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Serological Blood Grouping Reveals Genotypes and Family Relationship – A Case Report from Lemunnam District Clinic

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Abstract

Serological ABO and Rhesus blood groupings could be used to predict the possible ABO and Rhesus blood group genotype. In this study, ABO and Rhesus blood group genotypes of given six subjects were predicted and employed to establish possible family ties between them. Based on their ABO and Rhesus phenotype, five of the family members indicated a possible family lineage. The last child showed an ABO blood group that might not be a product from the apparent parents. As the case may be, the ABO antigens of the last child are not fully developed, hence the disagreeing result.

Keywords: Blood, genotype, family, traits, antigens.

Introduction

Prior to the discovery of genotyping, identification of family relation solely depends on physical resemblance such as eye colour, complexion, height and facial features. In 1800's, Mendel developed the formal scientific theories of genetic and inheritance. He projected that half of the offspring's characteristic features (traits) came from the mother and half came from the father (Guyer, 1907). Blood groups antigens are inherited characters which are determined and controlled by genes. Most genes that control particular blood group antigen differ from the other gene that determines the antithetical antigen by Single Nucleotide Polymorphism (SNP), except sex-linked genes, such as, Xg and Xk which are hemizygous. Blood group antigens such as ABO, Rh DCE, Duffy and Kell antigens are made up of proteins, glycoprotein or glycolipids (McBean *et al.*,

2014). In 1926, the phenotype of ABO blood group was first used to establish a non-paternity case in Vienna (Phelan and Morris, 2012). By blood grouping, individual's antigen can be employed to identify family tie.

Of the 35 blood group systems, ABO is the most clinically significant followed by Rh blood group system. Their importance is based on the fact that they are highly immunogenic (Contreras and Daniels, 2011) and can cause haemolytic transfusion reaction (HTR) and haemolytic disease of the foetus and newborn (HDFN). Blood group antigens play an important role in identifying family linkage. An offspring acquires or inherits genes from each parent that determine his or her blood antigen. Therefore, antigens are indirectly heritable traits and may provide important information in identifying possible biological relationships. In this study, the ABO and Rh blood groups of six subjects were serologically typed, their possible genotypes were predicted and compared to establish if there is a possible family linkage between them.

Materials and Methods

Venous blood was obtained from each subject into an EDTA tube and labelled respectively after verbal informed consent was obtained. The red cells were washed twice in phosphate buffered serological saline (Inverclyde Biologicals Ltd., UK). By tube technique, 3% red cell suspension of each subject was tested against Anti-A, Anti-B and AB serum (Sanguin Reagent, Netherlands). Those cells that reacted with Anti-A were further tested against Anti-



A₁ to differentiate A₁ from A₂ cells. The plasma was also tested against reagent red cells A₁, A₂, B and O red cell (NBS Liverpool) respectively. Using micro-tube technique, the Rh blood group of all the six members were determined by testing 3% red cell suspension of each subject against monoclonal anti-D, anti-C, anti-c, anti-E, anti-e, and AB serum (Bioscot Edinburgh, UK) respectively. Positive and negative controls were also included.

Results

From the analysis of the ABO and Rh blood grouping, five out of the six subjects were found to be biologically linked to the family lineage. The Rh

result of the last child (sample 6) also shows a possible link to the family lineage, but the ABO result indicated that the offspring might not be a product from the apparent parents. The red blood cells of sample 6 reacted with Anti-A but not with Anti-A₁, and the plasma (reverse grouping) did not react with any of the reagent red cell as shown in table 1 A and B. By cell grouping, sample 6 is said to be blood group A (A₂) red cell. The ABO/Rh phenotype and possible genotypes of all the subjects are shown in table 2. A family tree is also shown in Figure 1 with their probable genotype to depict a possible lineage between them.

