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Molecular basis for H blood group deficiency in Bombay (O_h) and para-Bombay individuals

(ABO blood group/Secretor blood group/oligosaccharide biosynthesis/fucosyltransferase/surface antigen)

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ABSTRACT **The penultimate step in the biosynthesis of the human ABO blood group oligosaccharide antigens is catalyzed by α-(1,2)-fucosyltransferase(s) (GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase, EC 2.4.1.69), whose expression is determined by the *H* and Secretor (*SE*) blood group loci (also known as *FUT1* and *FUT2*, respectively). These enzymes construct Fucα1 → 2Galβ-linkages, known as H determinants, which are essential precursors to the A and B antigens. Erythrocytes from individuals with the rare Bombay and para-Bombay blood group phenotypes are deficient in H determinants, and thus A and B determinants, as a consequence of apparent homozygosity for null alleles at the *H* locus. We report a molecular analysis of a human α-(1,2)-fucosyltransferase gene, thought to correspond to the *H* blood group locus, in a Bombay pedigree and a para-Bombay pedigree. We find inactivating point mutations in the coding regions of both alleles of this gene in each H-deficient individual. These results define the molecular basis for H blood group antigen deficiency in Bombay and para-Bombay phenotypes, provide compelling evidence that this gene represents the human *H* blood group locus, and strongly support a hypothesis that the *H* and *SE* loci represent distinct α-(1,2)-fucosyltransferase genes. Candidate sequences for the human *SE* locus are identified by low-stringency Southern blot hybridization analyses, using a probe derived from the *H* α-(1,2)-fucosyltransferase gene.**

The antigens determined by the human ABO blood group locus are oligosaccharide molecules constructed by the sequential action of specific glycosyltransferases. The final step in this pathway is catalyzed by allelic glycosyltransferases encoded by the ABO locus. These enzymes require a precursor oligosaccharide substrate (Fucα1 → 2Galβ-) known as the H blood group antigen, constructed by α-(1,2)-fucosyltransferase(s) [α(1,2)FTs; GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase, EC 2.4.1.69]. Genetic and biochemical observations are consistent with a hypothesis that the *H* and Secretor (*SE*) blood group loci (also known as *FUT1* and *FUT2*, respectively) correspond to distinct α(1,2)FT genes with tissue-specific expression patterns and close genetic linkage on chromosome 19 (1–6). Under this two-locus model (2), the human *H* blood group locus determines expression of the H antigen (as well as A and/or B antigens) in the erythroid lineage, whereas the *SE* locus controls H expression (and thus A or B antigen expression) in a variety of secretory epithelia and in saliva (Table 1). Nearly all humans maintain at least one functional *H* allele (1). Roughly 80% of these (Secretors) also maintain at least one functional *SE* allele, whereas the remainder (non-Secretors) are homozygous for null alleles at the *SE* locus. Individuals of the rare O_h (Bombay) phenotype (1, 7) lack H determinants in all tissues and are thought to be homozygous for null alleles at both loci (Table 1) (2). Other rare individuals, of the para-Bombay phenotype (1, 8), synthesize H determinants in their secretory epithelia but not in the erythroid lineage (1). These persons are believed to be homozygous for null alleles at the *H* locus (1, 2) but apparently have at least one functional *SE* allele.

Table 1. Phenotypes and predicted genotypes at the human *H* and *SE* blood group loci (see ref. 2)

Trivial name	Phenotype: Erythroid	Phenotype: Secretory epithelia	Genotype
Secretor	H positive	H positive	*H/H, SE/−*
Non-Secretor	H positive	H negative	*H/H, se/se*
para-Bombay (*h/h* Secretor)	H negative	H positive	*h/h, SE/−*
Bombay (*h/h* non-Secretor)	H negative	H negative	*h/h, se/se*

The basis for null alleles at these loci has not been defined, nor has the two-locus hypothesis been confirmed at the molecular level. We have previously isolated a human α(1,2)FT cDNA whose cognate enzyme is kinetically similar to that of the *H* α(1,2)FT and whose chromosomal location (number 19) is syntenic to the *H* blood group locus (9). To confirm the hypothesis that this cDNA corresponds to the *H* locus, and to identify the molecular basis for deficiency of H determinants in presumed *h/h* individuals, we determined the genomic structure and sequence of a wild-type allele and compared it to alleles taken from H-deficient individuals. Inactivating point mutations were identified within the coding portions of each such allele, including alleles in individuals with wild-type *SE* locus-determined H antigen expression. These results yield a molecular explanation for ABH blood group deficiency in Bombay and para-Bombay individuals, provide genetic support for assignment of this α(1,2)FT gene to the *H* locus, and indicate that the *SE* locus represents an α(1,2)FT gene distinct from the *H* locus.

