

Original article

Updated prevalence of the GP. Verweyst phenotype among Thai blood donors

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Abstract:

Introduction: GP.Verweyst (Vw), also known as Mi.I, is one of eleven antigens in the Miltenberger subclasses (Mi.I to Mi.XI). In the Thai population, only one case of Vw(+) phenotype has been reported. Currently, the National Blood Centre, Thai Red Cross Society (NBC, TRCS), has developed a monoclonal anti-Vw antibody, enabling its application for screening the Vw(+) phenotype in blood donors. **Objective:** To determine the phenotype frequency of GP.Vw in Thai blood donors in additional blood donors at the NBC, TRCS. **Material and Methods:** A total of 11,965 CPDA blood samples from Thai blood donors were analyzed. These included 2,224 (18.6%) group A, 4,152 (34.7%) group B, 4,488 (37.5%) group O, and 1,101 (9.2%) group AB. The samples were screened for GP.Vw using monoclonal anti-Vw from the NBC, TRCS via the conventional tube technique. GP.Vw-positive were confirmed using additional anti-Vw and Mi subclass antibodies from the Japanese Red Cross (anti-Vw, anti-Mur, anti-MUT, anti-Hil, anti-Hop, anti-Anek, and anti-Mi^a), employing both the conventional tube technique and the column agglutination technique (CAT). **Results:** This study found two donors (0.0167%) with the Vw(+): one male with blood group A and one female with blood group O. **Conclusion:** The discovery of the GP.Vw in Thai blood donors is highly valuable for blood bank laboratories. It serves as an important resource for testing in the production of anti-Mi^a blood grouping reagents and for evaluating antibodies against Mi subclasses derived from newly hybridoma cell lines.

Keywords : ● Miltenberger subclasses ● GP.Verweyst ● Mi.I

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นิพนธ์ต้นฉบับ

ความชุกของฟีโนไทป์ GP. Verweyst ในผู้บริจาคโลหิตชาวไทยในปัจจุบัน

สาริกา เมฆฉาย สรีญา แก้มทอง ชิมพิการ จันทูล กาญจนา เอี่ยมอัมพร กัลยา เกิดแก้วงาม และ อุดม ดึงต้อย
ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ

บทนำ GP. Verweyst (Vw) หรือ Mi.I เป็น 1 ใน 11 คลาสย่อยของมิลเทนเบอร์เกอร์ (Mi.I ถึง Mi.XI) ในคนไทย ซึ่งเคยตรวจพบเพียงรายเดียว ปัจจุบันศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ได้พัฒนาผลิตภัณฑ์โมโนโคลนัล anti-Vw ได้ และได้นำมาตรวจหา GP. Vw ในผู้บริจาคโลหิต **วัตถุประสงค์** เพื่อศึกษาความถี่ของ GP.Vw ในผู้บริจาคโลหิตคนไทย ของศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย **วัสดุและวิธีการ** ตัวอย่างโลหิตบริจาคจำนวน 11,965 ราย ประกอบด้วยหมู่ A 2,224 ราย (18.6%) หมู่ B 4,152 ราย (34.7%) หมู่ O 4,488 ราย (37.5%) และ หมู่ AB 1,101 ราย (9.2%) ทดสอบกับ anti-Vw ของศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ด้วยวิธีมาตรฐานหลอดทดลอง รายที่ให้ผลบวก ตรวจยืนยันด้วย anti-Vw และ แอนติบอดีต่อ Mi subclass ของสภากาชาดญี่ปุ่น ได้แก่ anti-Vw, -Mur, -MUT, -Hil, -Hop, -Anek และ anti-Mi^a ด้วยวิธีหลอดทดลอง และ column agglutination technique (CAT) **ผลการศึกษา** การศึกษานี้ตรวจพบ GP.Vw ให้ผลบวกจำนวน 2 ราย (0.0167%) เป็นเพศชายหมู่โลหิต A จำนวน 1 ราย และเพศหญิงหมู่โลหิต O จำนวน 1 ราย **สรุป** การตรวจพบ GP.Vw ในผู้บริจาคโลหิตไทย นับเป็นประโยชน์อย่างยิ่งในงานด้านห้องปฏิบัติการธนาคารเลือด และยังสามารถใช้ทดสอบในกระบวนการผลิตน้ำยาตรวจหมู่โลหิต anti-Mi^a รวมทั้งทดสอบเซลล์สายพันธุ์ใหม่ ที่สร้างแอนติบอดีต่อ Mi subclasses

คำสำคัญ : ● คลาสย่อยของมิลเทนเบอร์เกอร์ ● ไกลโคโปรตีนเวอร์เวส ● เอ็มไอวัน

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2569;36:99-104.