Table 1 A and B: ABO (tube) and Rhesus (micro-tube) typing result

A.

| S/No | Age (years) | ABO Cell Group Forward Group | | | | Reagent control | ABO Plasma group Reverse Group | | | | ABO Blood Group Results |
|------|------------------------------|------------------------------|---------------------|--------|-----------------|-----------------|--------------------------------|-------------------------|------------|---|-------------------------|
| | | Anti-A | Anti-A ₁ | Anti-B | Reagent control | | A ₁ cells rr | A ₂ cells rr | B cells rr | O cells R ₁ r | |
| 1 | 72 | 0 | NT | 0 | 0 | 5 | 5 | 5 | 0 | O | |
| 2 | 59 | 4 | 0 | 5 | 0 | 2 | 0 | 0 | 0 | A ₂ B | |
| 3 | 34 | 0 | NT | 5 | 0 | 5 | 5 | 0 | 0 | B | |
| 4 | 39 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | A ₁ A ₂ , A ₁ A ₁ , A ₁ O | |
| 5 | 4 | 0 | NT | 0 | 0 | 5 | 5 | 5 | 0 | O | |
| 6 | ⁵ / ₁₂ | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | A ₂ A ₂ , A ₂ O | |

Controls

| | | | |
|--------------------------|---|---|---|
| A ₁ cells rr | 5 | 5 | 0 |
| A ₂ cells rr | 5 | 0 | 0 |
| B Cells rr | 0 | 0 | 5 |
| O Cells R ₁ r | 0 | 0 | 0 |

Key NT; not tested; 0, 1, 2, 3, 4, 5 are varying degree of agglutination

B.

| S/No | Rh reagent | | | | | | Reagent control | Phenotype |
|------|------------|------------|--------|--------|--------|--------|-----------------|-----------|
| | Anti-D (1) | Anti-D (2) | Anti-C | Anti-c | Anti-E | Anti-e | | |
| 1 | 5 | 5 | 5 | 5 | 0 | 5 | 0 | DCce |
| 2 | 5 | 5 | 0 | 5 | 5 | 5 | 0 | DcEc |
| 3 | 5 | 5 | 5 | 5 | 0 | 5 | 0 | DCce |
| 4 | 5 | 5 | 5 | 0 | 0 | 5 | 0 | DCe |
| 5 | 0 | 0 | 5 | 5 | 0 | 5 | 0 | dCce |
| 6 | 5 | 5 | 5 | 5 | 0 | 5 | 0 | DCce |

Controls

| | | | | | | |
|----------|-----|-----|------|------|------|------|
| Positive | R1r | R1r | R1R2 | R1R2 | R1R2 | R1R2 |
| | 5 | 5 | 5 | 5 | 5 | 5 |
| Negative | Rr | rr | Rr | R1R1 | R1r | R2R2 |
| | 0 | 0 | 0 | 0 | 0 | 0 |

Key NT; not tested; 0, 1, 2, 3, 4, 5 are varying degree of agglutination

Table 2: ABO/Rh Phenotype and probable Genotype

| Sample | Phenotype | | Possible Genotypes | | Probable Genotype | |
|---------------------------------|------------------|------|---|--|-------------------|--|
| | ABO | Rh | ABO | Rh | ABO | Rh |
| 1 (Maternal Grandfather) | O | DCce | OO | DCe/dce (31.08%), Dce/dCe (0.16%), DCE/Dce (3.36%) | OO | DCe/dce (R ₁ r) |
| 2 (Maternal Grandmother) | A ₂ B | DcEe | A ₂ B | DcE/dce (10.36%), DcE/Dce (1.12%), Dce/dcE(0.08%) | A ₂ B | DcE/dce (R ₂ r) |
| 3 (Mother) | B | DCce | BB or BO | DCe/dce (31.08%), Dce/dCe (0.16%), DCE/Dce (3.36%) | BO | DCe/dce (R ₁ r) |
| 4 (Father) | A ₁ | DCE | A ₁ A ₂ , A ₁ A ₁ or A ₁ O | DCE/DCE (17.64%), DCE/dCe (1.68%) | A ₁ O | DCE/DCE (R ₁ R ₁) |
| 5 (Child A) | O | DCce | OO | dCe/dce (1.48%) | OO | dCe/dce (r'r) |
| 6 (Child B) | A ₂ | DCce | A ₂ A ₂ or A ₂ O | DCe/dce (31.08%), Dce/dCe (0.16%), DCE/Dce (3.36%) | A ₂ O | DCe/dce (R ₁ r) |

Rh % was calculated based on applications of Hardy-Weinberg equation (Carrucio, 2012).