MATERIALS AND METHODS

Cloning of α(1,2)FT Alleles. Genomic DNA sequences corresponding to a previously described human α(1,2)FT cDNA [pCDM7-α(1,2)FT; ref. 9] were isolated from phage genomic DNA libraries prepared from leukocyte DNA isolated from an H-positive individual and from an H-deficient

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Abbreviation: α(1,2)FT, α-(1,2)-fucosyltransferase.

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individual (X; Bombay phenotype) (10). Libraries were screened by plaque hybridization (11) using a 1.2-kb *Hin*fI fragment isolated from the insert in pCDM7-α(1,2)FT (9). Adjacent *Eco*RI fragments of 2.7 and 6.5 kb from a single phage were subcloned into the unique *Eco*RI site in the vector pWE15 (Stratagene). Both strands of these fragments were sequenced by using oligonucleotide primers corresponding to the insert sequence.

The coding sequences of the two alleles (Y_1 and Y_2) from a para-Bombay individual were generated with the PCR (11), using primer pairs flanking the mutation in Y_2 (Fig. 1). One pair of these primers amplifies a sequence between a position within the intron at a location 187–157 nt 5′ to the A of the initiation codon (GCGCAAGCTTCTCATCCCTGAAACCTAGGACTCAGGCTCT) and at nt 1035–1065 within the coding sequence (taking the A residue of the initiation codon as nt 1, numbered as in ref. 9; GCGCTCTAGAGTCTGCATTAATGCCCACCCACTCGGGCAG) at a site 3′ to the Y_2 mutation. The other primer pair amplifies a sequence between nt 657 and 685 of the coding sequence (5′ to the Y_2 mutation; GCGCAAGCTTGCGCCGTGGGGACTATCTGCAGGTTATGCC) and between nt 1135 and 1166 within the 3′ untranslated region (GCGCTCTAGACAGGCCTCTGAAGCCACGTACTGCTGGCTC). Genomic DNA was subjected to 30 cycles of amplification (denaturation at 94°C for 1.5 min; annealing/extension for 2.5 min at 72°C). Fragments generated with these primer pairs were subcloned into pTZ19R (Pharmacia LKB). Clones corresponding to allele Y_1 and to allele Y_2 were distinguished by digestion with *Bst*NI (cleaves Y_2, not wild type or Y_1, at this position). Multiple clones corresponding to each allele were sequenced to distinguish PCR errors from actual sequence polymorphisms. These analyses identified the mutation in the Y_1 allele corresponding to amino acid residue 164 and indicated that the Y_1 and Y_2 coding regions are otherwise identical to the wild-type coding region.

Expression Vector Construction and Analysis. Expression vectors containing single or multiple mutations were assembled with restriction fragment exchange procedures (11), using restriction sites in the 6.5-kb *Eco*RI fragment that encompasses the two α(1,2)FT exons (Fig. 1), cloned into the *Eco*RI site of vector pWE15 (12). Details of vector constructions are available from the authors. COS-1 cells were transfected by a DEAE-dextran procedure (11). A control vector (pCDM7-CAT) encoding chloramphenicol acetyltransferase [derived from pSV2-CAT (11) and pCDM7 (9)] was cotransfected to allow normalization for transfection efficiency. Cell extracts were prepared 72 hr after transfection (9) and were used in α(1,2)FT activity assays (12) performed in duplicate under linear reaction conditions.

