Molecular analysis to FUT–1,2,3 and ABO genotyping may instead of serological typing to diagnose para–Bombay in Chinese

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ABSTRACT

The aim of this study was to evaluate the consistency between serotyping and molecular analysis in Chinese with para–Bombay. The molecular analysis of gene fragments in FUT–1, FUT–2, FUT–3 and ABO genotyping and serotyping were used including a saliva test to examine the A, B, H substance and an absorption elution test to examine the A, B, H; and further routine tests including ABO, H and Lewis phenotype. From eleven samples with anti–H negative, 10 samples were confirmed with para–Bombay by sequencing to FUT–1, from which six samples were 547–548delAG, three samples were 880TT deletion, one sample was 649G>T heterozygous (h7, China) as carrier. The sequencing to FUT–2 confirmed 357C>T in 11 samples, meaning H, A and B substance was secreted in saliva except for one sample which occurred 385A>T (I129F) heterozygous, which is a weak secretor. The FUT–3 sequence result demonstrated four samples with heterozygous mutations to 59T>G (L20R) combined with 508G>A (G170S) and seven samples without mutations in FUT–3 gene fragment same as reference. The consistency between sequencing with FUT–1/FUT–2 and serotyping by anti–H reported an identical result, except for one sample, which interestingly showed the H/h7 carrier with serotyping negative to anti–H. The result of sequencing with FUT–2/FUT–3 and Lewis phenotyping also reported a complete consistency. The saliva test to A, B, H substance and absorption elution test examining the A, B, H antigens on the surface of red blood cells completely matched the ABO exon 6, 7 sequence results. The sequencing of FUT–1, FUT–2, FUT–3 and ABO exon 6, 7 may become a useful tool to confirm the para–Bombay blood type.

Keywords: para–Bombay, FUT–1, FUT–2, FUT–3, ABO blood group

INTRODUCTION

The ABO, H and Lewis blood group antigens are based on a common molecular mechanism, including fucosyltransferase–1(FUT–1). FUT–2, FUT–3. FUT–1 forms an H antigen on the red blood cell (RBC) membrane and FUT–2 regulates the H antigen secreted to saliva10. Lewis phenotype is produced by FUT–2 and FUT–321. The phenotype of Le(a‘b’) consists of FUT–3 non–expressed Lewis antigens, Le(a‘b’) consists of FUT–3 expressed Lewis antigens and FUT–2 is a secreted gene, and Le(a‘b’) in contrast consists of FUT–3 expressed Lewis antigens and FUT–2 is non–secreted gene3. The phenotype of Le(a‘b’), which only occurs in the Chinese population, consists of FUT–3 expressed Lewis antigen and FUT–2 is a weakly–secreted gene14,16. The ABO blood group is based on FUT–1 synthesizing the H antigen as a precursor and A transferase or B transferase to form ABO antigens on H antigen19.

The para–Bombay blood group expresses much fewer ABH antigens on the RBC membrane, with ABH substances present in the saliva. However, the anti–A/
anti-B and saliva proficiency and absorption-elution test easily leads to false positive or false negative results. Patients with a clinical status such as being in coma are unable to get saliva, which is also challenge to para-Bombay identification [10]. The aim of this study was to evaluate the consistency between serotyping and molecular analysis in Chinese with para-Bombay. We attempted to use molecular analysis to identify para-Bombay and this in turn may also offer more information about para-Bombay.

MATERIALS AND METHODS

Para-Bombay individual and DNA extraction

This study collected eleven individuals’ EDTA test-tube samples and saliva sent to the Beijing Red Cross Blood Center (BRCBC) Blood Group Laboratory (BGL); 5 samples were from Fujian (southeast China), 4 samples were from Beijing and 2 samples were from Taiwan. DNA was isolated from ethylene diamine-tetraacetic acid-anti-coagulated blood using a commercial kit (Prepito DNA Blood 250 Kit, Chemagen, PerkinElmer, Germany) based on magnetic separation on an automated system (Chemagic Prepito, Chemagen, PerkinElmer, Germany).