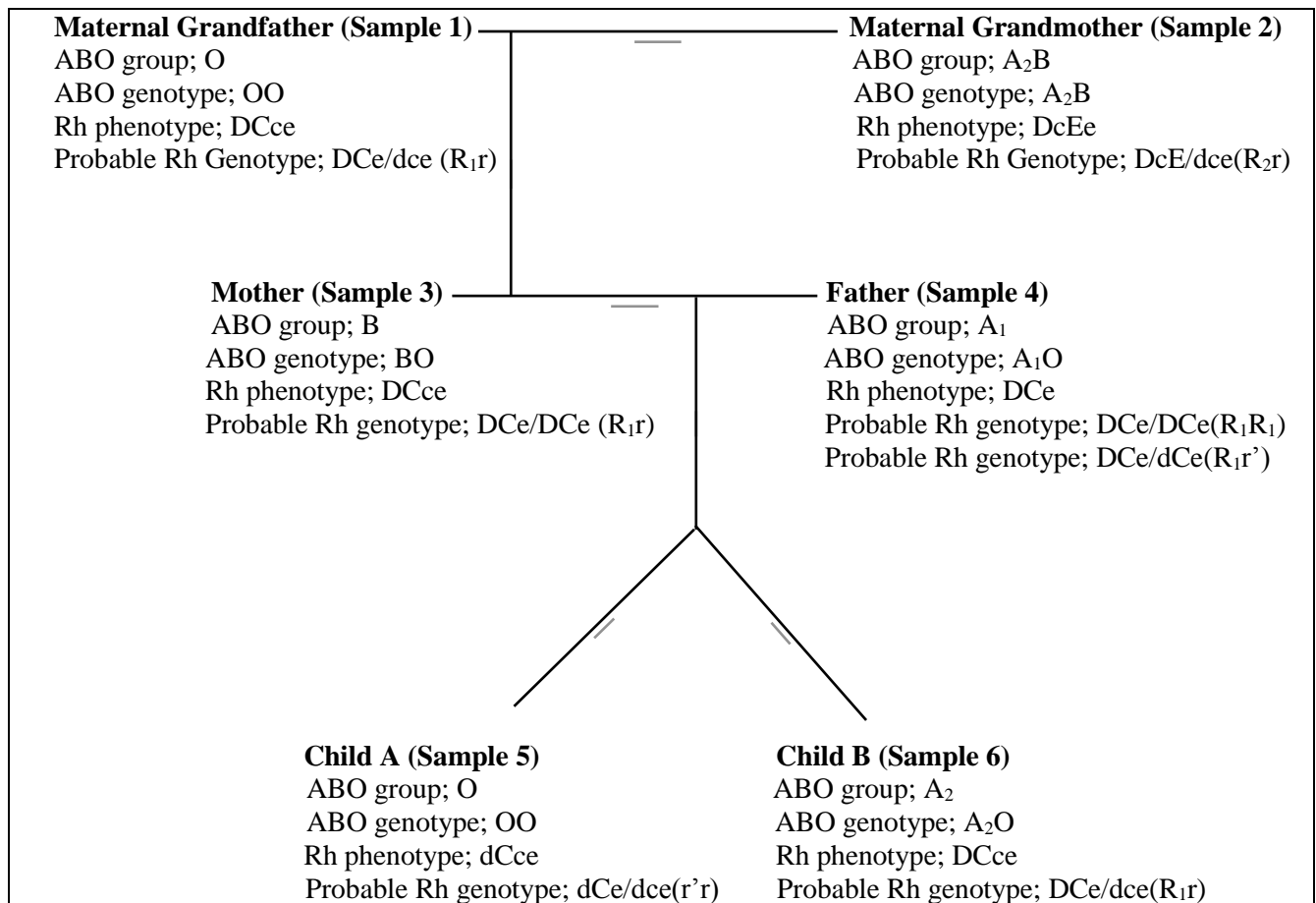


Figure 1: A Family Tree of the six members indicating their serological ABO/Rh Phenotype and Probable Genotype to establish a possible family lineage.

Discussion

The aim of the study was to establish a possible family linkage between the six individuals. Blood group systems, such as ABO and Rh have been in use for a very long time in paternity testing. Serological red cell agglutination methods with control reagents are a well-established way of determining blood group phenotypes (Tucker, 1975). In most instances, cell and serum grouping combined supports a common conclusion.

Of the six subjects involved in this study, five subjects are linked based on both the *ABO* and *RH* system while the 6th sample (Child B) differed based on the ABO blood group. However, the red blood cells of the 6th sample reacted with Anti-A but not with Anti-A₁, and the plasma (reverse grouping) did not react with any of the reagent red cell. By cell grouping (forward), sample 6 is said to be blood group A (A₂) red cell. Generally red blood cells that react with only Anti-A serum are supposed to contain Anti-B in their plasma. Some A₂ individuals produce antibodies against the A₁ antigen. About 2% of A₂ and 25% of A₂B red cells have Anti A₁ (Taylor *et al.*, 1942; Juel, 1959). The observation of a negative reaction on the reverse group with standard B cells might be due to the sample being tested was from a baby. Anti-A and anti-B antibodies are not present in the newborn and the low titres of these antibodies at this stage may fail to form a significant reaction with standard cells until the child reaches the age of 3 to 6 months (Hermening and Forneris, 2012).