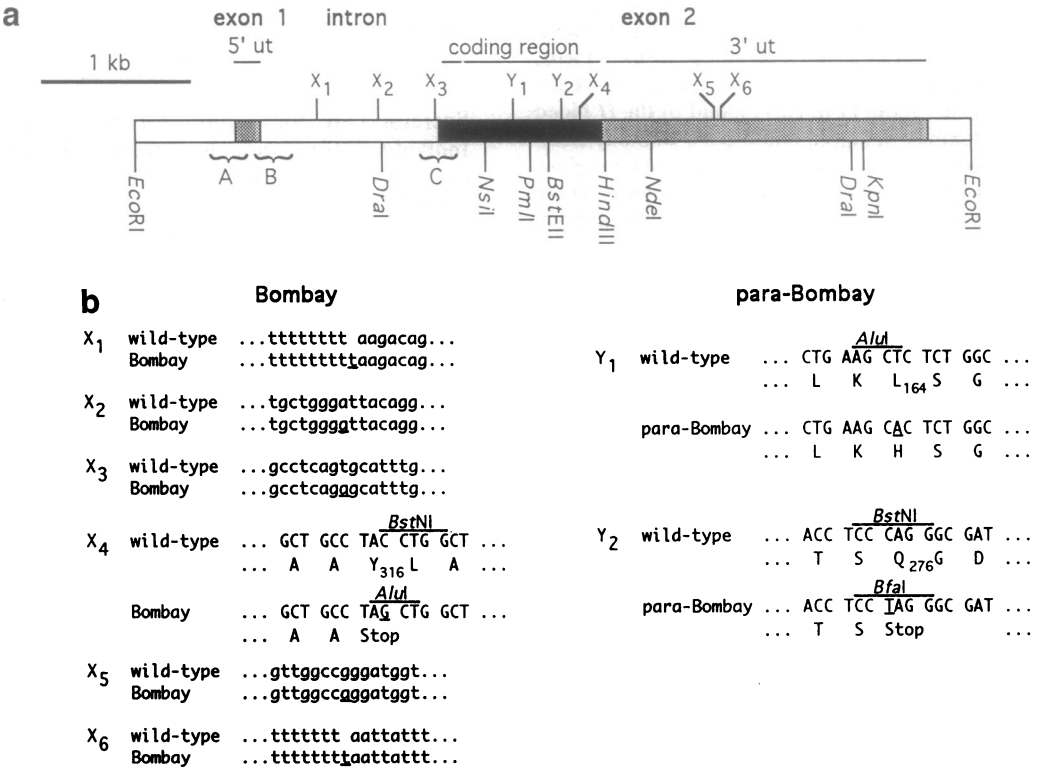

FIG. 1. Structure of wild-type and mutant α(1,2)FT alleles. (*a*) Scale and linear map of the gene. Coding portion of exon 2 is denoted by the solid area. Noncoding portions of exons 1 and 2 are shaded. Positions of DNA sequence differences between a wild-type allele and alleles isolated from H-deficient individuals (X_1–X_6, Bombay; Y_1 and Y_2, para-Bombay) are indicated above the schematic. Region indicated by A below the map corresponds to transcription initiation site. DNA sequence of this area is . . . tcctcccaagcCCTCGCTGCCAGTCCGGACAGGCTGCGCGGAGGGGAGGGCCTGCCGGGCCGGATAGCCGGACGCCTGGCGT . . . , where underlined nucleotides represent the first 9 bases of the human α(1,2)FT cDNA (9). Primer-extension analyses (data not shown) define the gene's transcription initiation site (boldface C) in a cell line (A431) that expresses this gene (9, 12). Region indicated by B corresponds to the 5′ splice junction between exon I and the intron (. . . AGCAGCTCGGgtaagtgg . . .; intronic sequence in lowercase letters). ut, Untranslated region. Region indicated by C in the schematic corresponds to the 3′ splice junction between the intron (lowercase letters) and exon 2 (uppercase letters) (. . .cagtgcatttgctaattcgcctttcctccctgcagCC ATG TGG CTC . . .). Underlined t residue in this sequence corresponds to mutation X_3 (see *b*). Underlined ATG residues correspond to the enzyme's initiator methionine codon (9). Restriction sites used to create chimeric α(1,2)FT vectors (see Fig. 2), and the *Eco*RI and *Dra* I sites used in Fig. 5, are shown below. (*b*) Sequence differences between the wild-type allele and the H-deficient alleles. Nucleotide sequence, predicted amino acid sequence, and amino acid number are shown for each sequence difference. Nucleotide sequence differences in H-deficient alleles are underlined. Sequence differences (X_1–X_6) identified in the Bombay propositus X are at left (Bombay). Polymorphic *Bst*NI and *Alu* I sites corresponding to the X_4 allele are overlined. Sequence differences (Y_1 and Y_2) identified in the para-Bombay individual Y are shown at right (para-Bombay). Polymorphic *Alu* I, *Bst*NI, and *Bfa* I sites corresponding to the Y_1 and Y_2 alleles are overlined.

