PIGG Defines the Emm Blood Group System

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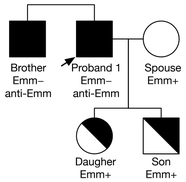
Whole Genomes Sequencing (WGS) of an **Emm− Proband** and family revealed a ***PIGG* loss of function variant**. *PIGG* loss of function variants **found in four other probands**. PIGG adds **EtNP onto GPI-anchor**.

Background

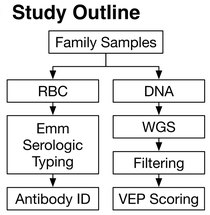
Emm is one of the few remaining blood groups whose basis is unknown. It is a high incidence red cell antigen with eight Emm− probands reported over the past 30 years. Anti-Emm appears to be naturally occurring yet responsible for a clinically significant acute hemolytic transfusion reaction. Previous work showed that Emm is located on a GPI-anchored protein, but the antigenic epitope and genetic basis have been elusive.

Identification of *PIGG* Deficiency In Emm− Proband 1

A Family Pedigree



B Study Outline



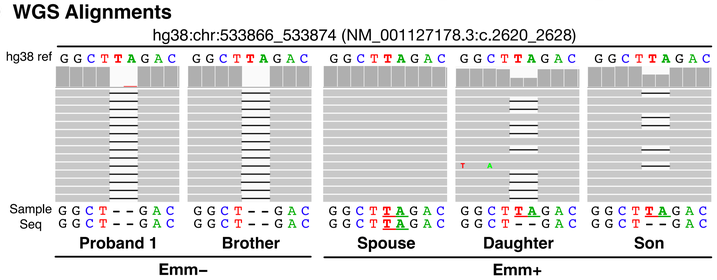
C Variant Identification

	Total Variants	Variant Effect Predictor (VEP) High	Moderate	Low
Emm− Proband 1 All Variants	5,149,248	1,564	15,296	19,822
Proband 1 Homozygous Variants	1,942,938	504	5,245	7,142
Homozygous in Emm− Brother	1,427,285	360	3,868	5,112
Heterozygous in Emm+ Children	425,464	106	989	1,466
gnomAD Allele Frequency <0.001	1,818	1	2	4

PIGG

2 bp deletion, frameshift, premature stop
NM_001127178.3:c.2624_2625delTA

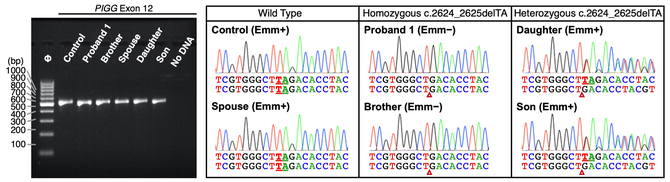
D WGS Alignments



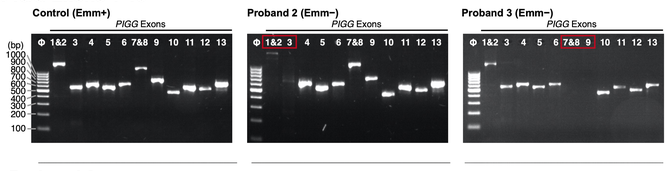
(A) Family Pedigree. Emm RBC phenotypes shown include the Proband and his brother, both with anti-Emm in the plasma, the Probands spouse and two children who are Emm+ and were shown to lack the antibody. (B) Study Outline. Emm serologic RBC typing was performed with in-house reagents and short read whole genome sequencing (WGS). (C) Variant Identification. Illustration of the strategy used to enrich for loss of function mutations associated with familial inheritance and variant effector prediction (VEP) scoring to identify the genetic cause of the Emm− phenotype. PIGG was the only candidate gene that passed our filtering strategy. (D) WGS alignments. IGV genomics viewer shows the wild type sequence (upper) and PIGG Exon 12 biallelic sequence for each family member (below). Individual sequence reads are shown in gray for positions corresponding to the hg38 reference sequence with bar plots above (dark grey) reflecting the relative number of reads at that position. Emm− family members were homozygous for a 2-bp deletion in *PIGG* Exon 12, predicted to cause a frameshift and premature stop, designated in as c.2624_2625delTA, p.(Leu875*), rs771819481, and chromosomal location hg38:chr4:533870_533871delTA. The spouse was wild type, and the daughter and son are heterozygous. The following *PIGG* reference sequences were used throughout the manuscript: NG_051621.1 (genomic) and NM_001127178.3 (transcript)

Identification of *PIGG* Variants by targeted NGS and Sanger

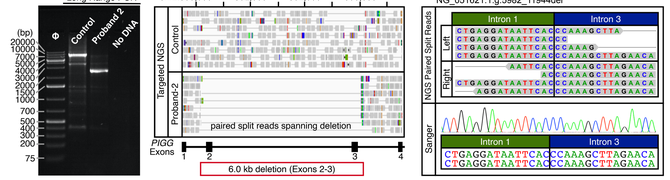
Proband 1 c.2624_2625delTA, p.(Leu875*), rs771819481



