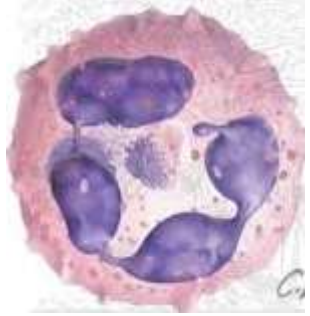

# *Molecular genotyping of Human Neutrophil Antigen-3 among Indian blood donors*

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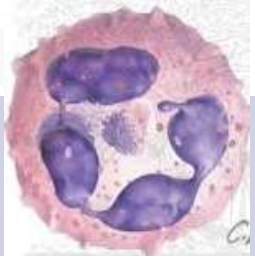
# *Introduction*



- Immune-mediated transfusion related acute lung injury (TRALI) is caused by antibodies against human leucocyte antigens (HLA) or human neutrophil antigens (HNA).

- These antibodies are transmitted to the patient via transfusion of blood products from donors who have been previously immunized against these antigens (during pregnancy or transfusion).

- In contrast to HLA, HNA-1 and HNA-2 antibodies, **HNA- 3a antibodies are usually involved in severe TRALI cases usually requiring artificial ventilation.**

- Despite their known clinical significance, screening for antibodies against HNA-3a is not performed in India.

- To identify individuals at the risk of producing anti-HNA-3 antibodies, it is essential to know the frequency of these antigens in Indian population.

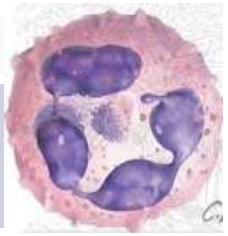
- Phenotyping of HNA-3 antigens and antibody detection were initially performed by granulocyte agglutination test and granulocyte immunofluoresence tests

- However, alloantisera specific to neutrophil antigens are not always available.

- HNA-3 system was identified by van Leeuwan et al in 1964 using antibodies from multiparous women that agglutinated with normal persons.
- The recent elucidation of the molecular properties of the HNA-3a and 3b alloantigens in 2010 has facilitated the detection of the HNA-3 antigens using DNA-based methods.
- HNA-3 is a bialleleic system consisting of two antigens-3a and 3b which are located on choline transporter-like protein 2 (CTL2).
- Single nucleotide polymorphisms (SNP) at nt 461 in exon 7 of SLC44A2 gene (Chromosome 19p13.1) in formation of HNA-3a/3b antigens

| Antigen | Allele name | Nuleotide at nt461 of exon 7 | Amino acid change at poition154 |
|---|---|---|---|
| HNA-3a | SLC44A2*1 | G | Arginine |
| HNA-3b | SLC44A2*2 | A | Glutamine |

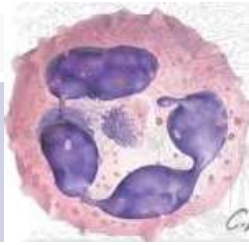
# *Aim*



- Since the frequencies of HNA-3 antigens may vary among different population groups and they are not defined in Indian population…

- The aim of this study was **to determine the frequency of human neutrophil antigens/alleles HNA-3a and HNA-3b** among Indian blood donors using a PCR-based method.

# Materials and methods



**Collection of samples**: Blood samples were collected from 294 blood donors

↓

**DNA Extraction**: Using standard phenol-chloroform method

↓

**PCR Amplification**: A 271bp fragment containing the HNA-3 SNP (461G and/or A) was amplified by polymerase chain reaction using specific primers.

↓

**Restriction Fragment Length Polymorphism (RFLP):** The PCR product was then digested with restriction enzyme Taqα1 (New England BioLabs, Beverly, MA) to distinguish between HNA-3a and 3b genotypes

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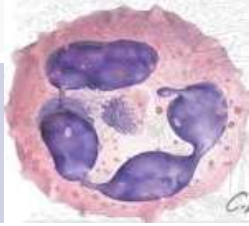
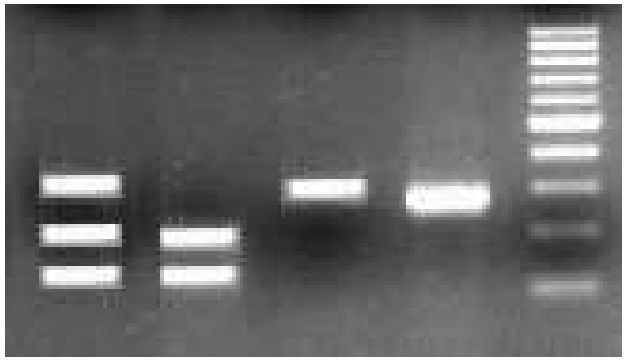
**Validation of RFLP by DNA Sequencing**: DNA Sequencing was carried out to confirm the PCR-RFLP genotyping results.