Serological typing, adsorption-elution test and saliva test

Monoclonal antibodies of anti-A (Inno-train GmbH, Germany), anti-B (Inno-train GmbH, Germany), and anti-H (Sanquin GmbH, Netherlands) were used for ABO forward blood typing, and A1, B, O cells (Shanghai Hemo Pharmaceutical Biological Co., Ltd, China) were used for reverse typing. Lewis monoclonal antibody phenotype was used for the diagnosis of Lea and Leb (Sanquin GmbH, Netherlands). An adsorption-elution test was used to examine A and B antigens on red blood cells and a saliva test (based on the haemagglutination inhibition test) to detect A, B and H substance. Other procedures such as sample reception, report documentation, and report writing were all based on the regulations and standard procedures specified by the BRCBC blood group laboratory.

Molecular analysis to FUT-1, FUT-2, FUT-3 and ABO exon 6, 7

Polymerase chain reaction with sequence primers was performed for the ABO, H and Lewis blood group by using H antigen whole genome kit (FUT-1 whole genome sequence kit, Jiangsu LiBio Biotech, China), Lewis blood group kit (FUT-2/FUT-3 whole genome sequence kit, Jiangsu LiBio Biotech, China) and ABO exon 6,7 sequence kit (Jiangsu LiBio Biotech, China). Sequencing of PCR purified products was done by Sangon Biotech (Beijing, China) and the results were analyzed using sequence analysis software (Geneious R9, New Zealand). The FUT-1 allele (GenBank No. M35531), FUT-2 allele (GenBank No. U17894), FUT-3 allele (GenBank No. X53578), A1.01.01 allele (GenBank No. AY805750) sequence template were used as reference to analyze and mark the mutations.

RESULTS

Serotyping and sequence analysis to FUT-1

For 4 of the 11 individuals, the saliva sample was not available, due to clinical status. We were able to examine the other seven samples by using H substance to neutralize anti-H. By sequencing the FUT-1 gene fragment, four variant types were identified, including homozygous 547AGdel in six samples, homozygous 880TTdel in three samples, 35C>T in one sample and heterozygous 649G>T in one sample. All eleven individuals believed to have para-Bombay blood type were able to be examined for polymorphisms in the FUT-1 gene fragment depending on their molecular sequences, and other information was shown on Table 1 and Fig.1.

Serotyping and sequence analysis to ABO blood group

The routine forward serotyping couldn’t detect A, B, H antigens except for one patient with h7 carrier reaction 1 w with anti-A, or w+ with anti-B. The absorption-elution test to examine ABO blood type also demonstrated an indistinguishable result, and the saliva test showed complete concordance to ABO exon 6 and 7 in seven samples. From sequencing to ABO exon, there are A/O in 3 individuals, A/B in 2 individuals and B/O in 6 individuals, other details are as shown on Table 1 and Fig.1.

Serotyping and sequence analysis to Lewis blood group

The Lewis phenotype was Le(a+b+) in our study. Sequencing with FUT-2 revealed Se/Se 357C>T (silent mutation) in seven samples, Se 357C>T homozygous (silent mutation) in three samples and Se/Se 357C>T, 385A>T (weak secretor) in one sample. All samples’ phenotype presented normal secretion (Table 1, Fig.2). For the FUT-3 sequence result, Le 59C>T with 508T>A unexpressed le antigens were detected on the RBC membrane. All subjects in our study expressed Le antigen with alleles Le/Le same as reference in seven samples. The other samples were Le/le59, 508 (Table1, Fig.3).
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Table 1  The forward serotyping, saliva test, phenotype and molecular analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>H</th>
<th>A</th>
<th>B</th>
<th>H</th>
<th>Lewis</th>
<th>FUT–1</th>
<th>FUT–2</th>
<th>FUT–3</th>
<th>ABO exon</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Se/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>A101/01</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>3</td>
<td>w²</td>
<td>w²</td>
<td></td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>a,b²</td>
<td>547AGdel/547AGdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>A101/01</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>880TTdel/880TTdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>a,b²</td>
<td>880TTdel/880TTdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>A102/01</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>880TTdel/880TTdel</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>A101/01</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b²</td>
<td>35C&gt;T/35C&gt;T</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>B101/01</td>
</tr>
<tr>
<td>11</td>
<td>w²</td>
<td>w²</td>
<td>w²</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>a,b²</td>
<td>H/649G&gt;T (h7, China)</td>
<td>Sc/Se 357C&gt;T</td>
<td>Le/Le</td>
<td>A102/0</td>
</tr>
</tbody>
</table>