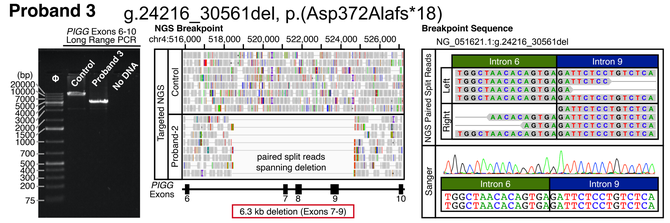
***PIGG* Exons 1-13**



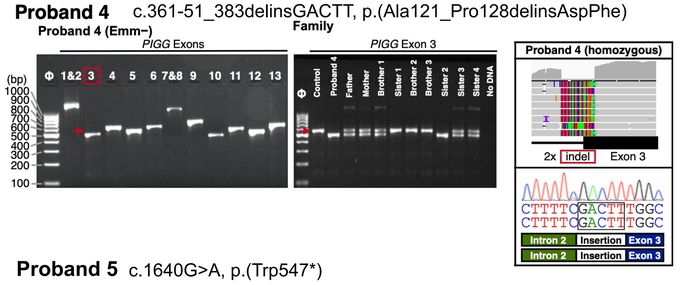
Proband 2 g.5982_11944del, p.(Ala53Glyfs*2)



Proband 3 g.24216_30561del, p.(Asp372Alafs*18)



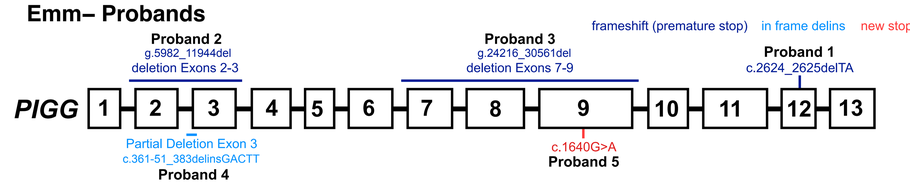
Proband 4 c.361-51_383delinsGACTT, p.(Ala121_Pro128delinsAspPhe)



Proband 5 c.1640G>A, p.(Trp547*)

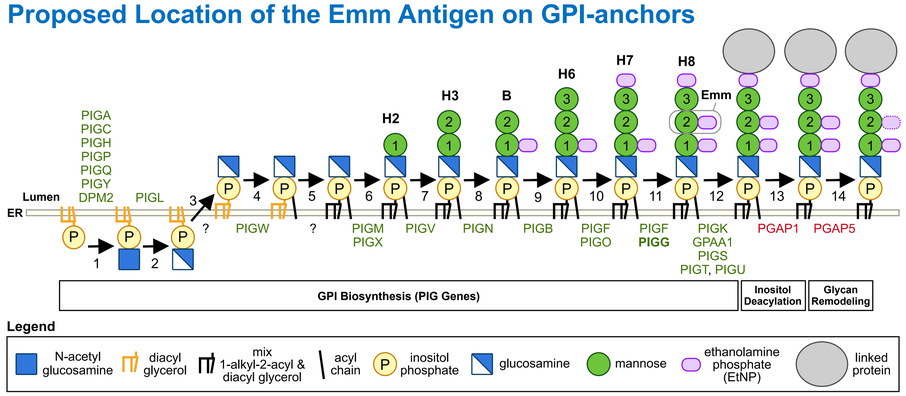
Red cells from proband 5 and her brother, previously reported to have a *PIGG* loss of function variant [c.1640G>A, p.(Trp547*), rs547951371] identified by WES, were found here to be also be Emm− by serologic testing.

PIGG Variants Found in Emm− Probands 1-5



The five loss of function variants reported or investigated here are shown. Three causes a frameshift and premature stop codon, one causes a deletion and insertion, and one introduces a stop codon.

Proposed Location of the Emm Antigen on GPI-anchors



The schematic shows the relevant part of the GPI anchor synthesis pathway. The proteins involved in each step are indicated below the line. PIGG transferase (bold) is reported to be expressed during erythropoiesis, but loss of function in Emm− individuals would mean EtNP is not added to mannose 2. EtNP on mannose 2 is the likely antigenic target responsible for anti-Emm.

Conclusion

The findings here illustrate the power of using WGS with family cohorts to uncover the genetic basis of blood group systems. Based on these findings the ISBT Red Cell Immunogenetics and Blood Group Terminology working party has designated Emm as the 42nd blood group system.

For more details see our recent paper

Lane WJ, et al. Sci Rep. 2021 Sep 17;11(1):18545. doi: 10.1038/s41598-021-98090-w. PMID: 34535746.
https://bit.ly/3ooLHII

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