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Genetic epidemiology of human neutrophil antigen variants suggest significant global variability

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Abstract

Human neutrophil antigens possess significant clinical implications especially in the fields of transfusion and transplantation medicine. Efforts to estimate the prevalence of genetic variations underpinning the antigenic expression are emerging. However, there lacks a precise capture of the global frequency profiles. Our article emphasizes the potential utility of maintaining an organized online repository of evidence on neutrophil antigen associated genetic variants from published literature and reports. This in our opinion, is an emerging area and would significantly benefit from the awareness and understanding of population-level diversities.

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Introduction

Granulocytes constitute the major proportion of human leukocytes and are an inevitable part of the innate immune system. As a significant majority (~99%) of the granulocyte population primarily comprises of neutrophils, a family of glycoprotein epitopes expressed on granulocytes have been collectively named as the human neutrophil antigens (HNAs) (Browne et al., 2021),(Flesch & Reil, 2018). Based on the serologically defined epitopes, these antigens are classified into five major groups/systems namely HNA-1 to HNA-5. Molecular characterization revealed that antigens of HNA class 1-5 are encoded by FCGR3B, CD177, CLT2, ITGAM and ITGAL genes respectively (Flesch & Reil, 2018). The FCGR3B gene encoding alleles of the HNA-1 system spans chromosome 1q23-24 and also carries a high degree of homology with the FCGR3A gene (Ravetch & Kinet, 1991), (Ravetch & Perussia, 1989). CD177 glycoprotein is produced by the CD177 gene located on chromosome 19q13.2 whereas the molecular functions of CD177P, a pseudogene located downstream of CD177 still remains unclear (Bettinotti et al., 2002), (Caruccio et al., 2006). Recently, a decade ago, the HNA-3 antigen was found localized to CTL2, encoded by SLC44A2 gene spanning chromosome 19p13.1 (Greinacher et al., 2010), (Curtis et al., 2010). HNA-4a was formerly termed "Mart^a" and is found to be expressed on the CD11b/α_M subunit of the $\alpha_M\beta_2$ - integrin (Simsek et al., 1996). Similarly, HNA-5a antigen which was previously described as "Onda" is expressed on the α_L subunit of the $\alpha_L\beta_2$ - integrin (Décary et al., 1979). Although HNA-1 and HNA-2 have been found specifically expressed on the neutrophil surfaces (Lalezari, 2017), classes HNA-3 to HNA-5 have a broad range of expression in other blood cells (Flesch et al., 2016).

Antibodies against HNA (auto-, iso- and allo-antibodies) have been found to possess significant clinical relevance especially in terms of transfusion and transplantation complications (Jug et al., 2021),(Key et al., 2018). Clinical disorders including alloimmune neonatal neutropenia and autoimmune neutropenia are caused by neutrophil specific antibodies (Porcelijn & de Haas, 2018),(Fung et al., 2003). In addition, almost all classes of HNA antigens (HNA-1a, 1b, 1c, 2, 3a, 4a and 5a) have been found to have significant implications in transfusion related acute lung injury (TRALI) cases (Vlaar, 2012), (Moritz et al., 2009), (Bayat & Sachs, 2012), (Caudrillier & Looney, 2012)

Limited studies have previously estimated the population frequencies of HNA antibodies(J. L. Gottschall et al., 2011),(J. Gottschall et al., 2013),(Nguyen et al., 2009),(Esmaeili et al., 2022). Various PCR-based HNA genotyping assays have been developed and continue to be the gold standard method of detection (Huvard et al., 2012), (Stein et al., 1995), (Cardone et al., 2006). Rapid advent of next generation sequencing technology has provided the opportunity to redesign genotyping strategies for identifying and evaluating new HNA alleles. The wide availability of population-scale genomic datasets as part of the 1000 genome ("A Global Reference for Human Genetic Variation," 2015) and gnomAD consortium (Karczewski et al., 2020) have enabled the deeper understanding of ethnic

