Genotyping confirms inheritance of the rare At(a−) type in a case of haemolytic disease of the newborn

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Abstract
The Ata blood group antigen (now AUG2 in the Augustine system) is a high-frequency antigen with negative phenotype At(a−) found only in individuals of African ancestry. In a twin pregnancy, the fifth pregnancy in a woman of African origin, serological investigations confirmed that the mother was At(a−) and anti-Ata was detected. DNA samples were exome sequenced and alignment was performed to allow variant calling. It was confirmed that the single nucleotide polymorphism, rs45458701, within the SLC29A1 gene encoding the ENT1 protein, recently reported to be a basis of the At(a−) phenotype was also the basis of the phenotype in this family. The reagents for serological analysis required to identify the rare blood type present in this mother are held in only a few reference laboratories worldwide. This case highlights the utility of genetic methods in resolving complex investigations involving blood grouping and demonstrates that genotyping of variants associated with blood types present in specific ethnic groups may be the fastest method available for identification of the basis of fetomaternal incompatibilities.

Keywords: Ata blood group antigen; fetomaternal incompatibility; genetic variant ENT1 transporter

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Introduction
Ata (proposed systematic terminology AUG2 in the Augustine system) is a high-frequency red blood cell (RBC) antigen found on the RBCs of over 99% of individuals [1]. The negative phenotype At(a−) is rare worldwide and has only been identified in individuals of West African or West Indies ancestry [2]. Examples of anti-Ata antibodies have been described in At(a−) individuals [3,4] although the clinical significance has remained largely uncharacterized due to the rarity of reagents to detect this phenotype.

Materials and methods
Standard serological procedures were used for indirect antiglobulin tests with a panel for antisera to rare blood group antigens available in the Australian Red Cross Blood Service Red Cell Reference Laboratory.

DNA samples from the mother, her husband, and their newborn twins (n = 4) were exome sequenced using the Agilent SureSelect DNA Human All Exon V5+UTRs on an Illumina HiSeq 2500. Sequence alignment was performed using the Illumina CASAVA1.8.2 pipeline and mapped to the reference genome hg19 to allow variant calling. Ethics approval for these genetic studies was from the Human Research Ethics Committee of the Australian Red Cross Blood Service.

Results
A case of haemolytic disease of the fetus and newborn (HDFN) in a twin pregnancy of parents of African ethnicity was referred to the Australian Red
Cross Blood Service Red Cell Reference Laboratory in June 2014. Cases of this type require rapid identification of the antibody for optimal management of suspected fetomaternal incompatibility and risk of bleeding at delivery. The full blood group phenotype in the mother in this case was Group AB and D+ C− E− c+ e+, M+N-S-s+, Jk(a+b−), Fy(a− b−), K− k+, Di(a−) P1+. The twins were born by Caesarean section prior to identification of the antibody and, fortunately, neither mother nor neonates required transfusion. Cord blood samples showed both twins were group A, RhD negative with a strong positive Direct Antiglobulin Test (DAT) of agglutination score 3+ on a scale with a maximum of 4+. An exchange transfusion was not required as one twin had a total bilirubin: 2.30 mg/dL, direct bilirubin: 0.21 mg/dL, Hct: 14.2 g/dL and the second twin had a total bilirubin: 2.27 mg/dL, direct bilirubin: 0.20 mg/dL, Hct: 39 and Hb: 13.5 g/dL. It was identified that the mother was At(a−) and, as no reaction was observed with a further example of At(-) RBCs that were ABO compatible, the implicated antibody was confirmed as anti-Ata.

DNA samples from the mother, her husband and their newborn twins (n = 4) were exome sequenced. Massively parallel sequencing (MPS) identified 126 575 variants, including single nucleotide variants and insertions and deletions within the four family members. Variants which did not fit the hypothesized inheritance model were excluded on the basis of allele count score. The next stage of the analysis was to identify variants in proteins in the currently accepted red blood cell proteome and further investigate candidate polymorphisms by serology. Simultaneous with this investigation it was reported that, for several other examples, a single nucleotide polymorphism, rs45458701, within the SLC29A1 gene encoding the ENT1 protein was associated with the At(a−) phenotype [5]. The rs45458701 variant was also found to be the basis of the At(a−) phenotype in this family (GenBank KT037686.1 partial CDS for the At(−) variant in this family) as the mother was homozygous for this variant, the twins were both heterozygous, and the father was homozygous for the “wild type” allele.

Discussion

The detection of the rs45458701 polymorphism in this case provides further supporting evidence for anti-Ata to be recognized as an antigen (AUG2) in the Augustine system, proposed by the International Society of Blood Transfusion as the 36th blood group system. Only one example of a case of mild HDFN caused by anti-Ata has been reported previously [3]. Also, as an example of a severe haemolytic transfusion reaction caused by anti-Ata has been reported [4] it is important to ensure that compatible blood is available should the mother require transfusion postdelivery. Pregnancies where there is a risk of HDFN in which the mother is At(a−) require careful monitoring and management to minimize any requirement for transfusion.

This case highlights the difficulties in identifying incompatibilities in populations in which there is significant blood group diversity such as contemporary Australia and the Asia/Pacific. The reagents for serological analysis required to identify the rare blood type present in this mother and the antibody implicated in the positive DAT are held in only a few reference laboratories worldwide. The genotyping method for exome sequencing currently takes an estimated 5–10 working days to perform. It comprises three key steps: library preparation (one day), sequencing (27 hours for 20 samples) followed by data analysis which, currently, is the rate-limiting step. Development and implementation of algorithms for the data analysis step, specifically targeting the exomes for the defined blood groups, provides a strategy for streamlining and making this analysis routine. When analysing a MPS data set from a large family group with a unique or unusual blood group phenotype, that includes members with and without the phenotype (as for the SARA antigen) [6], using linkage data greatly reduces the number of variants to be considered, accelerating the analytical process.

In conclusion, this case provides an example of the utility of genetic methods in resolving complex investigations in blood grouping and that genotyping of variants associated with blood types may be the fastest method available for identification of the basis of fetomaternal and other transfusion-related incompatibilities. MPS has become a powerful tool in the investigation of the genetic basis of RBC phenotypes that are novel and previously uncharacterized.

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Author contributions

RMcB conducted the genetic analysis and wrote the manuscript; YWL supervised serological analysis and
reviewed the manuscript; BW conducted serological analysis and reviewed the manuscript; PK conducted serological analysis and reviewed the manuscript; ME conducted serological analysis and reviewed the manuscript; CH supervised genetic analysis and reviewed the manuscript; RF supervised genetic analysis and co-wrote the manuscript.

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