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ORIGINAL RESEARCH

Distribution and Genetic Characterization of the MNS Blood Group in Multi-Ethnic Populations of East China

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Purpose: To investigate the distribution of antigen and allele frequencies of the MNS blood group system among multi-ethnic populations in East China, and to analyze the genetic polymorphism of uncommon phenotypes, thereby contributing to the enhancement of the regional blood type database.

Patients and Methods: A total of 8606 whole blood samples were randomly collected from voluntary blood donors in East China between October 2023 and June 2024. MNS blood group phenotypes were identified using serological methods, and allele frequencies were analyzed and compared across populations. Genetic sequencing was performed on samples with uncommon MNS phenotypes.

Results: The study primarily included the Han, Hui, and Manchu populations. Among the Han population, the most prevalent phenotypes were M+N+S-s+ (45.21%), M-N+S-s+ (25.94%), and M+N-S-s+ (19.84%), respectively. Phenotypic distributions in most other ethnic groups were comparable to that of the Han population, except for the Yi population, which showed a significantly different distribution ($P < 0.05$). Furthermore, a rare serological phenotype, S-s-, was identified with a frequency of 0.01%. The allele frequencies of the MNS blood group system among different population in East China were consistent with the Hardy-Weinberg equilibrium ($P > 0.05$).

Conclusion: The MNS blood group system in East China's multi-ethnic populations exhibits polymorphism and regional specificity. Notable allele frequency differences exist between certain minority populations and the Han population. Therefore, it is essential to enhance the development of a regional blood type database tailored to East China in order to support precise clinical transfusion with robust data, including informed pre-transfusion antibody screening for high-risk groups.

Keywords: MNS blood group, rare blood group, allele frequency, multi-ethnic populations

Introduction

Blood group is an inherited human trait, typically referring to the differences in specific antigens on the surface of red blood cells. Since the discovery of the ABO blood group system in the early 20th century, a total of 48 blood group systems and 371 blood group antigens have been identified, exhibiting high genetic polymorphism and regional diversity.[1] Among these, the MNS blood group system (ISBT 002) was the second to be identified after the ABO system and is second only to the Rh blood group system in terms of complexity. It is the only known blood group system encoded by three closely linked homologous genes, arranged in the order of 5′-*GYP*A-*GYPB*-*GYPE*-3′ on chromosome 4q31.21.[2] Due to their high sequence homology, gene rearrangements frequently occur, resulting in hybrid genes such as *GYP*(A-B) and *GYP*(B-A-B), which give rise to novel antigens like GP.Hil and GP.Mur.[3] Similarly, although *GPYE* is generally not believed to express proteins on the red blood cell membrane, its sequence similarity and close linkage with

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GYPA and *GYPB* make it susceptible to gene recombination, which can lead to hybrid genes such as *GYP*(B-E-B). These recombination events may result in loss of Ss antigen expression, as previously documented.[4] To date, 50 antigens have been identified within this system, with the five major antigens being M, N, S, s, and U. The MN antigens are located on glycophorin A (GPA), while the Ss antigens are located on glycophorin B (GPB).[1,5]

Rare blood groups are generally defined by the absence of common blood group antigens on the red cell surface or by uncommon antigen combinations. According to the Association for the Advancement of Blood and Biotherapies (AABB), an antigen is considered rare if its prevalence in the population is ≤ 1‰.[6] Individuals with such blood types are more prone to developing irregular antibodies after transfusion or pregnancy, which can result in hemolytic transfusion reactions (HTR), hemolytic disease of the fetus and newborn (HDFN), and other severe complications.[7] Within the MNS blood group system, anti-M is relatively common and usually regarded as a naturally occurring IgM antibody without clinical significance at 37 °C, but transfusion or pregnancy may induce clinically relevant cases, including HDFN.[8] Anti-S and anti-s, on the other hand, are predominantly IgG and more frequently associated with HTR and HDFN.[9] Although the MNS blood group system was once considered of limited clinical relevance, accumulating evidence now demonstrates its growing importance, with rising reports of alloimmunization underscoring its impact on transfusion safety.[10–12]

Globally, marked variability in MNS phenotype frequencies has been reported across populations.[13] In China, early large-scale serological surveys provided initial estimates but lacked detailed ethnic resolution,[14,15] while modern genomic panels such as the 1000 Genomes Project include only a very limited number of Chinese samples. More recent studies of specific ethnic groups (eg, Uygur in Xinjiang,[16] Yi in Yunnan,[17] and Li in Hainan[18]) remain small-scale and fragmented, and thus are not generalizable.[19] Moreover, local data highlight the clinical importance of MNS antibodies,[20] as a retrospective analysis of irregular antibodies identified at our blood center revealed that approximately one-third were anti-M. Taken together, these limitations underscore the need for large, multi-ethnic datasets to provide precise estimates for East China. To ensure the safety of clinical blood transfusions for individuals with uncommon blood types and to prevent alloimmune complications, it is crucial to investigate the antigen frequency distribution of the MNS blood group system in this region and establish a corresponding blood group database. Building on our previous establishment of an Rh blood group database that has supplied Rh-typed blood products for clinical use,[21] this study aims to characterize the antigen and allele frequency distribution of the MNS blood group system in multi-ethnic populations in East China.