Similarly, despite the fact that A and B antigen can be detected on the red blood cells of 5 to 6 weeks embryo, A and B antigens may not fully developed at birth (Bhat *et al.*, 2010; Brecher, 2005). This may provide a possible reason why red cells from sample 6, considered to be from a baby, failed to react with Anti-A₁ (Bhat *et al.*, 2010). Thus, since red blood cells of newborn babies who are genetically group A₁ may not necessarily react with Anti-A₁, sample 6 red blood cell cannot be completely ruled as not containing A₁ antigen, hence, the possible ABO genotype may be A₁A₂, A₁A₁ or A₁O. But for sample 5 (Child A) to be homozygous OO, the parents; sample 3 and 4 must be heterozygous such that sample 3 will be BO and sample 4 will be A₁O (cells reacted with Anti-A₁). From these probabilities, one may partly conclude that sample 6 is A₁O where the A₁ antigen is yet to be fully developed.

Furthermore, a fusion of *ABO* genes as a result of meiotic crossing over may result in sample 6 having

a disagreeing ABO blood group despite being possibly linked to the family. Previous report by Suzuki and colleagues (1997) indicated a similar case in which parents of blood group B and O produced a child of blood group A. Phenotypically, the child was thought not to be a product of mating between the B and O parents. However, sequencing of the *ABO* gene of the family revealed that the child possesses an *ABO* gene in which exon 6 and 7 had the sequence of B and O allele respectively. The fused *B-O* gene encodes for an enzyme with A-transferase activity. The author suggested that crossing-over between *B* and *O*₁ alleles occurred in the germ cell lineage of the mother, which created a *B-O* fused gene that encodes an enzyme with A-transferase activity. Several genes have been discovered that appear to be a hybrid gene, which may consist of some sequences with the slight characteristic of one *ABO* allele and some sequences with the slight characteristic of another allele (Daniels, 2002) and consequently produces different enzyme product (Gassner, 1996).