Introduction

The GP.Verweyst (GP.Vw) or MNS9 antigen is part of the Miltenberger classes (Mi subclasses) of the variants MNS phenotypes. It is a low-frequency antigen within the MNS blood group system, which is considered the second most complex after the Rh blood group system. The MNS system includes a wide range of variant alleles, resulting in a total of 50 known antigens.^{1,2} The Mi subclasses, also known as Mi^a, arises from a crossover event between the *GYPA* gene, which encodes glycoporphin A, and the *GYPB* gene, which encodes glycoporphin B. This recombination occurs during meiosis I phase in cell division and results in the formation of a hybrid chromosomes that are variant alleles.^{1,3} Initially, the Mi subclass was classified into five categories, Mi.I through Mi.V, based on red blood cells reactions with anti-Mi^a, anti-Vw, anti-Hut, anti-Mur, and anti-Hil. Subsequently, the classification expanded to 11 categories (Mi.I to Mi.XI), each associated with a specific glycoporphin (GP) variant: GP.Vw (Mi.I), GP.Hut (Mi.II), GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Hil (Mi.V), GP.Bun (Mi.VI), GP.Nob (Mi.VII), GP.Joh (Mi.VIII), GP.Dane (Mi.IX), GP.HF (Mi.X), and GP.JL (Mi.XI).^{4,5} GP.Vw or Mi.I was first reported by Hart, et al. in 1954⁶ and is classified under the glycoporphin A-B-A hybrid subgroup, GP.(A-B-A). This subgroup includes five antigens: GP.Vw, GP.Hut, GP.Nob, GP.Joh, and GP.Dane, all controlled by the *GYP(A-B-A)* hybrid gene. When tested with anti-Mi^a, these phenotypes typically yield a positive result (Mi^a-positive).⁷ GP.Vw phenotypes are often associated with the Ns allele, occasionally with NS, and rarely with Ms. The GP.Vw phenotype is found in approximately 1.43% of the southern Swiss population.^{8,9} Anti-Vw are clinically significant, as they have been reported to cause hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN), similar to anti-Mi^a.^{3,10-14} When anti-Mi^a was first detected in the serum of Mrs. Miltenberger, anti-Vw is part of a group of polyspecific antibodies that target various antigens in the Mi subsystem. Currently, it

is accepted that there is no single specific anti-Mi^a antibody.^{4,5} Anti-Mi^a found in patients or blood donors can be induced by pregnancy or blood transfusion and usually exhibit specificity for antigens such as anti-Mur, anti-MUT, anti-Hil, anti-Hop, and anti-Hut, based on tests using Mi subclass cells. When these plasma units are used to prepare polyclonal anti-Mi^a reagents, the specificity typically includes anti-Mur, anti-MUT, anti-Hil, anti-Hop, and anti-Hut. Currently, the anti-Mi^a reagent used by the National Blood Centre, Thai Red Cross Society (NBC, TRCS) is produced by using a mixture of two hybridoma cell lines: the 4E4 human hybridoma, which expresses anti-MUT and reacts with GP.Hut (Mi.II), GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Bun (Mi.VI), and GP.HF (Mi.X); and the 15D2 murine hybridoma, which expresses anti-Vw. This combination yields an anti-Mi^a reagent that is reactive with GP.Vw (Mi.I), GP.Hut (Mi.II), GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Bun (Mi.VI), and GP.HF (Mi.X). Not only the 15D2 hybridoma cell line expresses anti-Vw specificity, but it can also be used to identify the GP.Vw phenotype in Thai blood donors. The results are recorded in the blood donor database to facilitate the standardization of anti-Mi^a reagents produced by the NBC, TRCS. These reagents must effectively cover Mi subclasses that are found in Thai populations¹⁵

Materials and Methods

Blood and Reagent Samples

A total of 11,965 CPD blood donor samples were collected at the NBC, TRCS between October 2023 and March 2024. These samples remained from routine testing and consisted of the following blood groups: group A, 2,224 samples (18.6%); group B, 4,152 samples (34.7%); group O, 4,488 samples (37.5%); and group AB, 1,101 samples (9.2%). Blood grouping reagents from the NBC, TRCS included anti-Vw, anti-MUT, and anti-Mi^a, while blood grouping reagents from the Japanese Red Cross Society (JRCS) included anti-Vw, anti-Mur, anti-MUT, anti-Hil, anti-Hop, anti-Anek, and anti-Mi^a.