Extracts were also subjected to chloramphenicol acetyltransferase activity assays (11).

Pedigree Analyses. H and Lewis blood group phenotypes of each individual were determined by standard blood typing procedures (13). For PCR analyses, genomic DNA was prepared from peripheral blood (11), or from freshly plucked hairs (14), and was used as PCR template under the reaction conditions described above. PCR primers used to sample the X_4 and Y_2 positions (GCGCAAGCTTGCGCCGTGGGGACTATCTGCAGGTTATGCC and GCGCTCTAGAGTCTGCATTAATGCCCACCCACTCGGGCAG) correspond to positions 657–686 and 1035–1065, respectively, of the α(1,2)FT cDNA (9). Thirty nucleotides of each primer (underlined) are derived from the cDNA sequence. Ten flanking nucleotides include a restriction site (*Hin*dIII, AAGCTT; *Xba* I, TCTAGA). The Y_1 position was analyzed using nested PCR primers. The outermost pair corresponds to positions 335–364 (TCCTGCCTGCCATGCATGCCGCCCTGGCCC) and 657–686 (GGCATAACCTGCAGATAGTCCCCACGGCGC) of the α(1,2)FT cDNA (9). The inner pair corresponds to positions 366–395 (GGTATTCCGCATCACCCTGCCCGTGCTGGC) and 626–655 (CGTGGACGCCGACAAAGGTGCGCGGGCGGT).

PCR products were then subjected to restriction endonuclease digestion and acrylamide gel electrophoresis (see Figs. 3 and 4). Products encompassing the Y_1 position were also fixed to nylon hybridization membranes and probed with ^{32}P-labeled (11) allele-specific oligonucleotides (15) (wild-type probe, CTGAAGCTCTCTGGC; Y_1-specific probe, CTGAAGCACTCTGGC) (see Fig. 4). Filters were hybridized at 37°C in 5× standard saline phosphate/EDTA (SSPE)/5× Denhardt's solution/0.5% SDS/0.1 mg of sheared salmon sperm DNA per ml, rinsed in 2× SSPE/0.1% SDS at room temperature, washed for 10 min at 42°C in 2× SSPE/0.1% SDS, and subjected to autoradiography.

RESULTS

To identify DNA sequence differences that might inactivate the α(1,2)FT gene in Bombay individuals, we sequenced ≈6.5 kb of genomic DNA encompassing a wild-type allele and the corresponding 6.5-kb region in a Bombay allele (Fig. 1). The Bombay allele (individual X in Fig. 1) differs from the wild-type allele at six positions. Three of these single nucleotide differences are in the gene's single intervening sequence (X_1, X_2, X_3), one is within the coding region (X_4), and two are in the 3′ untranslated region (X_5, X_6). The sequence difference in the coding region (X_4) is a nonsense mutation that creates a termination codon corresponding to amino acid 316 of the wild-type α(1,2)FT (Tyr-316 → ter). This sequence alteration predicts a mutant polypeptide that is missing the 50 C-terminal amino acids of the wild-type enzyme.

A Nonsense Mutation Inactivates the α(1,2)FT Gene in a Bombay Individual. To confirm that the Tyr-316 → ter mutation inactivates this allele, this DNA sequence difference and each of the others were moved into the wild-type sequence background and tested for function by transfection into an α(1,2)FT-deficient mammalian host (COS-1 cells; ref. 9) (Fig. 2). The wild-type construct (plasmid pHHH; Fig. 2) encodes a substantial amount of α(1,2)FT activity, whereas the Bombay construct (plasmid pBBB) generates no enzyme activity. Introduction of the Tyr-316 → ter mutation into the wild-type background completely inactivates the wild-type gene (plasmid pHBH). By contrast, vectors containing each of the other DNA sequence differences in the Bombay allele express normal levels of α(1,2)FT activity in transfected cells (plasmids pBHH and pHHB). Correction of the Tyr-316 → ter mutation in the Bombay allele (plasmid pBHB) fully restores enzyme activity. These results indicate that the Tyr-316 → ter mutation is responsible for inactivity of this allele in the Bombay individual and indicate that the other DNA sequence differences represent functionally neutral DNA sequence polymorphisms.