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differences in genotypes. As population-scale genome programmes accelerate in pace across the world, the paucity of a comprehensive and systematic collection of genetic variants associated with human neutrophil antigens significantly limit the understanding of population differences. To fill in this gap, we compiled a comprehensive collection of Human Neutrophil Antigen variants from literature, adding to the collection maintained by the International Society of Blood Transfusion - Granulocyte Immunobiology Working Party (ISBT-GIWP). A brief summary of the compiled genetic variants is provided in **Table 1.** This collection has a total of 37 genomic variants encompassing the five major HNA classes and is available at the Human Neutrophil Antigen Resource http://hunar.genomes.in.

Methods

Data retrieval and analysis

Human genome variation catalogue generated by the 1000 Genomes phase 3 project which precisely comprised over 2500 whole genome sequences from 5 major global populations and 26 subpopulations was used in the study ("A Global Reference for Human Genetic Variation," 2015). Data was retrieved in variant call format (VCF) for chromosomes 1, 16 and 19. Information on allele counts, allele numbers and allele frequencies of each sub-populations were fetched from https://www.ensembl.org/. In addition, genetic variation information of global populations were also taken from gnomAD (version 3.1.2) (Karczewski et al., 2020). The retrieved HNA variant information was systematically documented in a pre-formatted template and was used for further downstream analysis. **Table 2.** provides a brief summary of global populations and subpopulations analyzed in the study.

Estimation of statistical significance

With the aim of identifying significantly distinct HNA variants, minor allele frequencies were compared among global sub-populations and statistical significance was observed using Fisher's exact test with a P-value < 0.05. Variants identified as significantly distinct were further checked for their clinical relevance in transfusion and transplantation procedures.

Results and Discussion

It is well established that neutrophil antigens play a central role in clinically significant transfusion and transplantation related complications in addition to other auto- and allo-immune conditions.Our analysis identified significant inter-population differences in the allele frequencies of the HNA variants. **Figure 1** provides a schematic overview of the differences in frequencies of HNA variants observed among various subpopulations of the 1000 Genomes project and gnomAD genomes. Interestingly, ITGAL_16:30506720G>C (rs2230433) variant, encoding the antithetical epitope of HNA-5a was found significantly distinct in all the 5 major global subpopulations with a frequency of 71.4% in South Asians, 13.4% in East Asians, 30.1% in Europeans, 42.1% in Africans and 18.9% in Americans. No allo antibodies against this form have been reported and characterized so far. In addition,

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ITGAM_16:31265490G>A substitution (rs1143679) which is a central variant in deciding the translation of either 61Arg or its antithetical form 61His was found to range from 0-0.15 in global subpopulations. This was found in concordance with the previous reports that states that HNA-4a allele frequencies in most global populations ranged from 0.9-1.0 (Xia et al., 2011), (Kline et al., 1986), (Sachs et al., 2004). Cases of fetal/neonatal alloimmune neutropenia induced by antibodies against HNA-4a/b and HNA-5a have also been reported (Curtis et al., 2016),(Porcelijn et al., 2011),(Mraz et al., 2016).

There also exists a handful of reports elaborating the fatal outcomes of transfusion related acute lung injury (TRALI) caused by alloantibodies against HNA-3a antigen (Chapman et al., 2009; Porretti et al., 2012),(Davoren et al., 2003). SLC44A2_19:10631494A>G substitution (rs2288904) leading to Arg152Gln marks the difference between HNA-3a and HNA-3b antigens. In compliance with previous findings, HNA-3a encoding allele frequencies were found to be 96.7% in Africans, 85.2% in Americans, 71.0% in East Asians, 76.4% in Europeans and 75.5% in South Asian populations, whereas the respective HNA-3b allele frequencies were 3.3%, 14.8%, 29.0%, 23.6% and 24.5%. The additional mutation SLC44A2_19:10631490C>T (rs147820753), which was found to impair HNA-3a epitope and abrogates the agglutination by inducing Leu151Phe amino acid change was found at a frequency ranging from 0 - 0.4% across global populations. Although concordant frequencies were observed between the two datasets used in the study, we were able to observe disparity in the frequency of FCGRB3_1:161629903T>C variant which can be possibly attributed to the fact that this variant was covered in fewer than 50% of individuals in gnomAD v3.1.2 genomes and can be a potential low-quality site.

