We had reported last year, screening for T725 G mutation in FUT 1 (H) gene on 38 serologically confirmed Bombay phenotype samples. During this year, 34 out of these 38 Bombay phenotype samples were screened for identification of FUT 2(Se) gene deletion as literature reports based on the molecular characterization study of a few Indian Bombay phenotypes settled in S.Africa and Re Union Island, have shown a T725 G mutation of FUT 1(H) gene along with gene deletion of FUT2 (Se) gene.

Amplification of DNA was performed to look for FUT 2(Se) gene deletion using a specific pair of primers; the position of one of the primers is within the fragment which is deleted. So if the deletion is present then this fragment is not amplified; if deletion is absent then this fragment of 1.05 Kb is amplified.

Thirty four Oh samples and 3 family members who were of normal O group were screened for FUT 2. Thirty three out of these 34 samples, failed to show any amplification suggesting deletion of FUT2(Se) gene. Only one Oh sample which showed amplification also happens to be heterozygous for T725G mutation in FUT 1(H) gene (Fig 24).
Specificity confirmation of a cell culture clone

A monoclonal antibody 3E8, produced by the Dept of Cell Biology, was tested with 120 random cells of A(26), B(45), O (36) and AB (13) groups to identify specificity. As the antibody reacted with all ABO groups, titration with A₁, A₂, A₁B, A₂B, B and O groups were performed which showed the following pattern:

- A₁  -  1:2
- A₂  -  1:32
- B   -  1:16
- A₁B -  1:2
- A₂B -  1:8
- O   -  1:64

This antibody when further tested with O group panel of red cells for antibody identification by saline and enzyme techniques at 37°C, 22°C and 4°C showed reactivity with all red cells. Hence it was tested with 12 Bombay phenotype red cells with which it did not show any agglutination reaction, suggesting its specificity to be anti-H. The antibody also gave differential titre values with different cells by showing maximum strength with O group red cells (1:64) and the least with A₁ and A₁B (1:2) as is the usual pattern of anti-H antibody.

Population screening for detection and molecular characterization of partial D variants

Year of Commencement : 1999
Year of Completion : 2004

Analysis of population screening with epitope specific monoclonal anti-D in various caste and communities and molecular analysis of partial D variant has been reported in earlier annual report. The main findings of this project are as follows:

1. The analysis showed ethnic variation in D epitopes in different caste and communities.
   a. Absence of D epitopes was predominately seen in some communities and tribal population.
   b. The percentage of Rh D positive subjects reactive with some monoclonal anti-D was less compared to others.

2. D antigenic sites/RBC were estimated successfully in partial D, weak D, rare Rh variants and normal RH phenotypes by sensitive flowcytometric method
   a. The technique could detect minor variation in D antigenic sites in weak D, partial D blood samples
   b. In partial D variants studied, DVI category had minimum and Dv\(^a\) had maximum number of D antigenic sites/RBC.
   c. Anti-D quantitation by flow cytometry showed that none of the partial D variants identified in the present study produced anti-D

1. PCR for Rh D typing was standardized and used for prenatal determination of fetal Rh D status. There was complete correlation between fetal DNA tying by serological and molecular techniques.

4. Molecular analysis of 48 partial D variants was carried out by Multiplex PCR (M-PCR)
   a. 29.16% of partial D variants belonged to DFR category and 14.6% were of DVI category.
   b. Eight partial D variants not classified by serological methods were classified by M-PCR to be of DFR category.

5. Panel of 30 monoclonal anti-D and partial D kit could classify 65% and 69.5% of partial D variants respectively. 79.2% of partial D were classified into different categories by M-PCR.
Study of partial D variants with commercial anti-D reagents

Polyclonal anti-D reagents have been largely replaced by monoclonal (MAb) anti-D as reagents, which are epitope specific. Several laboratories worldwide have produced large number of human monoclonal anti-D which are used as Rh D typing reagents. However they exhibit some variations in reactivity in detection of weak D and partial D variants. These reagents are usually a combination of MAb derived from several clones to ensure reactivity with broad spectrum of Rh positive cells, but have led to confusion while testing donors in blood banks.

Forty two cases of partial D variants (confirmed with partial D kit) were tested with seven different commercial monoclonal anti-D reagents by serological methods according to manufacturer’s instruction. Out of these reagents two were IgM, one IgG and four were blend of IgM and IgG. The different reagents showed wide variation in the results (Table 22).

Total 83% of partial D (35/42) exhibited weak reactions with polyclonal anti-D and no sample gave negative reaction. Monoclonal reagents number 3,4 and 5 showed negative results more frequently. The highest reactivity rate was seen with reagent number 6.

To select the MAb reagents which could be suitable for identification of partial D variants, the reactions obtained by a pair of reagents used simultaneously were analysed. All partial D variants could be detected as Rh (D) positive using two pair of anti-D reagents i.e 1&2 or 1&6 used simultaneously.

For Rh(D) typing of patients, detection of weak expression of D antigen is essential. Analysis was performed with two commercial anti-D reagents were simultaneously which either gave weak reaction or discrepant results which would lead to identification of partial D variants. None of the pairs could identify all the partial D variants in our population an were not ideal for both donor and recipient testing.

Table 22 : Reactivity of partial D cases with different anti-D reagents.
<table>
<thead>
<tr>
<th>Positive</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Strong reaction</strong></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>25</td>
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<tr>
<td><strong>Weak reaction</strong></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

**b. Family study of partial D variants**

The clinical importance of partial D variants is known as these subjects when exposed to normal D antigen can produce anti-D against missing epitopes. Hence the detection of partial D in blood donors and patients is important.

In the present study families of fifteen partial D variants were studied with serological and molecular method. The family of these subjects realized the clinical importance or partial D and agreed to get themselves tested to avoid confusion in Rh(D) status in future. In 7 families, 10 more individuals with the same partial D variant were identified. All partial D variants identified were of R1r (Dce/dce) genotype and the hybrid gene (RHD –CE-D) was inherited from one of the parents who were mostly of R1R1 or R1R0/ R1r genotype. The linkage of all partial D hybrid gene is the family studied was the Ce gene.

Figure 25 shows family tree of nine members of partial D variant family (DFR). All other members except propositus, her brother and son were Rh D positive showing presence of all epitopes of D antigen. Multiplex PCR was performed for molecular characterization of partial D variant in the family. Absence of exon 4 specific band of Rh D gene in 3 members of the family is shown in Fig 26. All other members were Rh D positive showing presence of all RH D specific exons.

**Fig 25: Family tree of AFP (DFR partial D variant subject**
Figure 26: Multiplex PCR Analysis of Partial DFR Family (AFP)

From Left to Right:
- Lane 1 = Propositus (AFP) DFR Partial D (absence of RHD specific exon 4)
- Lane 2 = Son DFR Partial D (absence of RHD specific exon 4)
- Lane 3 = Brother Partial D DFR (absence of RHD specific exon 4)
- Lane 4 = Mother RhD Negative
- Lane 5 = Father RhD Positive
- Lane 6 = Sister RhD Positive
- Lane 7 = DNA Molecular Weight Marker V (pBR322 DNA cleaved with Hae III)
- Lane 8 = Husband RhD Positive
- Lane 9 = Maternal Uncle RhD Positive
- Lane 10 = Paternal Aunt RhD Positive