# Results & Discussion



- PCR-RFLP was standardized for genotyping HNA-3a/3b antigens.

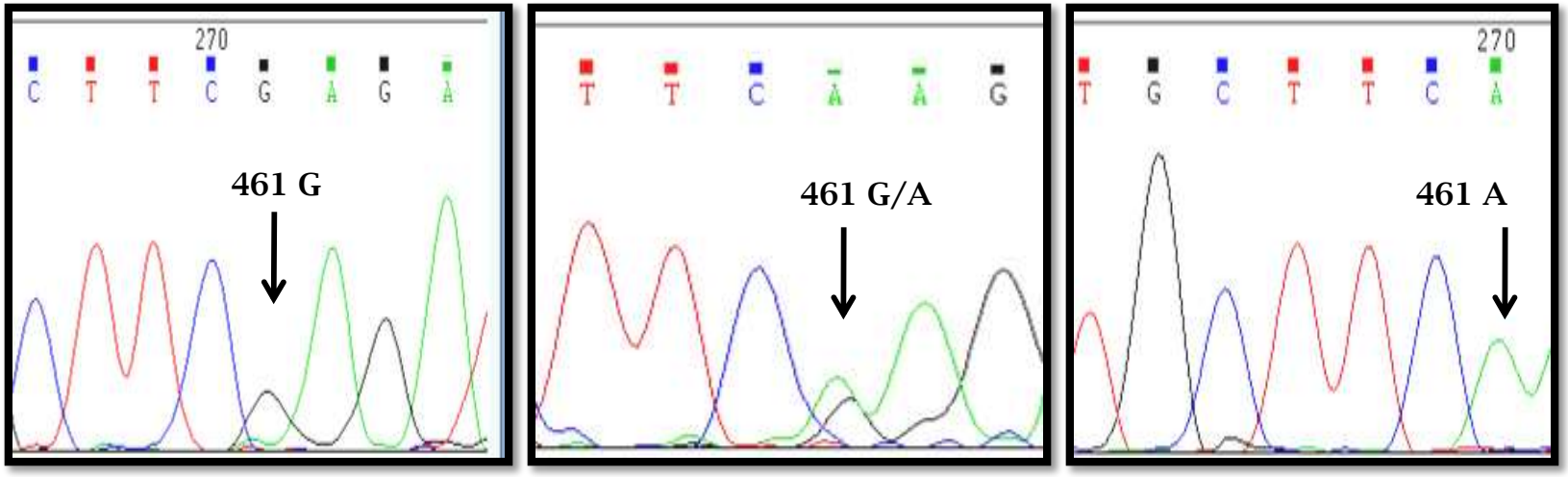
- Figure: RFLP analysis for HNA-3 genotyping.




**Lane1: HNA-3 (a+b+); 461G/A**
(271bp+171bp+100bp)
**Lane2: HNA-3 (a+b-); 461G**
(171bp+100bp)
**Lane3: HNA-3 (a-b+); 461A**
(271bp)
**Lane4: Undigested PCR product**