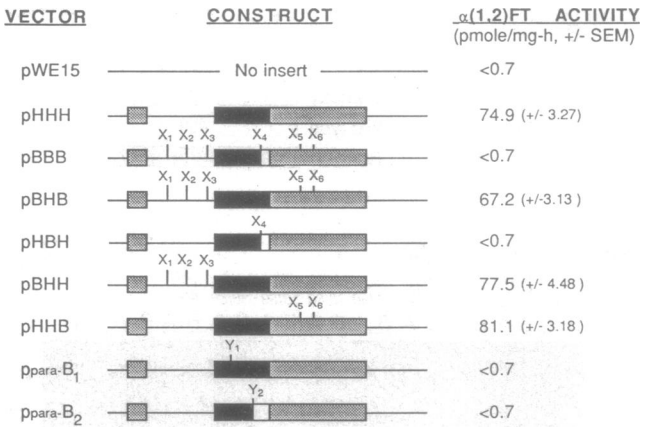

FIG. 2. Functional analysis of α(1,2)FT alleles isolated from wild-type and H-deficient individuals. Expression vectors contain, singly or in combination, the DNA sequence differences associated with the H-deficient phenotype (plasmids pBBB, pBHB, pHBH, pBHH, p_{para}-B_1, and p_{para}-B_2) on a wild-type sequence background (plasmid HHH). Point mutations are indicated above each vector diagram. Single lines indicate intronic or flanking sequence. Stippled boxes correspond to the 5′ noncoding exon and 3′ untranslated region. Solid box denotes coding sequence. Open areas within the solid box correspond to regions of the protein predicted to be truncated by nonsense mutations (vectors pBBB, pHBH, and p_{para}-B_2). Each vector, or a control plasmid (pWE15), was transfected into COS-1 cells, and extracts from the transfected cells were subjected to α(1,2)FT activity determinations. α(1,2)FT activity levels are mean values ± SEM determined for at least three independent transfections, each normalized for transfection efficiency as described.

The Bombay Propositus Is Homozygous for the Nonsense Mutation. Because of the rarity of the recessive Bombay phenotype (1), and because consanguinity is not uncommon in Bombay pedigrees (1, 16), we sought to determine whether individual X was homozygous for the Tyr-316 → ter mutation. This sequence difference yields restriction site cleavage polymorphisms for *Bst*NI (cleaves the wild-type allele) and *Alu* I (cleaves the Bombay allele) (Fig. 1). Allele assignment at this position was therefore accomplished by restriction analysis of the corresponding segment of the coding region generated by the PCR (Fig. 3). These analyses indicate that the propositus is homozygous for the Tyr-316 → ter mutation as a result of inheriting the corresponding DNA sequence alteration from each heterozygous parent (Fig. 3). Each of his living siblings is not erythroid H deficient and is either heterozygous for this allele or is homozygous for the wild-type allele (Fig. 3). This is a rare allele in the general population, since this DNA sequence alteration was not detected in an analysis of >100 alleles from unrelated H-positive individuals (data not shown). These data indicate that the Tyr-316 → ter mutation is responsible for the H-deficient phenotype of the propositus. When considered together with enzyme kinetic data and chromosomal localization results, the data also strongly support the hypothesis that this gene corresponds to the human *H* blood group locus (9).

