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.

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. 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). In addition, clinical disorders including alloimmune neonatal neutropenia and autoimmune neutropenia are caused by neutrophil specific antibodies (Porcelijn & de Haas, 2018),(Fung et al., 2003).

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). 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 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.

Materials and methods

Minor allele frequencies of the compiled collection of HNA variants were systematically fetched from 1000 genomes phase 3 project which comprises whole genome data of over 2500 samples from various global populations. Statistically distinct differences in frequencies among the populations were estimated using Fisher's exact test with p-value ≤ 0.05.

Results

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. ITGAL_16:30506720G>C variant of HNA class 5 was found significantly distinct in all the 5 major global subpopulations with a frequency of 71.4% South Asians, 13.4% in East Asians, 30.1% in Europeans, 42.1% in Africans and 18.9% in Americans.

HNA-1						
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						
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)
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						
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						
Chr	**Pos (Hg38)**	**Ref**	**Alt**	**RSID**	**Category**	**Reference**
16	31265490	G	A	rs1143679	ISBT approved variants	-
HNA-5						
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.

Discussion and Conclusion

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. There 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). Cases of fetal/neonatal alloimmune neutropenia induced by antibodies against other classes of HNAs including HNA-4a/b and HNA-5a have also been reported (Curtis et al., 2016),(Porcelijn et al., 2011),(Mraz et al., 2016). Efforts to estimate the genotype frequencies of neutrophil antigens among global populations provides an overview of population - specific prevalence.

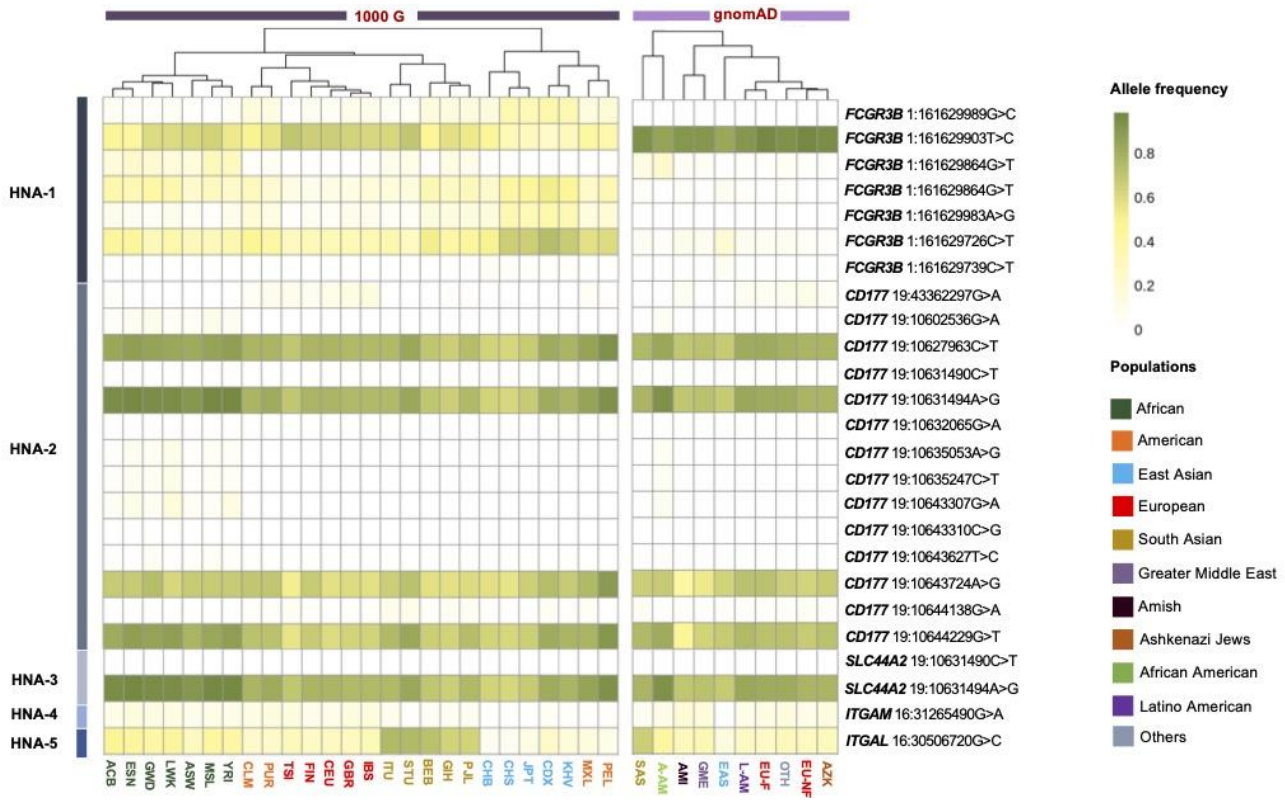

Figure 1. Comparison of frequencies of human neutrophil antigen variants among various subpopulations of the 1000 Genomes project and gnomAD

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

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 reaction method for HNA-2 genotyping: homozygous c.843A>T mutation predicts the absence of CD177. *Transfusion*, *56*(8), 2127–2132.
3. Browne, T., Dearman, R. J., & Poles, A. (2021). Human neutrophil antigens: Nature, clinical significance and detection. *International Journal of Immunogenetics*, *48*(2), 145–156.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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
10. Flesch, B. K., Reil, A., & Bux, J. (2011). Genetic variation of the HNA-3a encoding gene. *Transfusion*, *51*(11), 2391–2397.
11. 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.
12. 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

13. 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
14. 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.
15. 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.
16. 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.
17. 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
18. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.

24. 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