Such efforts to estimate the genotype frequencies of neutrophil antigens among global populations provides an overview of population - specific prevalence.

Conclusion

In conclusion, we suggest significant population differences in the HNA variants which could potentially impact clinical presentations and transfusion reactions. With the availability of genomic data from more populations which have been traditionally under-represented in global sequencing efforts, much clearer inter-population differences in HNA variant frequencies could be potentially uncovered. We sincerely hope this data could impact transfusion practices and provide much deeper insights into the genetic underpinnings of HNA related reactions and clinical presentations.

Figures and Tables

Table 1. Tabulation of list of ISBT approved and other curated variants of HNA classes 1-5
Table 2. Tabulation of global populations and subpopulations analyzed in the study.

Figure 1. Comparison of frequencies of human neutrophil antigen variants among various subpopulations of the 1000 Genomes project and gnomAD

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HNA-1 : Antigens encoded by FCGR3B gene						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
1	161629781	T	C	rs2290834	ISBT approved variant	-
1	161629853	T	C	rs147574249	ISBT approved variant	-
1	161629864	G	T	rs5030738	ISBT approved variant	-
1	161629903	T	C	rs448740	ISBT approved variant	-
1	161629983	A	G	rs527909462	ISBT approved variant	-
1	161629989	G	C	rs200688856	ISBT approved variant	-
1	161629726	C	T	rs201867931	Curated variant	(He et al., 2014)
1	161629739	C	T	rs202089363	Curated variant	(He et al., 2014)
HNA-2 : Antigens encoded by CD177 gene						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
19	43361169	A	T	rs201821720	Curated variant	(Wu et al., 2019)
19	43362297	G	A	rs78718189	Curated variant	(Wu et al., 2019)
19	43361225	A	T	NA	Curated variant	(Bayat et al., 2016)
19	43361509	G	-	NA	Curated variant	(Bayat et al., 2016)
19	43361175	A	C	NA	Curated variant	(Moritz et al., 2010)
19	43362090	G	A	NA	Curated variant	(Moritz et al., 2010)
19	43353934	A	T	NA	Curated variant	(Moritz et al., 2010)
19	43353956	G	A	NA	Curated variant	(Moritz et al., 2010)
19	43362339	G	A	NA	Curated variant	(Moritz et al., 2010)
19	10602536	G	A	rs115559926	Curated variant	(Chen et al., 2016)
19	10627963	C	T	rs3087969	Curated variant	(Chen et al., 2016)
19	10631490	C	T	rs147820753	Curated variant	(Chen et al., 2016)
19	10631494	A	G	rs2288904	Curated variant	(Chen et al., 2016)
19	10632065	G	A	rs146127348	Curated variant	(Chen et al., 2016)
19	10632155	C	T	rs141667578	Curated variant	(Chen et al., 2016)
19	10635053	A	G	rs35362308	Curated variant	(Chen et al., 2016)
19	10635247	C	T	rs7255431	Curated variant	(Chen et al., 2016)