Methods

Detection of individuals with the Vw(+) phenotype was performed as follows: blood samples were prepared at a concentration of 2-5% red cells suspended in normal saline. One drop of this red blood cells suspension was mixed with one drop of anti-Vw reagent from the NBC, TRCS. The mixture was incubated at room temperature (22-24°C) for 10 minutes, then centrifuged at 1,000 ×g for 15 seconds. The agglutination reaction was graded are 1+, 2+, 3+, 4+, and 0 for negative. Samples with a positive reaction were further tested for confirmation using anti-Vw, anti-MUT, and anti-Mi^a reagents from the NBC, TRCS via the conventional tube technique.¹⁶

Subsequently, confirmation was performed using reagents from the JRCS (anti-Vw, anti-Mur, anti-MUT, anti-Hil, anti-Hop, anti-Anek, and anti-Mi^a) using the column agglutination technique (CAT) from Bio-Rad Laboratories, Inc., Hercules, California, USA. Using CAT method, blood samples were prepared at a concentration of 0.8% red cell suspension, and 50 µL of the suspension was added to the column along with 25 µL of antibodies. The mixture was incubated at 37°C for 15 minutes and centrifuged for 10 minutes.

The study is part of a research project aimed at developing blood grouping reagents. The project was approved by the Research Ethics Committee of the NBC, TRCS (COA No. NBC 6/2016) on July 15, 2023.

Results

Among the 11,965 blood donor samples tested for the GP.Vw (Mi.I) phenotype, only two donors were positive. The distribution of GP.Vw-positivity according to ABO blood group is summarized in Table 1. One positive case was observed in group A donors and one in group O donors, while no positive cases were detected in groups B or AB. Overall, the prevalence of the GP.Vw phenotype in this donor population was 0.0167% (2/11,965). Both GP.Vw-positive donors were middle-aged adults (42 and 50 years). Serologic testing showed positive reactions with anti-Vw and anti-Mi^a using both NBC, TRCS and Japanese antisera, while no reactivity was observed with anti-Mur, anti-MUT, anti-Hil, anti-Hop, or anti-Anek. Based on the reaction pattern, both donors were classified as GP.Vw (Mi.I), as shown in Table 2.

Discussion

A total of 11,965 blood donor samples from the NBC, TRCS were tested for GP.Vw antigen. It was found that two samples tested positive with anti-Vw. Both detected samples were tested for Mi subclasses using following antibodies: anti-Vw, anti-MUT, and anti-Mi^a reagents from the NBC, TRCS, as well as reagents from the JRCS including anti-Vw, anti-Mi^a, anti-Mur, anti-MUT, anti-Hil, anti-Hop, and anti-Anek showed positive results for anti-Vw

Table 1 The detection of GP. Verweyst in Thai blood donors

Blood group	Number of donors		
	GP. Vw (positive)	GP. Vw (negative)	Total
A	1	2,223	2,224 (18.6%)
B	0	4,152	4,152 (34.7%)
O	1	4,487	4,488 (37.5%)
AB	0	1,101	1,101 (9.2%)
Total	2	11,963	11,965 (100.0%)

Table 2 The characteristics of GP. Verweyst positive blood donors

Donor No.	Sex	Age	Reactions with NBC antisera			Reactions with Japan antisera							Mi Classes
			Vw	MUT	Mi ^a	Vw	Mi ^a	Mur	MUT	Hil	Hop	Anek	
1	Female	42	+	0	+	+	+	0	0	0	0	0	GP.Vw
2	Male	50	+	0	+	+	+	0	0	0	0	0	GP.Vw