Materials and Methods

Blood Samples

Between October 2023 and June 2024, a total of 8606 whole blood samples (3–5 mL, EDTA-K2 anticoagulated) were randomly collected from voluntary blood donors at a blood center in East China. Among the participants, 6000 were male and 2606 were female, with an age range of 18–60 (34.35 ± 10.16) years. Ethnic groups with more than 50 individuals included Han (n = 8034), Hui (n = 116), Manchu (n = 62), Tujia (n = 61), Miao (n = 59), and Yi (n = 56) populations, while those with fewer than 50 individuals (218 donors in total, mainly Zhuang (n = 42), Mongol (n = 25), and Dong (n = 24)) were excluded from statistical analysis. Ethnicity was determined based on the information provided by donors at the time of registration and confirmed through the donor's Resident Identity Card. Each donor was uniquely identified by their card number, and only the first eligible donation was included to prevent duplicate entries. Donors with inconsistent or missing ethnicity information were excluded from the analysis.

Phenotyping of MNS Blood Group Antigens

The whole blood samples were centrifuged at 1200 g for 3 minutes. Blood typing was performed using the automated immunohematology analyzer Galileo NEO (Immucor, Norcross, GA, USA) to identify the MNS blood group phenotypes. Samples with ambiguous results, defined as weak agglutination (≤1+), mixed-field agglutination, or discrepancies with expected serological patterns, were further confirmed by manual methods for final determination. The reagent concentrations were strictly prepared according to the manufacturer's instructions. For manual confirmation, 50 μL of the corresponding antibody reagent (Monoclonal anti-IgM serum: anti-M, 20231221; anti-N, 20230607; Shanghai Blood Biomedical Co, China. Human IgG monoclonal serum: anti-S, OSM216-1; anti-s, OsM302-1; CE-Immundiagnostika GmbH, Germany) was added

to a clean test tube along with and 50 μL of a 2–4% red blood cell suspension. The mixture was gently mixed and incubated at room temperature (20–25°C) for 15 minutes, followed by centrifugation at 1000 g for 15 seconds. The tube was then gently rocked, and the presence or absence of agglutination was observed to interpret the result.

Sequencing of MNS Blood Group

Samples with uncommon phenotypes identified by serological testing were subjected to genetic sequencing, which was conducted by Jiangsu Weihe Biotechnology Co., Ltd. The analysis was based on the Names for MNS (ISBT 002) Blood Group Alleles provided by the International Society of Blood Transfusion (ISBT).[22] Specific exonic regions (Exons 2–5) of *GYPA* and *GYPB* were amplified using polymerase chain reaction (PCR), generating products of approximately 4.3 kb. After verification by gel electrophoresis and subsequent purification, the PCR products were subjected to Sanger sequencing, covering Exons 2–4 of *GYPA* and Exons 2, 4, and 5 of *GYPB*. To ensure the accuracy of the genotype calls, validation was performed through bidirectional sequencing. Then the final sequence is generated from the alignment of these two independent reads.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics software, version 25 (IBM Corp., Armonk, NY, USA). Allele frequencies of MNS blood group antigens were calculated and Hardy-Weinberg equilibrium (HWE) test was performed. A P-value > 0.05 in the HWE test is indicated that the gene was stably present within the population and representative of the group. Chi-square (χ^2) tests were used to compare the MNS blood group phenotypes and allele frequencies among different ethnic groups within the voluntary blood donor population in East China, as well as to evaluate differences between regional populations. A P-value < 0.05 is considered to be statistically significant. For the estimation of the prevalence of uncommon phenotypes, the 95% confidence interval (CI) was calculated using the Clopper-Pearson exact method.