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19	10643307	G	A	rs34625417	Curated variant	(Chen et al., 2016)
19	10643310	C	G	rs151043533	Curated variant	(Chen et al., 2016)
19	10643627	T	C	rs116639546	Curated variant	(Chen et al., 2016)
19	10643724	A	G	rs1053007	Curated variant	(Chen et al., 2016)
19	10644138	G	A	rs74795234	Curated variant	(Chen et al., 2016)
19	10644229	G	T	rs10948	Curated variant	(Chen et al., 2016)
HNA-3 : Antigens encoded by CLT2 gene						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
19	10631490	C	T	rs147820753	ISBT approved variants	-
19	10631494	A	G	rs2288904	ISBT approved variants	-
19	10635012	C	T	NA	Curated variant	(Flesch et al., 2011)
19	10631666	C	T	NA	Curated variant	(Flesch et al., 2011)
HNA-4 : Antigens encoded by ITGAM gene						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
16	31265490	G	A	rs1143679	ISBT approved variants	-
HNA-5 : Antigens encoded by ITGAL gene						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
16	30506720	G	C	rs2230433	ISBT approved variants	-

Table 1. Tabulation of list of ISBT approved and other curated variants of HNA classes 1-5.

1000 Genomes Phase 3 dataset		
Population	**Sub-population**	**Description**
	ACB	African Caribbean in Barbados
	ASW	African Ancestry in Southwest US
	ESN	Esan in Nigeria
	GWD	Gambian in Western Division, The Gambia
African - AFR	LWK	Luhya in Webuye, Kenya

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	MSL	Mende in Sierra Leone
	YRI	Yoruba in Ibadan, Nigeria
American - AMR	CLM	Colombian in Medellin, Colombia
	MXL	Mexican Ancestry in Los Angeles, California
	PEL	Peruvian in Lima, Peru
	PUR	Puerto Rican in Puerto Rico
East Asian - EAS	CDX	Chinese Dai in Xishuangbanna, China
	CHB	Han Chinese in Beijing, China
	CHS	Southern Han Chinese, China
	JPT	Japanese in Tokyo, Japan
	KHV	Kinh in Ho Chi Minh City, Vietnam
European - EUR	CEU	Utah residents with Northern and Western European ancestry
	FIN	Finnish in Finland
	GBR	British in England and Scotland
	IBS	Iberian populations in Spain
	TSI	Toscani in Italy
South Asian - SAS	BEB	Bengali in Bangladesh
	GIH	Gujarati Indian in Houston, TX
	ITU	Indian Telugu in UK
	PJL	Punjabi in Lahore, Pakistan
	STU	Sri Lankan Tamil in the UK
gnomAD v3.1.2 dataset		
	AZK	Ashkenazi Jewish
	EU-NF	European Non-Finnish
	EU-F	European Finnish
	L-AM	Latino/Admixtured American
	EAS	East Asian
	GME	Middle Eastern
	AMI	Amish
	A-AM	African/African American
	SAS	South Asian

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	OTH	Others

Table 2. Tabulation of global populations and subpopulations analyzed in the study.

