

Original Article

Frequency of MNS hybrid glycoporphins in Mi(a+) blood donors at the National Blood Centre, Thai Red Cross Society

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Abstract:

Introduction: The MNS hybrid glycoporphins represent a group of low frequency antigens associated with MNS blood group system. Mi^a antigen has various structures which can be classified by the reactions with mono-specific antisera in Miltenberger (Mi classes) subsystem. Some MNS hybrid glycoporphins are not reactive with anti-Mur which is commonly found in anti- Mi^a . So, to detect anti-Mur, $Mi(a+)$ red cells should be GP.Mur ($Mi.III$) or GP.Bun ($Mi.VI$). Therefore, we carefully selected MNS hybrid glycoporphins to produce antibody screening cells and antibody identification panel cells because we found that anti-Mur is the most common antibody in Thais.

Objective: To study, characterize and determine the frequency of MNS hybrid glycoporphins in $Mi(a+)$ Thai blood donors in order to produce antibody screening cells. **Materials and Methods:** A total of $Mi(a+)$ 1,216 blood samples from blood donor's database were phenotyped by using saline standard tube technique with anti-Mur, anti-MUT, anti-Vw and column agglutination technique (Bio-Rad) with anti-Hil, anti-Hop and anti-Hop+anti-Nob (anti-Anek). These mentioned antibodies were kindly supplied by Kanto-Koshinetsu Block Blood Center, Japanese Red Cross, Tokyo, Japan. **Results:** Phenotyping with a panel of antisera revealed that 1 donor (0.082%), 8 donors (0.658%), 1,122 donors (92.270%), and 74 donors (6.090%) were GP.Vw ($Mi.I$), GP.Hut ($Mi.II$), GP.Mur ($Mi.III$), and GP.Bun ($Mi.VI$), respectively. A new phenotype, we named from blood donor name GP.Nuk, was identified in 11 blood donors. **Conclusion:** The study showed that GP.Mur ($Mi.III$) was the most common MNS glycoporphin variants in $Mi(a+)$ Thai blood donors. GP.Vw ($Mi.I$), GP.Hut ($Mi.II$), and GP.Bun ($Mi.VI$) were rare. A novel phenotype, proposed name GP.Nuk, was found in this study. Further studies are required to characterize the structure of the gene that encodes GP.Nuk.

Keywords : ● $Mi(a+)$ ● MNS blood group ● Hybrid glycoporphins

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นิพนธ์ต้นฉบับ

ความถี่ของ MNS hybrid glycoporphorins ในผู้บริจาคโลหิตที่มีหมู่โลหิต Mi(a+) ณ ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

กัลยา เกิดแก้วงาม จินตนา ทัพรอด และ อุดม ตั้งต้อย

ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ

บทนำ MNS hybrid glycoporphorins เป็นแอนติเจนหายากที่มีความเกี่ยวข้องกับหมู่โลหิตระบบ MNS แอนติเจน Mi^a มีโครงสร้างที่หลากหลายสามารถจัดจำแนก โดยใช้การเกิดปฏิกิริยากับ monospecific antisera ในระบบย่อย Miltenberger (Mi classes) แต่บางชนิดไม่เกิดปฏิกิริยากับ anti-Mur ซึ่งพบได้บ่อยใน anti- Mi^a เพื่อให้ตรวจพบ anti-Mur เม็ดเลือดแดง $Mi(a+)$ ควรจะเป็น GP.Mur (Mi.III) หรือ GP.Bun (Mi.VI) ดังนั้นจึงต้องคัดเลือก $Mi(a+)$ hybrid glycoporphorins สำหรับนำมาผลิต antibody screening cells อย่างระมัดระวังเพราะ anti-Mur เป็นแอนติบอดีที่พบได้บ่อยในคนไทย **วัตถุประสงค์** เพื่อศึกษาลักษณะและหาความถี่ของ $Mi(a+)$ hybrid glycoporphorins ในผู้บริจาคโลหิตไทย เพื่อให้สามารถเลือก $Mi(a+)$ hybrid glycoporphorins สำหรับการผลิต antibody screening cells **วัสดุและวิธีการ** ตัวอย่างเลือด $Mi(a+)$ hybrid glycoporphorins จำนวนทั้งหมด 1,216 ตัวอย่างจากฐานข้อมูลผู้บริจาคโลหิต ตรวจโดยใช้วิธี standard tube technique กับ anti-Mur, anti-MUT, anti-Vw และวิธี column agglutination technique (BioRad) กับ anti-Hil, anti-Hop และ anti-Hop+anti-Nob (anti-Anek) โดยแอนติบอดีที่กล่าวมานี้ ได้รับความอนุเคราะห์จาก Kanto-Koshinetsu Block Blood Center สภากาชาดญี่ปุ่น กรุงโตเกียว ประเทศญี่ปุ่น **ผลการศึกษา** ผลการตรวจ phenotyping ด้วยชุดแอนติบอดี พบว่ามี 1 ราย (ร้อยละ 0.082), 8 ราย (ร้อยละ 0.658), 1,122 ราย (ร้อยละ 92.270) และ 74 ราย (ร้อยละ 6.090) เป็น GP.Vw (Mi.I), GP.Hut (Mi.II), GP.Mur (Mi.III) และ GP.Bun (Mi.VI) ตามลำดับ และพบ phenotype ใหม่ ซึ่งขอเสนอชื่อว่า GP.Nuk ตามชื่อผู้บริจาคโลหิตที่พบ จำนวน 11 ราย **สรุป** การศึกษานี้แสดงให้เห็นว่า GP.Mur (Mi.III) เป็น MNS glycoporphorin variant ที่พบมากที่สุดในผู้บริจาคโลหิตไทยที่เป็น $Mi(a+)$ ส่วน GP.Vw (Mi.I), GP.Hut (Mi.II) และ GP.Bun (Mi.VI) พบได้ยาก นอกจากนี้ได้พบ phenotype ใหม่ ที่ขอเสนอชื่อว่า GP.Nuk ซึ่งจำเป็นต้องมีการศึกษาลักษณะของ GP.Nuk เพิ่มเติมในระดับโครงสร้างยีน