Nonsense and Missense Mutations Inactivate the α(1,2)FT Gene in para-Bombay Individuals. As a direct test of the hypothesis that the *H* and *SE* loci are distinct α(1,2)FT genes, and to further support the assignment of this gene to the *H* locus, we examined both alleles of this gene in a Secretor-positive, erythroid H-deficient individual [para-Bombay phenotype (1, 2, 8); individual Y in Fig. 1]. We found that the coding region of each allele in this para-Bombay person contains a single base sequence alteration relative to corresponding positions in the wild-type sequence (Fig. 1). One

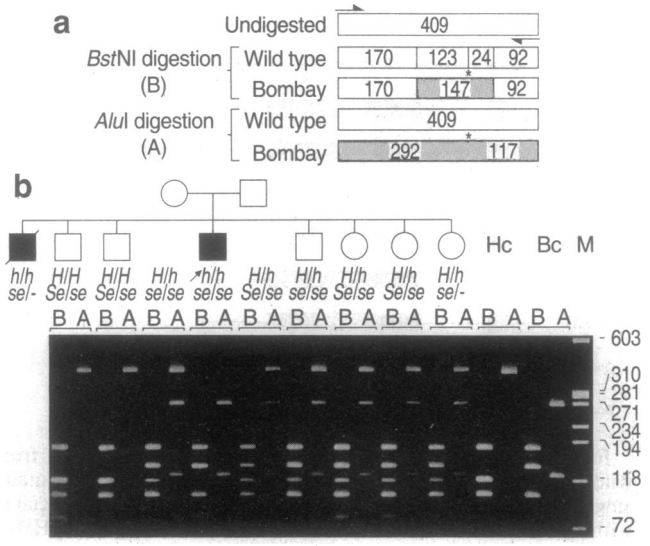

FIG. 3. Analysis of the X_4 mutation in a Bombay pedigree (10). (*a*) Allele-specific restriction site polymorphisms at the X_4 position. The single base change (C → G) corresponding to the X_4 Bombay allele (indicated by *) destroys a *Bst*NI restriction site and creates an *Alu* I site (see Fig. 1*b*). PCR was used to amplify 409 bp of the α(1,2)FT gene encompassing this position (top), using primers (indicated by arrows) described in *Materials and Methods*. *Bst*NI cleavage of this segment (middle, labeled B) from the wild-type allele yields 123- and 24-bp fragments, whereas a single diagnostic *Bst*NI-resistant 147-bp fragment is obtained with the X_4 Bombay allele (shaded). Conversely, *Alu* I digestion (bottom, labeled A) of the PCR fragment generated from the X_4 Bombay allele yields 292- and 117-bp fragments (shaded), whereas the 409-bp segment from the wild-type allele is not cleaved by *Alu* I. (*b*) Analysis of allele-specific restriction site polymorphisms. Genomic DNA was subjected to PCR amplification and restriction endonuclease cleavage as outlined in *a*. Cloned DNA segments containing wild-type sequence (Hc) or the X_4 mutation (Bc) served as control templates. PCR fragments were digested with either *Bst*NI (lanes B) or *Alu* I (lanes A) and were fractionated by acrylamide gel electrophoresis. Molecular size standards (bp) are in lane M. Solid symbols within the pedigree denote an individual with the Bombay phenotype. Bombay propositus is indicated by an arrow. Open symbols denote an individual with H-positive erythrocytes. *H* locus genotypes under each symbol are inferred from the individual's erythrocyte H antigen status and restriction fragment patterns. Genotypes at the *SE* locus are inferred from each individual's Lewis blood group phenotype (1) and/or with consideration for the Secretor status of the parents and siblings. Dash indicates that the status of one allele at the *SE* locus is not known.

allele contains a missense mutation at codon 164 that predicts the substitution of a histidine residue for the wild-type leucine residue at this position (Y_1; Leu-164 → His). The other allele contains a termination codon at a position corresponding to amino acid residue 276 (Y_2; Gln-276 → ter) and predicts a protein 90 amino acids shorter than the wild-type enzyme. Both mutant alleles yield undetectable α(1,2)FT activity when tested by transfection (Fig. 2).