**Lane5: 100bp step up ladder**

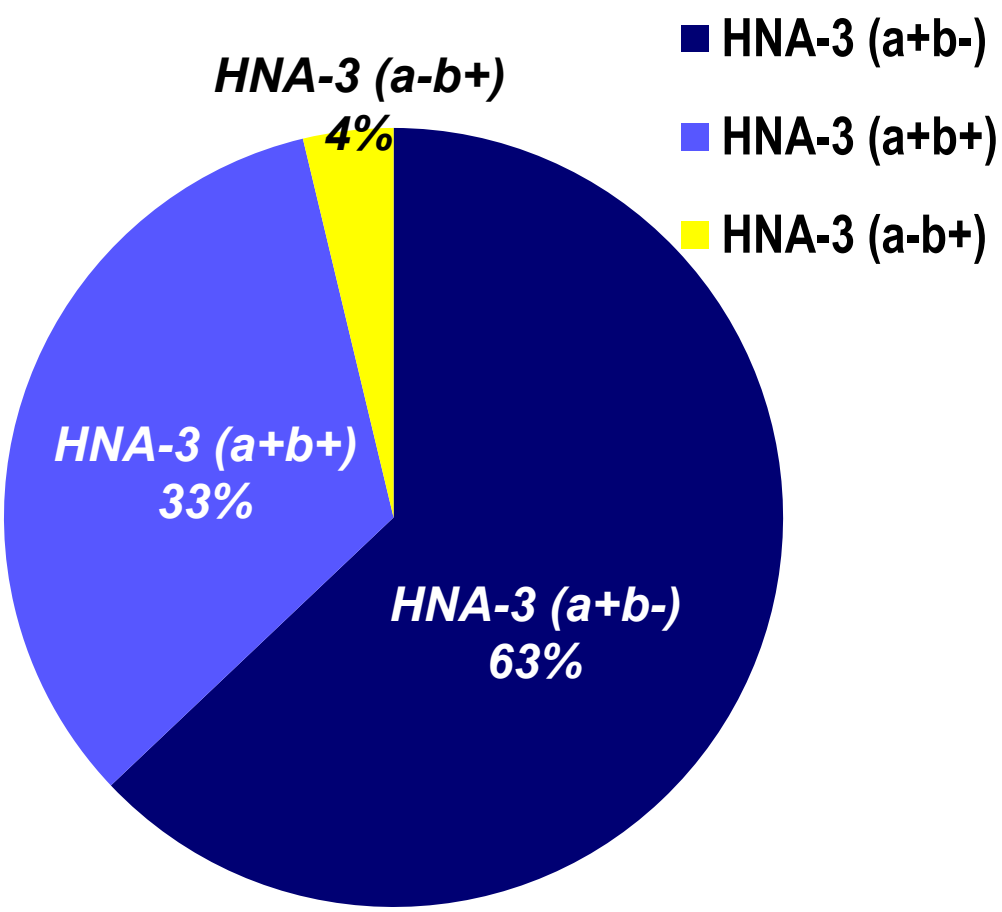
- PCR-RFLP results were confirmed by carrying out DNA sequencing of 10 samples.
- HNA-3a-positive genotypes (a+b-) showed 461G polymorphism
- HNA-3a-negative genotypes (a-b+) showed 461A polymorphism
- HNA-3a heterozygous samples(a+b+) showed both 461G and 461A polymorphisms.