คำสำคัญ : ● เอ็มไอ (เอบวก) ● หมู่เลือดเอ็มเอ็นเอส ● ไฮบริดไกลโคพอริน

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2567;34:27-32.

Introduction

There are currently 50 antigens in the MNS blood group system.¹ These antigens are expressed on glycoporphin A (GPA), glycoporphin B (GPB) or hybrid glycoporphins. Hybrid glycoporphins express several MNS antigens including Mi^a (MNS7) and have been reported in about 9.1% in central and about 20% in the north and northeast of Thailand. Therefore, anti-Mi^a is commonly found in Thai patients both as single antibody and combined antibodies.² Antibodies to MNS antigens carried on hybrid glycoporphins can cause hemolytic transfusion reaction and hemolytic disease of the fetus and newborn.¹ The antibodies against MNS hybrid glycoporphins in patients at Phramongkutklao Hospital have been investigated and found that the most common single red cell antibody is anti-Mi^a 65.16% (647/993) and the most common multiple antibodies are anti-E + anti-Mi^a 28.77% (42/146) of alloimmunized patients.³

The report about MNS glycoporphin variants in Thai blood donors by *Chandanyingyong and Pejrachandra* (1975) showed that 243 of 2,500 subjects (9.7%) are Mi(a+), consisted of 1 GP.Vw (Mi.I), 1 GP.Hut (Mi.II) and 241 GP.Mur (Mi.III).⁴ The incidence of 9.72% Mi(a+) in the Thai population is the highest in the world.⁵ In Caucasians, GP.Vw (Mi.I) and GP.Hut (Mi.II) are about six times as common as GP.Mur (Mi.III), whereas most Mi^a-positive Thai subjects belong to GP.Mur (Mi.III).⁴ The frequency of GP subclasses that often found in Thais and Asian people mostly is GP.Mur (Mi.III) (> 80%), the second is GP.Bun (Mi.VI) (> 10%), the rest are GP.Hut (Mi.II) and GP.Vw (Mi.I), so anti-Mi^a that mostly found in Thai patients is anti-Mur but anti-Hop and anti-Hil are rarely found. The classification of GP subclasses antibodies required the used of specific screening cells and panel cells that the GP subclasses were known.