Results

Distribution of MNS Blood Group Phenotypes

A total of 8606 samples were analyzed, with the primary ethnic groups in East China including Han, Hui, Manchu, Tujia, Miao, and Yi populations. The frequencies of MNS phenotypes for each ethnic group are presented in Table 1. Among the Han population, the most prevalent phenotype was M+N+S-s+ (45.21%, 95% CI: 44.12–46.30%), followed by M-N+S-s+ (25.94%, 95% CI: 24.99–26.91%) and M+N-S-s+ (19.84%, 95% CI: 18.98–20.73%). The distribution of phenotypes among other ethnic groups within East China was found to be generally similar to that of the Han population. Chi-square (χ^2) analysis revealed a statistically significant difference ($P < 0.05$) only between the Yi and Han populations.

Allele Frequencies of MNS Blood Group

Allele frequencies of the MNS blood group among different ethnic groups are presented in Table 2. In East China, the allele frequencies of M, N, S, and s in the Han population were 0.4793, 0.5207, 0.0465, and 0.9535, respectively. Chi-square (χ^2) analysis showed that both MN and Ss allele distributions conformed to the Hardy-Weinberg equilibrium ($P > 0.05$), indicating genetic stability in these populations. Notably, the MN allele frequencies in the Miao and Yi populations were significantly different from those in the Han population ($P < 0.05$).

Uncommon MNS Blood Group Phenotypes

In this study, a total of 26 uncommon phenotypes were identified, including 24 cases of S+s- (0.30%, 95% CI: 0.19–0.44%) and one case of S-s- among the Han population (0.0125%, 95% CI 0.0003–0.0694%), as well as one case of S+s- among the Hui population (0.862%, 95% CI: 0.02–4.71%). The specific serological results are presented in Table 1. To investigate the underlying genetic basis, three randomly selected samples (or the only available sample) from each uncommon phenotype group within the Han population were sent to Jiangsu Weihe Biotechnology Co., Ltd. for genetic sequencing. One M−N+ sample exhibited homozygous mutations in *GYPA* Exon 2 at c.59C>T, c.71G>A, and c.72T>G (p.Ser20Leu, p.Gly24Glu),

Table 1 Comparison of MNS Blood Group Phenotype Frequencies Among Different Ethnic Groups in the Voluntary Blood Donor Population of East China and Other Regional Ethnic Populations

	Han (%)	Hui (%)	Manchu (%)	Tujia (%)	Miao (%)	Yi (%)	India[23] (%)	White[24] (%)	African[25] (%)
	n = 8034	n = 116	n = 62	n = 61	n = 59	n = 56	n = 317	n = 1000	n = 1322
M+N-S+s-	13(0.16)	0(0)	0(0)	0(0)	0(0)	0(0)	25(7.89)	57(5.7)	28(2.1)
M+N-S+s+	240(2.99)	3(2.59)	4(6.45)	2(3.28)	2(3.39)	4(7.14)	47(14.83)	140(14)	92(7)
M+N-S-s+	1594(19.84)	25(21.55)	7(11.29)	12(19.67)	20(33.9)	16(28.57)	50(15.77)	101(10.1)	205(15.5)
M+N+S+s-	10(0.12)	1(0.86)	0(0)	0(0)	0(0)	0(0)	11(3.47)	39(3.9)	29(2.2)
M+N+S+s+	365(4.54)	11(9.48)	2(3.23)	5(8.2)	0(0)	2(3.57)	62(19.56)	224(22.4)	172(13)
M+N+S-s+	3632(45.21)	55(47.41)	30(48.39)	25(40.98)	25(42.37)	29(51.79)	44(13.88)	226(22.6)	442(33.4)
M-N+S+s-	1(0.01)	0(0)	0(0)	0(0)	0(0)	0(0)	4(1.26)	3(0.3)	21(1.6)
M-N+S+s+	94(1.17)	1(0.86)	0(0)	0(0)	0(0)	0(0)	30(9.46)	54(5.4)	59(4.5)
M-N+S-s+	2084(25.94)	20(17.24)	19(30.65)	17(27.87)	12(20.34)	5(8.93)	44(13.88)	156(15.6)	254(19.2)
M+N-S-s-	1(0.01)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(0.4)
	p	0.069	0.41	0.776	0.18	0.043*	0.000***	0.000***	0.000***

Note: P-value was obtained by comparing with the Han Chinese population in this study. *$P < 0.05$, ***$P < 0.001$.