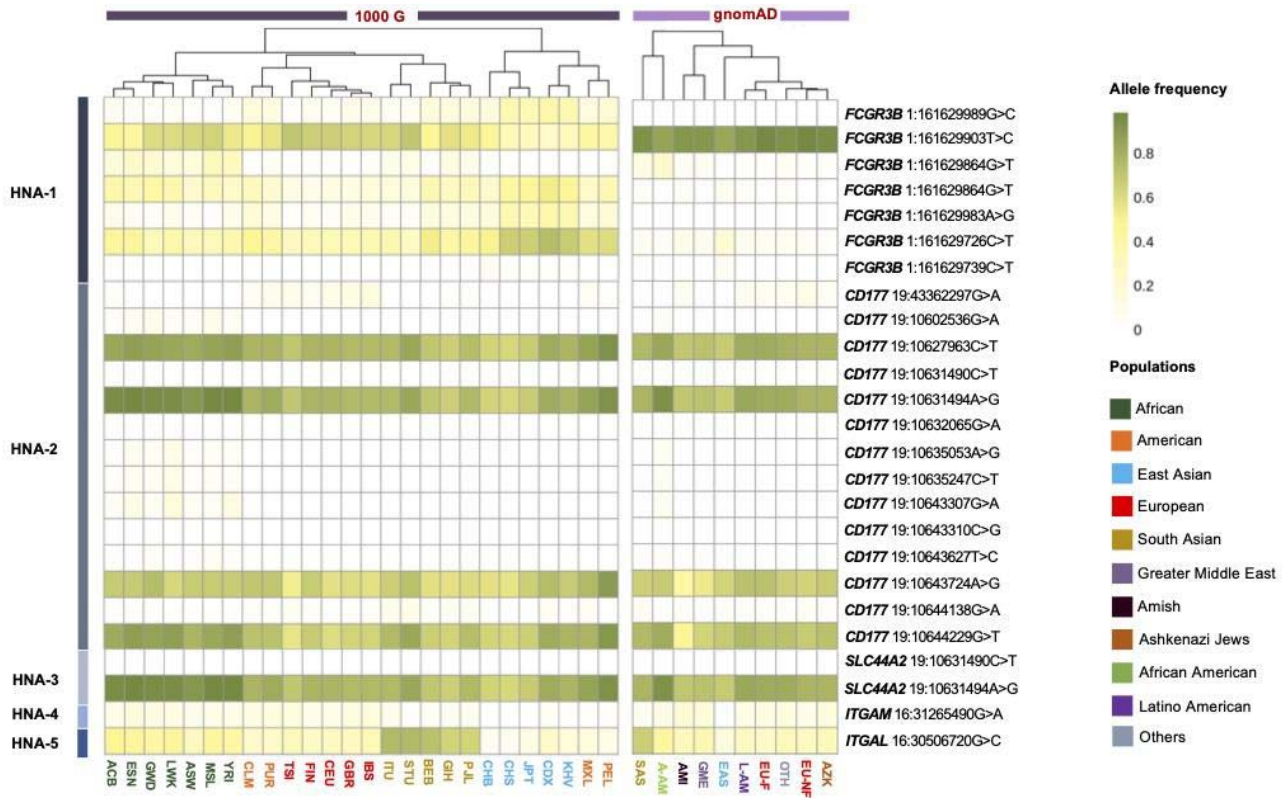

Figure 1. Comparison of frequencies of human neutrophil antigen variants among various subpopulations of the 1000 Genomes project and gnomAD

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Conflicts of Interest

None declared

References

1. A global reference for human genetic variation. (2015). *Nature*, *526*(7571), 68–74.
2. Bayat, B., Bein, G., & Sachs, U. J. (2016). A sequence-specific polymerase chain

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reaction method for HNA-2 genotyping: homozygous c.843A>T mutation predicts the absence of CD177. *Transfusion*, *56*(8), 2127–2132.