Inheritance of these alleles was examined in this para-Bombay pedigree, using restriction site cleavage polymorphisms for *Bst*NI, *Bfa* I, and *Alu* I (see Fig. 1) and by allele-specific oligonucleotide hybridization analysis of the Y_1 allele. These analyses demonstrate that the para-Bombay sibling of the propositus is also a compound heterozygote for the Y_1 and Y_2 alleles (Fig. 4). All other family members examined are not erythroid H deficient and are either heterozygous for one of the two mutant alleles (parents and children of the para-Bombay individuals) or maintain the wild-type sequence at both positions (other siblings). Taken together with the results from the Bombay alleles, these data indicate that inactivating point mutations within the coding sequence of this α(1,2)FT gene are responsible for H blood

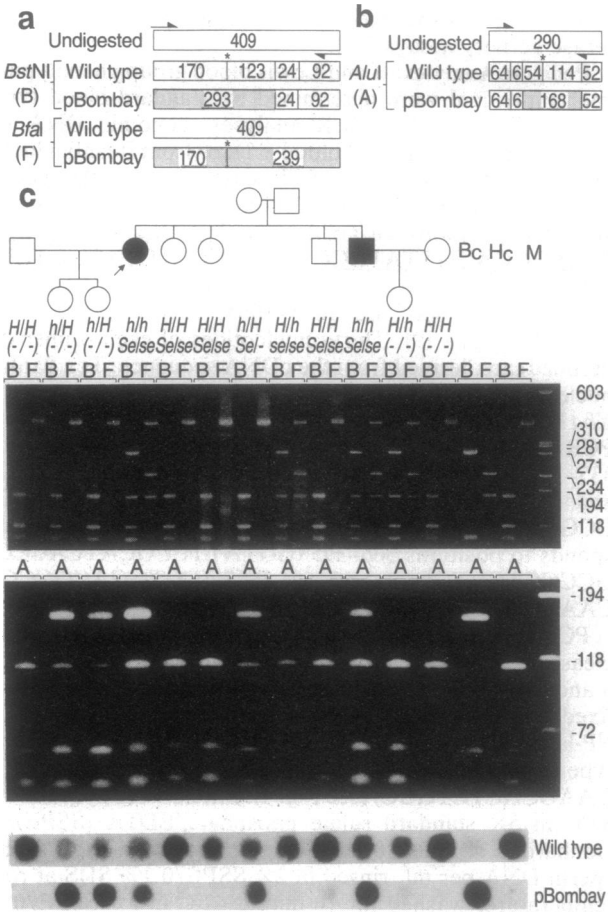

FIG. 4. Analysis of Y_1 and Y_2 mutations in a para-Bombay pedigree (8). (*a*) Allele-specific restriction site polymorphisms at position 826 (Y_2). The single base change (C → T) in allele Y_2 in the para-Bombay person (indicated by *) destroys a *Bst*NI restriction site and creates a *Bfa* I site (see Fig. 1*b*). PCR was used to amplify 409 bp of the α(1,2)FT gene encompassing this position (top), using primers (indicated by arrows) described in *Materials and Methods*. *Bst*NI cleavage of this segment (middle, labeled B) from the wild-type allele yields diagnostic 170- and 123-bp *Bst*NI fragments, whereas a single diagnostic *Bst*NI-resistant 293-bp fragment (shaded) is obtained with the Y_2 allele in the para-Bombay individual. Conversely, *Bfa* I digestion (bottom, labeled F) of the PCR fragment from the Y_2 allele yields fragments that are 170 and 239 bp long (shaded), whereas the 409-bp segment from the wild-type allele is not cleaved by *Bfa* I. (*b*) Allele-specific restriction site polymorphism at position 491 (Y_1). The single nucleotide change (T → A) at position 491 (indicated by *) in allele Y_1 of the para-Bombay person destroys an *Alu* I site (see Fig. 1*b*). PCR primers flanking this site generate a 290-bp wild-type fragment that yields diagnostic 54- and 114-bp *Alu* I fragments. Absence of the *Alu* I site at this position in the Y_1 allele yields a diagnostic 168-bp fragment (shaded). (*c*) Analysis of allele-specific sequence polymorphisms. The para-Bombay propositus is indicated by an arrow; both para-Bombay individuals are indicated by solid symbols. The rest of the pedigree is labeled as in Fig. 3. PCR fragments shown in *a* and *b* were generated by using genomic DNA from family members or by using cloned DNA segments containing wild-type sequence (Hc) or the mutations in the para-Bombay individuals (pBc; allele Y_2 in top gel; allele Y_1 in bottom gel). PCR fragments were digested with *Bst*NI (lanes B) or *Bfa* I (lanes F) (top gel), or *Alu* I (lanes A) (bottom gel) and were fractionated by acrylamide gel electrophoresis. Molecular size standards (bp) are in lane M. Alternatively, for allele-specific oligonucleotide analysis of the Y_1 position, PCR products diagrammed in *b* were generated for each individual and for control templates (pBc, Y_1 allele; Hc, wild-type allele). These fragments were then probed with a radiolabeled oligonucleotide specific for either the wild-type sequence at the Y_1 position (Wild type) or for the mutant Y_1 sequence (pBombay).