Table 2 Comparison of MNS Blood Group Allele Frequencies Among Different Ethnic Groups in the Voluntary Blood Donor Population of East China

Ethnic background	n	Allele frequency							
		M	N	χ^2	*P*	S	s	χ^2	*P*
Han	8034	0.4793	0.5207	–	–	0.0465	0.9535	–	–
Hui	116	0.5302	0.4698	2.3680	0.1240	0.0733	0.9267	3.671	0.055
Manchu	62	0.4355	0.5645	0.9480	0.3300	0.0484	0.9516	0.01	0.921
Tujia	61	0.4754	0.5246	0.0070	0.9310	0.0574	0.9426	0.323	0.57
Miao	59	0.5847	0.4153	5.2150	0.0220*	0.0169	0.9831	2.317	0.128
Yi	56	0.6339	0.3661	10.6490	0.0010**	0.0536	0.9464	0.125	0.723

Note: P-value was obtained by comparing with the Han Chinese population in this study. *$P < 0.05$, **$P < 0.01$.

Table 3 Sequencing Results of S+s− and S−s− Phenotypes

Phenotype	Number of Samples	*GYPB*
S+s-	7	Mutation: Exon 4 143C > T
S-s-	1	-

while three M+N+ samples showed the same combination of heterozygous mutations, and three M+N− samples did not present any mutations. All seven analyzed samples exhibited a homozygous c.143C>T mutation in *GYPB* Exon 4, which corresponds to the molecular basis of *GYPB**03 (p.Thr48Met) and is associated with the S+ phenotype. While one rare sample with the M+N-S-s- phenotype did not present any mutations. The detailed sequencing results are presented in Table 3 and Figure 1.

Discussion

This study provides the first large-scale and multi-ethnic characterization of MNS blood group phenotypes and alleles in East China. We demonstrated that the MNS system exhibits considerable polymorphism and regional specificity, with significant allele frequency differences between Han and minority groups.

Referring to the ABO blood group screening approach, this study utilized the Galileo NEO to develop an MNS blood group screening program, enabling rapid and efficient MNS blood group screening. A total of 8606 samples from multi-ethnic populations in East China were screened in this study. Ethnic groups with more than 50 individuals included Han, Hui, Manchu, Tujia, Miao, and Yi population, while ethnic groups with fewer than 50 individuals were excluded from

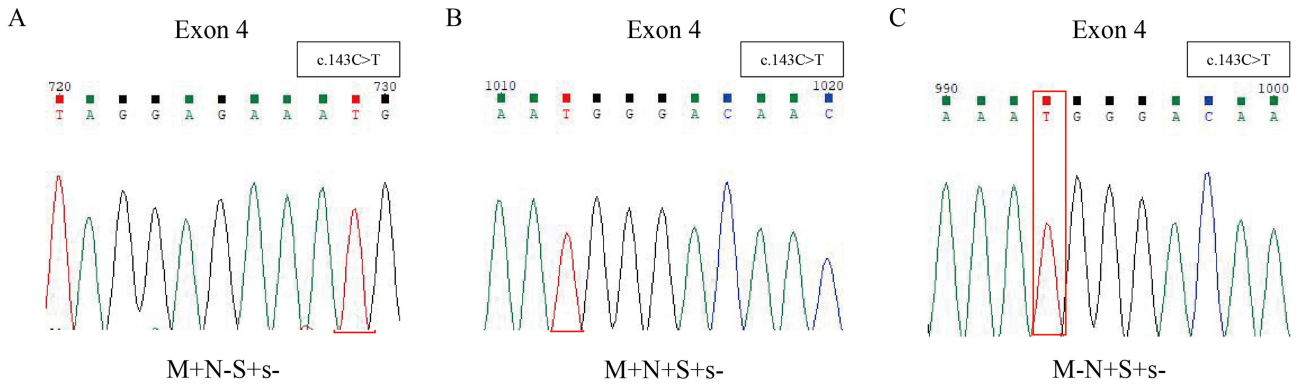

Figure 1 Representative sequencing results of S+s- phenotype. (**A–C**) Sequencing results of different MN phenotypes with S+s- samples consistently showed a c.143C>T point mutation located in Exon 4.

statistical analysis. The Han population accounted for 8034 cases, with the predominant phenotypes being M+N+S-s+, M-N+S-s+, and M+N-S-s+. The allele frequencies of M, N, S, and s were 0.4793, 0.5207, 0.0465, and 0.9535, respectively. Importantly, uncommon phenotypes were also identified. The uncommon SS phenotype accounted for only 0.30%, and one case of the rare S-s- phenotype was identified, which has been rarely reported in China. Most ethnic groups exhibited MNS phenotypic distributions similar to that of the Han population. However, the Yi population showed a statistically significant difference compared to the Han population. Furthermore, the MNS phenotype distribution observed in the Han population was found to be statistically different from that of other major global populations, including Indian,[23] White,[24] and African[25] populations. These results emphasize the distinct regional characteristics of MNS blood group distribution in East China's voluntary blood donor population. This distinct profile has a critical implication for transfusion strategy: it suggests that blood stocks from other regions within China may not be suitable for patients in East China requiring specific antigen-negative blood, given the documented geographic and ethnic variability in MNS frequencies across the country.[19] This highlights the necessity of establishing a regional blood type database tailored to the local population's specific phenotypic characteristics.