and anti-Mi^a but negative for anti-MUT. Anti-MUT is known to react with Mi.II, Mi.III, Mi.IV, Mi.VI, and Mi.X. Negative reactions were also observed with anti-Mur, anti-Hil, anti-Hop, and anti-Anek, which were used as negative controls. When tested with anti-Mi^a which reacts with Mi.I, Mi.II, Mi.III, Mi.IV, Mi.VI, and Mi.X both samples were positive. Therefore, it was confirmed that both samples truly carried the Vw(+) antigen. In a previous study of the Miltenberger subsystem in the Thai population by Chandanayingyong D, only one case of GP.Vw was found among 243 Mi^a(+) individuals out of a total of 2,500 participants, representing a frequency of 0.0415%.¹⁷ In Australia, a study involving 5,098 blood donors found 11 individuals with Mi^a(+) (0.22%), and 3 of them had GP.Vw.¹⁸ Another study by Kerdkaewngam K. reported the frequency of MNS hybrid glycoporphins in Mi(a+) donors was one Vw(+) individual out of 1,216 samples, or 0.082%.¹⁵ This current study identified two blood donors of GP.Vw. The first was a 50-year-old male, blood group A, who was donating for the 77th time and previously tested negative for anti-Mi^a. The second was a 42-year-old female, blood group O, who had donated blood 8th times and had a history of a weak Mi^a antigen reaction. Follow-up revealed that this donor was previously reported by Kerdkaewngam K. In this study, the frequency of the Vw antigen was determined to be 0.0167%. The previously weak anti-Mi^a reaction in this donor may be attributed to the natural occurring human polyclonal anti-Mi^a reagents produced by the NBC, TRCS. Initially, this anti-Mi^a was produced as a polyclonal antibody derived from human serum pooled from 4-6 donors known to have anti-Mi^a. Each batch may have had different specificities, such as anti-MUT, anti-Mur, anti-Hil, or anti-Hop. At the time, subclass-specific reagents for the Mi system were not available, limiting the ability to identify and distinguish between Mi subclasses. As a result, anti-Mi^a human polyclonal reagents may have lacked reactivity with certain subclasses, such as GP.Vw, leading to negative or weak reactions. Moreover, due to limited understanding of the Mi subsystem and the lack of standardized

reagent red cells covering all Mi subclasses, detecting GP.Vw using polyclonal anti-Mi^a was challenging. The amount of anti-Vw present in a reagent batch could be very low or diluted by plasma from donors without anti-Vw, leading to reduced or absent reactivity. The inability to accurately detect GP.Vw can result in serious clinical consequences. In the absence of accurate identification patients who possess both Vw and Mi^a antibodies may receive incompatible transfusions, leading to intravascular HTRs, which can result in mortality.¹⁹ Additionally, there are reports of HTRs and HDFN caused by antibodies against hybrid glycoporphins such as GP.Vw and GP.Mur.²⁰⁻²³ Within the framework of collaborative project between the NBC, TRCS, and the JRCS, the NBC, TRCS has received specific antibodies and hybridoma cell lines for the development of blood group reagents using human monoclonal hybridoma technology. These include anti-M, anti-N, anti-P1, and anti-Di^a, among others. Furthermore, the NBC, TRCS has successfully produced anti-Mi^a (IgM) using the human hybridoma method, resulting in anti-Mi^a (human MoAb). The JRCS confirmed that clone 4E4 expresses anti-MUT specificity, and clone 15D2 expresses anti-Vw specificity. These monoclonal antibodies can collectively detect all Mi subclasses, as reported by Aiem-umporn K.²⁴ The two GP.Vw(+) samples identified in this study can be used to evaluate the performance of anti-Mi^a (human monoclonal antibody), ensuring its reactivity with Mi.I, Mi.II, Mi.III, Mi.IV, Mi.VI, and Mi.X without reacting with the known GP.Vw(+) samples. This development is significant for improving Mi^a antigen detection in both patients and blood donors, ensuring the provision of compatible blood products. For blood donors, especially those used to produce panels cells at the NBC, TRCS, it is essential that Mi subclasses reflect the antibody specificities most commonly found in the Thai population. GP.Mur or Mi.III is predominant among Thais and reacts with anti-Mi^a, anti-MUT, anti-Mur, and anti-Hil. Using such red cells in antibody identification ensures consistency between screening and identification results.

Conclusion

The discovery of GP.Vw (Mi.I) antigen-positive Thai blood donors not only supports safe blood transfusion practices but also plays an important role in the development of anti-Mi^a blood grouping reagents and in the evaluation of antibodies against Mi subclasses derived from newly developed hybridoma cell lines produced by the NBC, TRCS. These reagents are capable of detecting all Mi subclasses in the Thai population, thereby enhancing the accuracy of antibody screening and identification. Additionally, this advancement reduces the need for imported reagents, fostering self-reliance in the production of rare blood group testing materials.