Note: ? Saliva test didn’t be operated because of patient’s unconsciousness.

**Fig.1. Molecular analysis to FUT–1.** A: 35C>T homozygous. B: 547AG deletion homozygous. C: 880TT deletion homozygous. D: 649G>T (h7, China) heterozygous.

**Consistency of serotyping and molecular analysis**

The saliva test to examine A, B, H substance was done in concordance with FUT–1, FUT–2, FUT–3 and ABO exon 6, 7 in seven individuals. For the remaining four individuals, the saliva specimen was not available. The absorption elution test and serotyping test showed indistinguishable results of ABO typing with the saliva test and molecular analysis, however, a serotyping reaction with anti–H is the golden rule to index para–Bombay blood type.

**DISCUSSION**

Our study used molecular sequence analysis of FUT–1, FUT–2, FUT–3 and ABO exon 6, 7 to successfully
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identify para–Bombay blood type. The estimated frequency of para–Bombay from the blood donor database is less than 1/100,000 in the north of China, 1/8,500 in Fujian (the Southeast of China) and 1/8,000 in Taiwan[11]. On the other hand, para–Bombay blood type is identified every month in medical centers located in South East Asia. Taking FUT–1 and FUT–2 genomic information could identify Bombay or para–Bombay[12], plus the ABO exon 6, 7 molecule could accurately confirm the ABO blood type.

Because of the molecular mechanism of synthesizing the Lewis blood group, this phenotype could offer as an assistant tool to diagnose the secretion status through Le(a–b+) formed by secretor in FUT–2 which is also the most frequent type in the Lewis blood group[1]. However, some reasons challenge the Lewis phenotype, including it being a weak secretor only found in Chinese, the monoclonal antibody showed weak reactions with the Lewis phenotype and transfusion interference. We believe molecular analysis of FUT–1, FUT–2 and ABO exon 6, 7 could offer a powerful tool to diagnosis para–Bombay blood type.

Hu J. et al. [13] published that ABO genotyping could be used as an assistant tool to diagnose samples with result discrepancies. As one sample in our study was a para–Bombay carrier, we used the ABO exon 6, 7 sequence to identify their ABO blood type and genomic information. From our study, we found ABO genotyping may be a faster tool than sequence analysis to diagnose ABO blood type.

Interestingly, in one individual we observed the FUT–1 sequence was H/h7 with serotyping negative reaction with anti–H, with the ABO exon 6, 7 showing normal A101/B101. This patient with cardiovascular disease underwent open heart surgery and excluded hematological disease through peripheral venous blood examination. Although we were unable to find obvious molecular evidence of the cause of para–Bombay, two possible hypotheses in this case were: ① disease caused weak expression of antigen and ② A transferase and B transferase compete emulous reacted with H antigen and h7 caused less H antigen expression on the RBC membrane. Further study is needed to analyze the molecule and serum examination to confirm if the h7 carrier indeed has an anti–H negative result.

The advantage of using the experimental molecular tool is that the access to the specimen wasn’t limited by the patient’s clinical status. The more information from the gene can be obtained and it avoids the bias of human resourced polyclonal anti–A and anti–B and false positive or false negative results caused by staff judgment. Our study offers evidence that molecular analysis of FUT–1, FUT–2, FUT–3 and ABO exon 6, 7 may offer useful information to identify para–Bombay, compared to traditional serotyping including the saliva test and the absorption–elution test.
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References


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