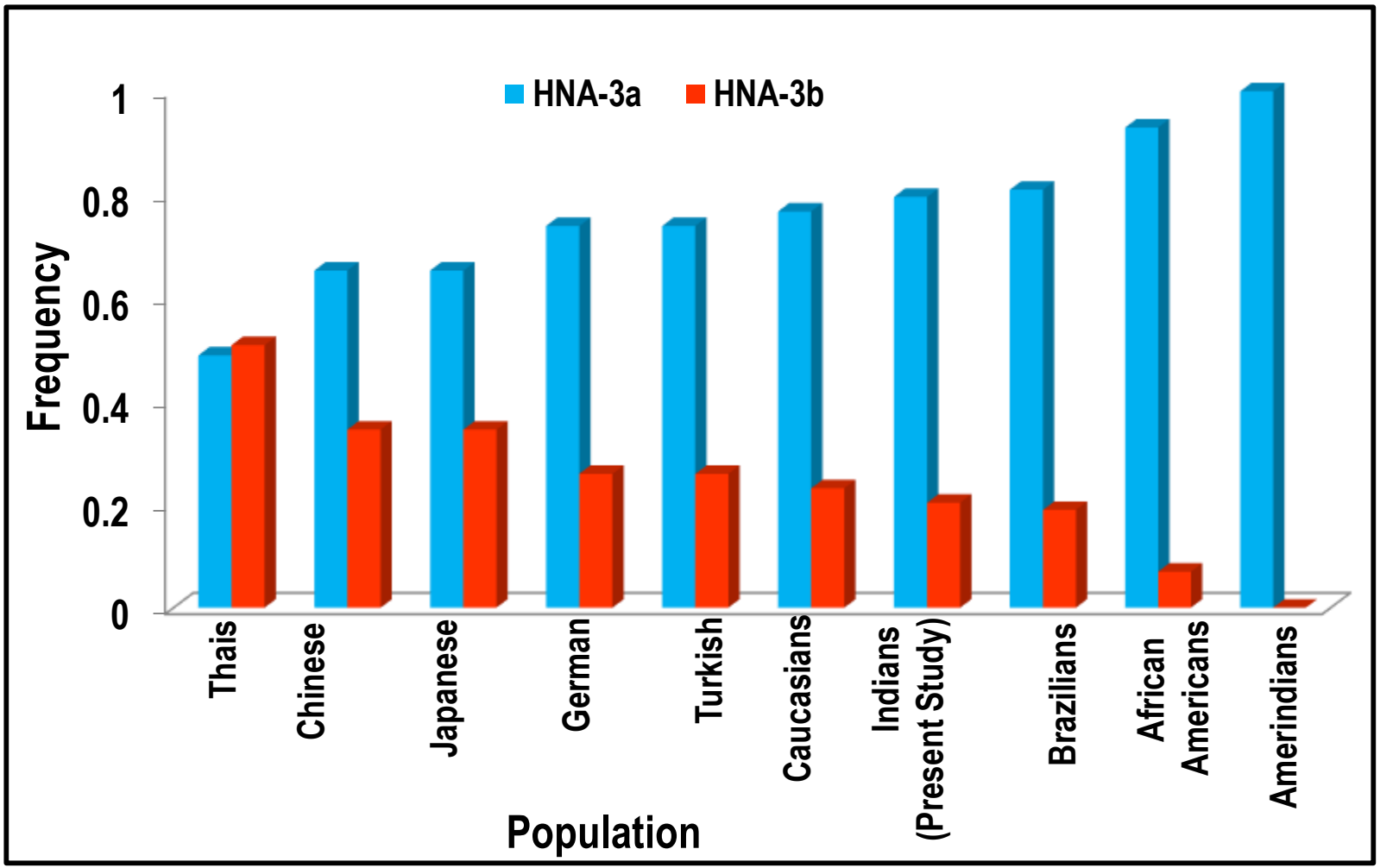

*HNA-3(a+b-)* *HNA-3(a+b+)* *HNA-3(a-b+)*

**Figure: DNA sequencing of HNA-3**

- Among 294 donors, 62.9% donors were homozygous for HNA-3a antigen (aa) i.e. HNA-3 (a+b-).
- 33.3% were heterozygous (ab) i.e. HNA-3 (a+b+).
- 3.7% donors were HNA-3a-negative genotype (bb) i.e. HNA-3 (a-b+).



- The frequency of HNA-3a (0.7959) found in our study was higher as compared to that of Thais, Chinese and Japanese and comparable to that of Caucasians, Germans and Turkish. However, it was lower than Brazilians.



- In addition to 461G>A polymorphism, 100 samples showing HNA-3a homozygous genotype were sequenced for presence of additional nonsynonymous polymorphism c.457C>T.

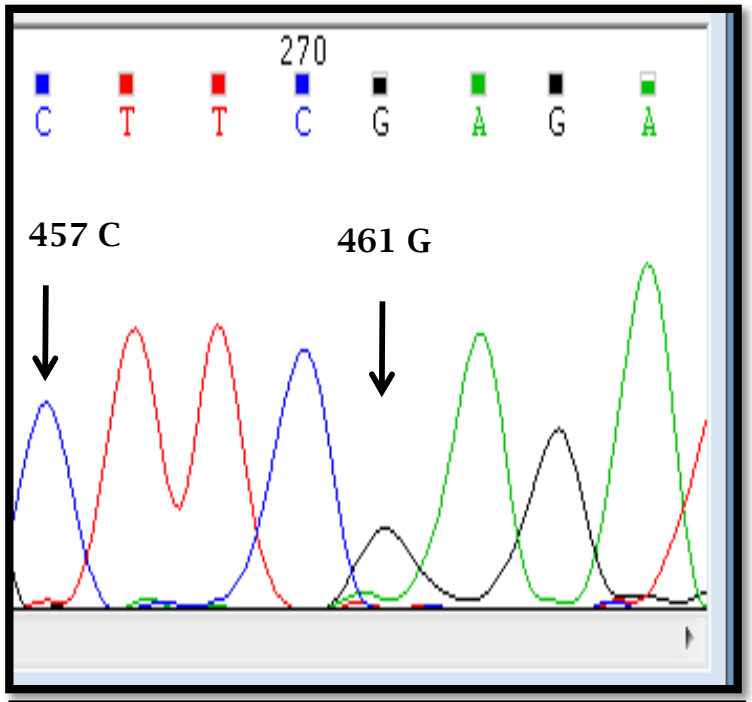



**Figure: DNA sequencing showing 457C (homozygous) in a HNA-3a homozygous individual**

- This polymorphism alters the allogenic determinants of some anti-HNA-3a antibodies.

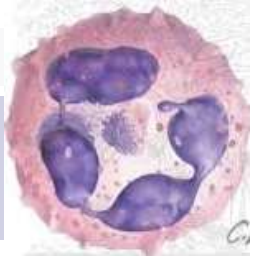
- All 100 HNA-3a homozygous samples did not show presence of c.457T polymorphism.

- Our findings suggest that about 4% individuals are at a risk of producing anti-HNA-3a that may cause severe cases of TRALI.

- Genotyping of donors for HNA-3 will thereby contribute to reduction of the frequency of TRALI cases.

- Also, typed donors for HNA-3a/3b can be included in screening and identification panels for HNA antibody detection.

# *Conclusion*



- This is the **first study** to report the frequency of HNA-3 antigens among Indian blood donors.
- The allele frequency of HNA-3a and HNA-3b was found to be 0.7959 and 0.2041 respectively.
- Rapid identification of HNA-3a–negative individuals using a simple PCR-based technique (such as PCR-RFLP) can be used as a part of diagnostic assessment for TRALI and also to identify persons at the risk of producing anti–HNA-3a.

# Thank You!!