While serological phenotyping remains the standard for routine blood group identification, it has inherent limitations, such as the potential for ambiguous results due to reagent variability, dosage effects, or the presence of variant antigens that may not be detected by standard antisera. Genetic sequencing overcomes these challenges by providing definitive molecular information. It is indispensable for resolving weak or discrepant serological findings and for elucidating the molecular basis of rare or null phenotypes, as was necessary for the uncommon phenotypes identified in our study. The MNS blood group antigens are located on the red blood cell membrane glycoproteins GPA and GPB, encoded by *GYPA* (Exons 2–7) and *GYPB* (Exons 3–5), respectively. *GYPA* consists of seven exons, while *GYPB* comprises five exons and one pseudogene exon. In this study, a total of 26 uncommon phenotypes were identified. Due to budgetary constraints, eight samples were randomly selected for genetic sequencing to investigate their molecular characteristics. In seven samples exhibiting the S+s− phenotype, the mutation site combinations in *GYPA* and *GYPB* were consistent with entries reported by ISBT, confirming concordance between serological phenotyping and genotyping. Interestingly, one sample with the rare M+N−S−s− phenotype showed no detectable mutations in either *GYPA* or *GYPB*. The S−s− phenotype is predominantly observed in African populations and is likely associated with the absence or weak expression of the U antigen (U− or U+^{var}). This apparent conflict between serology and genotyping is likely explained by the scope of our molecular analysis, which was limited to the coding regions of these genes. The genetic basis for the S-s- phenotype often involves mutations outside of the exons. Previous reports suggest that genetic mechanisms such as c.37+4_8delAGTGA in *GYPB* intron 1 can lead to the absence of s antigen expression.[26,27] These rare phenotypes are therefore likely driven by underlying genetic mechanisms including *GYPA–GYPB* recombination, point mutations, or intronic variants affecting antigen expression, which are consistent with prior studies. Therefore, further investigation is required to elucidate the molecular basis underlying this particular case. These findings suggest that the MNS blood group in the population of East China exhibit a certain degree of genetic polymorphism, highlighting the necessity of combining serological screening with targeted sequencing. Serology enables

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efficient large-scale testing, while sequencing resolves uncommon or ambiguous cases at the molecular level. Such an integrated approach not only enhances the accuracy of uncommon phenotype identification but also provides the essential basis for building and expanding a comprehensive regional blood type database.

From a clinical perspective, these phenotypes are of particular concern because alloantibodies directed against S or s antigens are predominantly IgG and have been associated with hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. Among them, the S+s- phenotype was linked to the 143C>T substitution in *GYPB* Exon 4. The critical functional consequence of this mutation is the absence of the high-frequency 's' antigen, which places these individuals at high risk for alloimmunization. Therefore, proactively identifying these patients and providing compatible s-negative blood is essential to preventing life-threatening complications. However, several limitations should be noted. The functional consequences of the identified mutations, including the unresolved S–s– genotype, were not experimentally validated and require further molecular clarification. In addition, the prospective clinical utility of the expanded regional blood type database was not evaluated and will be the subject of future studies. Ultimately, these findings underscore the necessity of establishing and continuously expanding a comprehensive regional blood type database to ensure transfusion safety.

Conclusion

The MNS blood group antigen and allele frequencies among multi-ethnic populations in East China region exhibit distinct regional characteristics, with significant differences when compared to other ethnic groups in different geographic regions. Moreover, the genes associated with uncommon blood types in this population exhibit polymorphic variations. Our blood center has previously established an Rh blood type database and conducted CcEe antigen phenotyping, providing clinically relevant Rh-typed blood products. These findings not only expand the regional blood type database but also inform rare donor registry development by identifying clinically relevant phenotypes (eg, S+s– and S–s–) for proactive inclusion, while guiding pre-transfusion antibody screening policies to ensure targeted monitoring and improved transfusion safety for high-risk groups. Moving forward, comprehensive studies of multiple blood group systems among the multi-ethnic populations in this region should be conducted to enhance transfusion safety and ensure the availability of compatible blood for clinical use.

Data Sharing Statement

Data will be made available on request.

Ethics Approval and Informed Consent

The study complies with the Declaration of Helsinki and was reviewed and approved by the Medical Ethics Committee of the Suzhou Blood Center (approval number: 202202, dated May 6, 2022). Written informed consent was obtained from all participants prior to their inclusion in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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