group antigen deficiency in these Bombay and para-Bombay individuals and thus provide genetic support for biochemical

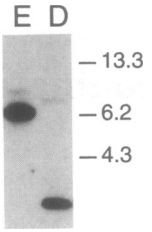

FIG. 5. Human DNA sequences that cross-hybridize with the H α(1,2)FT coding region. Human genomic DNA was digested with *Eco*RI (lane E) or *Dra* I (lane D) (see Fig. 1*a*) and subjected to Southern blot analysis (11) using the coding region of the α(1,2)FT cDNA as a probe (9). Hybridization was done at 42°C, with a final wash at 65°C in 2× standard saline citrate/0.2% SDS. Molecular size standards (kb) are indicated on the right.

(9, 12) and chromosomal localization (9) data, indicating that this gene corresponds to the human *H* blood group locus.

The *H* α(1,2)FT Gene Identifies Candidates for the Human *SE* Locus. Since the two para-Bombay individuals maintain a functional *SE*-determined α(1,2)FT, but not an *H*-encoded α(1,2)FT, it can be concluded that the *SE* locus must therefore correspond to an α(1,2)FT gene distinct from the one examined here. These results provide additional support for genetic (2) and biochemical (3–5) data indicating that the human *H* and *SE* blood group loci correspond to distinct α(1,2)FT genes. Indeed, when used in Southern blot analyses, the coding portion of the *H* gene identifies cross-hybridizing human DNA sequences distinct from those corresponding to the *H* α(1,2)FT gene (Fig. 5). By analogy to the structurally related family of human α-(1,3)-fucosyltransferase genes (17), such sequences represent candidates for another α(1,2)FT gene that may correspond to the *SE* locus.

DISCUSSION

Bombay and para-Bombay individuals display no apparent deleterious phenotype (1) except in circumstances requiring blood transfusion, wherein they are cross-match incompatible with all donors except other H-deficient individuals (1). Since the coding sequence mutations identified here yield nonfunctional α(1,2)FTs, it follows that the fucosyltransferase activity encoded by the *H* gene is not essential in humans [barring processes that suppress nonsense codons (18) or other RNA editing events (19)]. Given the high frequency of non-Secretor individuals (≈20% of Caucasians; ref. 1), the α(1,2)FT encoded by the *SE* locus is apparently also not essential (at least in the adult human), although a definition of the molecular basis for null alleles at this locus must await the isolation and characterization of this gene.

These observations suggest that any evolutionary constraints that may have once selected for functional alleles at these α(1,2)FT loci have since ceased to be operative in adult humans. It remains possible, however, that related loci maintain compensating functional redundancy sufficient to sustain a normal phenotype. Indeed, by analogy to the family of human α-(1,3)-fucosyltransferase genes (17), it remains possible that other α(1,2)FTs (20), encoded by as yet uncloned genes, may fulfill one or more essential roles. Moreover, such roles (like embryo implantation in the mouse; ref. 21) may be operative in tissues, or at developmental stages, not yet examined in *Homo sapiens*. Alternatively, the functions maintained by terminal α(1,2)fucose linkages may be accomplished by other terminal sugar moieties that are, in turn, encoded by nonpolymorphic glycosyltransferases (discussed in ref. 22). These issues are not idiosyncratic to the α(1,2)FTs. Null alleles are rather common within other glycosyltransferase genes (the ABO, Lewis, P, and Sda blood group systems, for example; see ref. 1) and in some instances have been shown to correspond to inactivating coding sequence mutations (23). Experimental tests of hypotheses concerning a function(s) of these and other mammalian glycosyltransferases, and their cognate oligosaccharide products, should be facilitated with the use of cloned glycosyltransferase genes in conjunction with transgenic and gene disruption technologies (24).

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