames after the deleted nucleotides and create stop codons in the coding region. The *h3* and *h4* alleles, comprising missense mutations, also appear to express nonfunctional enzymes, based on the serological picture of the typical para-Bombay phenotype produced in TP1, JYT and HST, but awaits confirmation by functional analysis of the two alleles. However, HYTP was quite different to the other 7 para-Bombay individuals, as the RBC contains easily detectable A antigen but no H antigen (see Sample Preparation of Materials and Methods). This phenomenon implicates that there is some weak activity of the *H* α(1,2)fucosyltransferase enzyme present in HYTP which synthesizes a small amount of H antigen, most of which is transformed to the A antigen by a normal blood group A transferase [2, 3]. Thus the *h5* allele with a T^{460} to C missense mutation, which together with an *h1* allele composes the heterozygous genotype of HYTP, appears to result in the expression of an α(1,2)fucosyltransferase enzyme with weak activity. Therefore the *h5* allele may not be a null *h* allele but a weak variant of the *H* allele. Functional analysis of the *h5* allele and comparison of the enzyme activity resulting from the *h5* allele with those from the other alleles will hopefully confirm or disprove this hypothesis.

Five different *h* alleles were identified in 8 Taiwanese with the para-Bombay phenotype, and therefore together with the three null *h* alleles reported by Kelly et al. [10] and

the three mutations of the *H* genes not found in the Taiwanese reported by Johnson et al. [11], a total of eleven different *h* alleles have now been demonstrated. It is not surprising that so many polymorphisms at the *H* locus exist in para-Bombay Taiwanese, since heterogeneity of the para-Bombay phenotype in Taiwanese has been suggested previously as a result of different agglutination patterns by monoclonal antibodies observed in this phenotype [16]. It is quite possible that other variants of the *H* allele, including both null alleles or weak variants, still await identification in Taiwanese.

It has also been of interest that out of a total of 25 cases of the para-Bombay phenotype among the Taiwanese analyzed, only the Lewis (a–b+; 92%) and the Lewis (a–b–; 8%) phenotypes have been observed, but no cases of the Lewis (a+b+) phenotype. In addition, of 55 Hong Kong para-Bombay individuals 88% were of the Le(a–b+) phenotype, 8% were of the Le(a–b–) phenotype and only 4% were of the Le(a+b+) [Mak, person. commun.]. Since the Lewis phenotype frequencies are Le(a–b+) 67%, Le(a+b+) 25% and Le(a–b–) 8% among Taiwanese [17] and similar frequencies are also observed among Hong Kong Chinese [18], the data from both Taiwan and Hong Kong para-Bombay individuals show significant discrepancies in the incidences of the various Lewis phenotypes, compared with those of the general population.

So far, no biological relevance of the blood group H antigen has been discovered, and the H antigen deficiency of Bombay and para-Bombay individuals does not appear to result in any deleterious effects. The same situation has been found at the *Se* locus, which is closely related to the *H* locus and also synthesizes blood group H determinants in epithelia derived from embryonic endoderm. Many polymorphisms have also been identified at the *Se* locus [19–26], but no obvious selective disadvantages associated with the nonsecretor or partial secretor phenotypes, resulting from these polymorphisms in the *Se* gene, have been observed. There may be a better understanding of the evolutionary basis for the polymorphisms at these loci after correlation of the different phenotypes with the polymorphisms at these two loci in different ethnic populations has been systematically studied, or after any associations between specific phenotypes determined by these loci and susceptibility to specific diseases has been demonstrated, or when the biological relevance of the oligosaccharide molecules constructed by the two loci is better understood.

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