The study by Karl Landsteiner and Wiener in Europe (Andresen, 1947) has contributed to the universal acceptance of the immutability of a person's blood type. The application of ABO and Rh phenotypes to deduce genotype and evaluate possible family's lineage has been firmly established. These traits or characters are not controlled by diet, disease or age (Sussman, 1953). ABO and Rh phenotype are reliable information to some extent in identifying the true family lineage; however, gene mapping or genotyping is far superior to phenotyping. Though a gene may undergo mutation, genotyping is far more precise and reliable than serological grouping for determining family relationships. In addition, the higher the number of immutable antigens included in the procedure the higher the reliability of determining a precise family lineage. Serological ABO and Rh blood grouping alone is not fully reliable to conclude the family lineage of these six members.

References

1. Andresen, P.H. (1947). Reliability of the exclusion of paternity after the MN and ABO systems as elucidated by 20,000 mother-child examinations and its significance to the medicolegal conclusion. *Acta Pathologica Microbiologica Scandinavica*; **24**, 545–553.
2. Bhat, S., Singh, K., and Shastri, S. (2010). Is Wait and Watch Policy More Appropriate in Newborn

- Grouping? *The Internet Journal of Laboratory Medicine*; **4**(1) 1.
3. Brecher, M.E. (2005). Blood Groups Antigen *In AABB Technical Manual 15th*, Chapter 13:293-294.
 4. Carrucio, L. (2012). Basic Genetics in *Modern Blood Banking & Transfusion Practices. 6th Edition*. Harmening DM (Eds) FA Davis Publishing Company: 29-30.
 5. Contrares, M. and Daniels, G. (2011). Antigens in human blood in *Postgraduate Haematology 6th Edition*. Hoffbrand, A.V., Catovsky, D., Tuddenham, E.G.D. and Green, A.R. (Eds). Blackwell Publishing: 244-245.
 6. Daniels, G. (2002). ABO, Hh, and Lewis System *In Human Blood Group 2nd Edition*. Blackwell Publishing: 21.
 7. Gassner, C., Schmarada, A., Nussbaumer, W. *et al.* (1996). ABO glycosyltransferase genotyping by polymerase chain reaction using sequence-specific primers. *Blood*; **88**: 1852–1856.
 8. Guyer, M.F. (1907). Do Offspring Inherit Equally from Each Parent? *Science*; **25**(652), 1006-1010.
 9. Harmening, D.M. and Forneris, G. (2012). The ABO blood group system. *In Modern Blood Banking and Transfusion Practices 6th Edition*. FA Davis Publishing Company: 112.
 10. Juel, E. (1959) Anti-A agglutinins in sera from A2B individuals. *Acta Pathology and Microbiology of Scandinavia*; **46**:91–95.
 11. McBean, R., Hyland, C. and Flower, R. (2014) Molecular genotyping platforms for blood group antigen prediction. *Pathology-Journal of the Royal College of Pathology of Australia*; **46**: S87.
 12. Phelan, D.L. and Morris, G.P., (2012). The HLA system. *In Modern blood banking and transfusion practices 6th Edition*. FA Davis Publishing Company: 496.
 13. Sussman, L.N. (1953). The Rh Blood Factor: Genetic Aspects of Erythroblastosis. *Eugene. News*; **38**: 102-106.
 14. Suzuki, K., Iwata, M., Tsuji, H., *et al.* (1997). A de novo recombination in the ABO blood group gene and evidence for the occurrence of recombination products. *Human Genetics*; **99**: 454–461
 15. Taylor, G.L., Race, R.R., Prior, A.M., *et al.* (1942). The frequency of the iso-agglutinin a1 in the serum of the subgroups A₂ and A₂B. *Journal of Pathology and Bacteriology*; **54**:514–516.
 16. Tucker, E. M. (1975). Genetic markers in the plasma and red blood cells. *In The blood of sheep*. Springer Berlin Heidelberg:123-153.