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References

- Kerdkaewngam K, Tubrod J. The differences of nine blood groups system in the three major ethnicities of human. *J Med Tech Assoc Thailand*. 2015;43:5127-40.
- Reid ME. MNS blood group system: a review. *Immunohematology*. 2009;25:95-101.
- Hamning DM. *Modern blood banking and transfusion practice*. 4th ed. Baltimore: F.A. Davis Company; 1999.
- Bejrachandra S, Nathalang O. Miltenberger subsystem. *J Hematol Transfus Med*. 2015;25:93-8.
- Lomas-Francis C. Miltenberger phenotypes are glycoprotein variants: a review. *ISBT Sci Ser*. 2016:296-301.
- Van der Hart M, Bosman H, van Loghem JJ. Rare blood groups. *Maandschr Kindergeneesht*. 1954;21:357-66.
- Poole J, King MJ, Mark KH, Liew W, Leong S, Chua M. The Mi III phenotypes among Chinese donors in Hongkong immunochemical and serological studies. *Transfus Med*. 1991;1:169-75.
- Dahr W. Miltenberger subsystem of the MNSs blood group system. Review and outlook. *Vox Sang*. 1992;62:129-35.
- Race RR, Sanger R. *Blood group in man*. 6th ed. Oxford: Blackwell; 1975.
- Taylor AM, Knighton GJ. A case of severe hemolytic disease of the newborn due to anti-Verweyst (Vw). *Transfusion*. 1982;22:165-6.
- Waldvogel S, Natterer J, Canellini G, Truttmann A, Tissot JD. Severe neonatal hyporegenerative anemia due to anti-Vw (anti-MNS9) alloantibody. *J Perinat Med*. 2009;37:422-4.
- Moncharmont P, Buclet D, Trouiloud C, Peyrard T, Rigal D. Severe hemolytic disease of the fetus and the newborn associated with anti-Vw (Vw). *J Matern Fetal Neonatal Med*. 2010;23:1066-8.
- Broadberry RE, Lin M. The incidence and significance of anti-Mi^a in Taiwan. *Transfusion*. 1994;34:349-52.
- Chiewsilp O, Yodpornpin W, Cherdchai S. Delayed hemolytic transfusion reaction due to anti-Mi^a. *Thai J Hematol Transfus Med*. 1996;6:289-90.
- Kerdkaewngam K, Tubrod J and Tingtoy U. Frequency of MNS hybrid glycoproteins in Mia (+) blood donors at the National Blood Centre, Thai Red Cross Society. *J Hematol Transfus Med*. 2024;34:27-32.
- Cohn CS, Delaney M, Johnson ST, Katz LM, Schwartz J, editors. *Technical manual*. 21st ed. Bethesda, MD: AABB; 2023.
- Chandanyingyong D, Pejrachandra S. Studies on the Miltenberger complex frequency in Thailand and family studies. *Vox Sang*. 1965;28:152-5.
- Lopez GH, Wilson B, Turner RM, Millard GM, Fraser NS, Roots NM, et al. Frequency of Mi^a (MNS7) and classification of Mi^a-positive hybrid glycoproteins in an Australian blood donor population. *Transfus Med Hemother*. 2020;47:279-86.
- Molthan L. Intravascular hemolytic transfusion reaction due to anti-Vw+Mi^a with fetal outcome. *Vox Sang*. 1981;40:105-8.
- Heathcote DJ, Carroll TE, Flower RL. Sixty years of antibodies to MNS system hybrid glycoproteins: what have we learned? *Transfus Med Rev*. 2011;25:111-24.
- Bakhtary S, Gikas A, Glader B, Andrews J. Anti-Mur as the most likely cause of mild hemolytic disease of the newborn. *Transfusion*. 2016;56:1182-4.
- Lin M, Broadberry RE. An intravascular hemolytic transfusion reaction due to 'anti-Mi(a)' in Taiwan. *Vox Sang*. 1994;67:320.
- Mallari RA, Chan A, Powers RJ, Pandipati S, Bensing KM, Biese D, et al. Fetal inheritance of GP*^{Mur} causing severe HDFN in an unrecognized case of maternal alloimmunization. *Transfusion*. 2020;60:870-4.
- Aiem-umporn K, Makechay S and Tingtoy U. Innovation in monoclonal anti-Mi^a production. *J Hematol Transfus Med*. 2024;34:149-54.