3. Bayat, B., & Sachs, U. J. (2012). Transfusion-related acute lung injury: an overview. *Current Pharmaceutical Design*, *18*(22), 3236–3240.
4. Bettinotti, M. P., Olsen, A., & Stroncek, D. (2002). The use of bioinformatics to identify the genomic structure of the gene that encodes neutrophil antigen NB1, CD177. *Clinical Immunology* , *102*(2), 138–144.
5. Browne, T., Dearman, R. J., & Poles, A. (2021). Human neutrophil antigens: Nature, clinical significance and detection. *International Journal of Immunogenetics*, *48*(2), 145–156.
6. Cardone, J. D. B., Bordin, J. O., Chiba, A. K., Norcia, A. M. M. I., & Vieira-Filho, J. P. B. (2006). Gene frequencies of the HNA-4a and -5a neutrophil antigens in Brazilian persons and a new polymerase chain reaction-restriction fragment length polymorphism method for HNA-5a genotyping. *Transfusion*, *46*(9), 1515–1520.
7. Caruccio, L., Bettinotti, M., Director-Myska, A. E., Arthur, D. C., & Stroncek, D. (2006). The gene overexpressed in polycythemia rubra vera, PRV-1, and the gene encoding a neutrophil alloantigen, NB1, are alleles of a single gene, CD177, in chromosome band 19q13.31. *Transfusion*, *46*(3), 441–447.
8. Caudrillier, A., & Looney, M. R. (2012). Platelet-neutrophil interactions as a target for prevention and treatment of transfusion-related acute lung injury. *Current Pharmaceutical Design*, *18*(22), 3260–3266.
9. Chapman, C. E., Stainsby, D., Jones, H., Love, E., Massey, E., Win, N., Navarrete, C., Lucas, G., Soni, N., Morgan, C., Choo, L., Cohen, H., Williamson, L. M., & Serious Hazards of Transfusion Steering Group. (2009). Ten years of hemovigilance reports of transfusion-related acute lung injury in the United Kingdom and the impact of preferential use of male donor plasma. *Transfusion*, *49*(3), 440–452.
10. Chen, Q., Srivastava, K., Ardinski, S. C., Lam, K., Huvard, M. J., Schmid, P., & Flegel, W. A. (2016). Full-length nucleotide sequences of 30 common SLC44A2 alleles encoding human neutrophil antigen-3. *Transfusion*, *56*(3), 729–736.
11. Curtis, B. R., Cox, N. J., Sullivan, M. J., Konkashbaev, A., Bowens, K., Hansen, K., & Aster, R. H. (2010). The neutrophil alloantigen HNA-3a (5b) is located on choline transporter-like protein 2 and appears to be encoded by an R>Q154 amino acid substitution. *Blood*, *115*(10), 2073–2076.
12. Curtis, B. R., Roman, A. S., Sullivan, M. J., Raven, C. S., Larison, J., & Weitekamp, L. A. (2016). Two cases of maternal alloimmunization against human neutrophil alloantigen-4b, one causing severe alloimmune neonatal neutropenia. *Transfusion*, *56*(1), 101–106.
13. Davoren, A., Curtis, B. R., Shulman, I. A., Mohrbacher, A. F., Bux, J., Kwiatkowska, B. J., McFarland, J. G., & Aster, R. H. (2003). TRALI due to granulocyte-agglutinating human neutrophil antigen-3a (5b) alloantibodies in donor plasma: a report of 2 fatalities. *Transfusion*, *43*(5), 641–645.
14. Décary, F., Verheugt, F. W., van Helden-Henningheim, L., von Riesz, E., Schreuder-van

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Gelder, R., von dem Borne, A. E., & Engelfriet, C. P. (1979). Recognition of a non-HLA-ABC antigen present on B and T lymphocytes and monocytes only detectable with the indirect immunofluorescence test. *Vox Sanguinis*, *36*(3), 150–158.

15. Esmaeili, B., Bayat, B., Alirezaee, A., Delkhah, M., Mehdizadeh, M. R., & Pourpak, Z. (2022). Human Neutrophil Antigen Genotype and Allele Frequencies in Iranian Blood Donors. *Journal of Immunology Research*, *2022*, 4387555.
16. Flesch, B. K., Curtis, B. R., de Haas, M., Lucas, G., & Sachs, U. J. (2016). Update on the nomenclature of human neutrophil antigens and alleles. *Transfusion*, *56*(6), 1477–1479.
17. Flesch, B. K., & Reil, A. (2018). Molecular Genetics of the Human Neutrophil Antigens. In *Transfusion Medicine and Hemotherapy* (Vol. 45, Issue 5, pp. 300–309). https://doi.org/10.1159/000491031
18. Flesch, B. K., Reil, A., & Bux, J. (2011). Genetic variation of the HNA-3a encoding gene. *Transfusion*, *51*(11), 2391–2397.
19. Fung, Y. L., Pitcher, L. A., Willett, J. E., Reed, C., Mison, L., Bux, J., Eiber, G., & Minchinton, R. M. (2003). Alloimmune neonatal neutropenia linked to anti-HNA-4a. *Transfusion Medicine* , *13*(1), 49–52.
20. Gottschall, J. L., Triulzi, D. J., Curtis, B., Kakaiya, R. M., Busch, M. P., Norris, P. J., Glynn, S. A., Carrick, D., Wright, D. J., & Kleinman, S. (2011). The frequency and specificity of human neutrophil antigen antibodies in a blood donor population. In *Transfusion* (Vol. 51, Issue 4, pp. 820–827). https://doi.org/10.1111/j.1537-2995.2010.02913.x
21. Gottschall, J., Triulzi, D., Kakaiya, R., Carrick, D., Roback, J. D., Carey, P., Kleinman, S., & For the NHLBI Retrovirus Epidemiology Donor Study-II. (2013). Human neutrophil antibodies in a blood donor population: a lookback study. In *Vox Sanguinis* (Vol. 104, Issue 2, pp. 166–170). https://doi.org/10.1111/j.1423-0410.2012.01651.x
22. Greinacher, A., Wesche, J., Hammer, E., Fürll, B., Völker, U., Reil, A., & Bux, J. (2010). Characterization of the human neutrophil alloantigen-3a. *Nature Medicine*, *16*(1), 45–48.
23. He, J., Zhang, W., Wang, W., Chen, N., Han, Z., He, J., Zhu, F., & Lv, H. (2014). Genotyping of human neutrophil antigens by polymerase chain reaction sequence-based typing. *Blood Transfusion = Trasfusione Del Sangue*, *12 Suppl 1*, s292–s298.
24. Huvard, M. J., Schmid, P., Stroncek, D. F., & Flegel, W. A. (2012). Frequencies of SLC44A2 alleles encoding human neutrophil antigen-3 variants in the African American population. *Transfusion*, *52*(5), 1106–1111.
25. Jug, R., Anani, W., & Callum, J. (2021). A possible case of recipient anti-neutrophil and anti-human leukocyte antigen antibody-mediated fatal reverse transfusion-related acute lung injury. *Transfusion*, *61*(4), 1336–1340.
26. Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., … MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), 434–443.

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27. Key, T., Carter, V., Goodwin, P., Goodwin, J., Harmer, A., Knight, A., Mather, F., McKane, W., Poles, A., & Rigg, K. (2018). Human Neutrophil Antibodies are Associated with Severe Early Rejection in Kidney Transplant Recipients. In *Transplantation* (Vol. 102, Issue Supplement 7, p. S214). https://doi.org/10.1097/01.tp.0000542873.30386.b8
28. Kline, W. E., Press, C., Clay, M., Keashen-Schnell, M., Hackel, E., & McCullough, J. (1986). Three sera defining a new granulocyte-monocyte-T-lymphocyte antigen. *Vox Sanguinis*, *50*(3), 181–186.
29. Lalezari, P. (2017). Neutrophil-specific antigens, immunobiology, and implications in transfusion medicine and blood disorders. *Transfusion*, *57*(9), 2066–2073.
30. Moritz, E., Chiba, A. K., Kimura, E. Y., Albuquerque, D., Guirão, F. P., Yamamoto, M., Costa, F. F., & Bordin, J. O. (2010). Molecular studies reveal that A134T, G156A and G1333A SNPs in the CD177 gene are associated with atypical expression of human neutrophil antigen-2. *Vox Sanguinis*, *98*(2), 160–166.
31. Moritz, E., Norcia, Â. M. M., Cardone, J. D. B., Kuwano, S. T., Chiba, A. K., Yamamoto, M., & Bordin, J. O. (2009). Human neutrophil alloantigens systems. In *Anais da Academia Brasileira de Ciências* (Vol. 81, Issue 3, pp. 559–569). https://doi.org/10.1590/s0001-37652009000300019
32. Mraz, G. A., Crighton, G. L., & Christie, D. J. (2016). Antibodies to human neutrophil antigen HNA-4b implicated in a case of neonatal alloimmune neutropenia. *Transfusion*, *56*(5), 1161–1165.
33. Nguyen, X. D., Flesch, B., Sachs, U. J., Kroll, H., Klüter, H., & Müller-Steinhardt, M. (2009). Rapid screening of granulocyte antibodies with a novel assay: flow cytometric granulocyte immunofluorescence test. *Transfusion*, *49*(12), 2700–2708.
34. Porcelijn, L., Abbink, F., Terraneo, L., Onderwater-vd Hoogen, L., Huiskes, E., & de Haas, M. (2011). Neonatal alloimmune neutropenia due to immunoglobulin G antibodies against human neutrophil antigen-5a. *Transfusion*, *51*(3), 574–577.
35. Porcelijn, L., & de Haas, M. (2018). Neonatal Alloimmune Neutropenia. *Transfusion Medicine and Hemotherapy: Offizielles Organ Der Deutschen Gesellschaft Fur Transfusionsmedizin Und Immunhamatologie*, *45*(5), 311–316.
36. Porretti, L., Cattaneo, A., Coluccio, E., Mantione, E., Colombo, F., Mariani, M., Bottelli, G., Mazzucchelli, S., Pappalettera, M., Speranza, T., Migliari, M., Cambié, G., Prati, D., & Rebulla, P. (2012). Implementation and outcomes of a transfusion-related acute lung injury surveillance programme and study of HLA/HNA alloimmunisation in blood donors. *Blood Transfusion = Trasfusione Del Sangue*, *10*(3), 351–359.
37. Ravetch, J. V., & Kinet, J. P. (1991). Fc receptors. *Annual Review of Immunology*, *9*, 457–492.
38. Ravetch, J. V., & Perussia, B. (1989). Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *The Journal of Experimental Medicine*, *170*(2), 481–497.
39. Sachs, U. J. H., Chavakis, T., Fung, L., Lohrenz, A., Bux, J., Reil, A., Ruf, A., & Santoso, S. (2004). Human alloantibody anti-Mart interferes with Mac-1-dependent leukocyte

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adhesion. *Blood*, *104*(3), 727–734.

40. Simsek, S., van der Schoot, C. E., Daams, M., Huiskes, E., Clay, M., McCullough, J., van Dalen, C., Stroncek, D., & von dem Borne, A. E. (1996). Molecular characterization of antigenic polymorphisms (Ond(a) and Mart(a)) of the beta 2 family recognized by human leukocyte alloantisera. *Blood*, *88*(4), 1350–1358.
41. Stein, E. L., Santoso, S., Behrens, G., Mueller-Eckhardt, C., & Bux, J. (1995). Genotyping of the granulocyte-specific NA antigens from small quantities of blood or serum. *Tissue Antigens*, *45*(1), 69–72.
42. Vlaar, A. P. J. (2012). Transfusion-related acute lung injury: Current understanding and preventive strategies. In *Transfusion Clinique et Biologique* (Vol. 19, Issue 3, pp. 117–124). https://doi.org/10.1016/j.tracli.2012.03.001
43. Wu, J., Li, Y., Schuller, R. M., Li, L., Litmeyer, A., Bein, G., Sachs, U. J., & Bayat, B. (2019). The nonconservative*CD177*single‐nucleotide polymorphism c.1291G>A is a genetic determinant for human neutrophil antigen‐2 atypical/low expression and deficiency. In *Transfusion* (Vol. 59, Issue 5, pp. 1836–1842). https://doi.org/10.1111/trf.15222
44. Xia, W., Bayat, B., Sachs, U., Chen, Y., Shao, Y., Xu, X., Deng, J., Ding, H., Fu, Y., Ye, X., & Santoso, S. (2011). The frequencies of human neutrophil alloantigens in the Chinese Han population of Guangzhou. *Transfusion*, *51*(6), 1271–1277.