MNS low incidence antigens are glycoporphin A (GPA) and glycoporphin B (GPB) hybrid proteins of hybrid gene on *GYP*A* and *GYP*B*. GP variant phenotypes polymorphism arisen from various genetic mechanisms, for

example single nucleotide substitution, unequal crossing over, gene conversion and gene deletion, resulting in base alignment that affected antigens expression on red blood cells.⁵ In 1993 Reid and Tippett summarized 11 classes Miltenberger subsystem (Mi.I to Mi.XI) using GP initials to name antigens and classification of the 11 subclasses.⁵

Antiserum and Standard Cell Preparation Section, National Blood Centre, Thai Red Cross Society is a manufacturer of antibody screening cells for use in Thailand and The Lao People's Democratic Republic. Currently, there are eight Mi(a+) hybrid glycoporphins and each has a unique combination of MNS antigens. Therefore, we carefully selected Mi(a+) hybrid glycoporphins to produce antibody screening cells and antibody identification panel cells in order to detect anti-Mur which is the most common antibody in Thais. Anti-Mur can not be detected by antibody screening cell Mi(a+) bearing only hybrid glycoporphins GP.Hut (Mi.II). Therefore, we select only GP. Mur (Mi.III) and GP.Bun (Mi.VI) for our screening cells and antibody identification panel cells production. Most hospital blood banks in Thailand use screening cells and panel cells produced by National Blood Centre, Thai Red Cross Society that show only Mi^a antigen but do not show the GP subclasses so some antibodies of GP subclasses may not be detected. This study aims to increase the efficiency of antibody identification by testing donor's blood samples to select suitable Mi(a+) phenotype for producing screening cells and panel cells.

Materials and Methods

Study populations

A total of 1,216 known phenotype Mi(a+) hybrid glycoporphins blood donors that have been selected for screening cells and panel cells production were classified into GP subclasses between April to September 2023, after that the results were kept in donor's database of National Blood Centre, Thai Red Cross Society.

Methods

1. Phenotyping using saline standard tube technique with anti-Mur, anti-MUT and anti-Vw kindly provided from Kanto-Koshinetsu Block Blood Center, Japanese Red Cross, Tokyo, Japan were performed. One hundred ul of antibodies and 50 ul of 3% red blood cells suspension were mixed in tube and incubated for 15 minute at room temperature, after that centrifuged for 15 seconds and interpreted the results.

2. Phenotyping using column agglutination technique (CAT) from Bio-Rad Laboratories, Inc Hercules, California, USA with anti-Hil, anti-Hop and anti-Hop+anti-Nob (anti-Anek) kindly provided from Kanto-Koshinetsu Block Blood Center, Japanese Red Cross, Tokyo, Japan. Twenty five ul of antiserum and 50 ul of 0.8% red blood cells suspension were mixed in column and incubated for 15 minutes after that centrifuged for 10 minutes and interpreted the results.

This study was conducted in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived by Research Ethics Committee, National Blood Centre, Thai Red Cross Society, Bangkok, Thailand. (COA No. NBC 8/2023).

Results

Classification of GP. subclasses is showed in Table 1. The distribution of variant MNS hybrid glycoporphorins in 1,216 Mi(a+) Thai blood donors is showed in Table 2. This study used 6 antisera including anti-Mur, anti-MUT, anti-Vw, anti-Hil, anti-Hop and anti-Hop+anti-Nob (anti-Anek) to classify 1,216 Mi(a+) hybrid glycoporphorins, revealing that 1 donor (0.082%), 8 donors (0.658%), 1,122 donors (92.270%), and 74 donors (6.086%) were GP.Vw (Mi.I), GP.Hut (Mi.II), GP.Mur (Mi.III), and GP.Bun (Mi.VI), respectively. MNS phenotyping results from National Blood Centre donor database is showed in Table 2. In addition, red blood cells typing from 11 donors (0.905%) were positive with anti-Mi^a, anti-Mur, anti-MUT but failed to react with anti-Vw, anti-Hil, anti-Hop and anti-Hop+anti-Nob (anti-Anek) (Table 2). Based on seven anti-sera, this pattern of reaction is distinct from the previous reports. We propose to call this phenotype as GP.Nuk that is the name of the first donor that found to have this phenotype.

Table 1 Terminology and antigens presented in Miltenberger subsystem⁵

Terminology		Reaction of red cells with anti-										
Mi.	GP.	Mi ^a	Vw	Hut	Mur	MUT	Hil	TSEN	MINY	Hop	Nob	DANE
Mi.I	GP.Vw	+	+	0	0	0	0	0	0	0	0	0
Mi.II	GP.Hut	+	0	+	0	+	0	0	0	0	0	0
Mi.III	GP.Mur	+	0	0	+	+	+	0	+	0	0	0
Mi.IV	GP.Hop	+	0	0	+	+	0	+	+	+	0	0
Mi.V	GP.Hil	0	0	0	0	0	+	0	+	0	0	0
Mi.VI	GP.Bun	+	0	0	+	+	+	0	+	+	0	0
Mi.VII	GP.Nob	0	0	0	0	0	0	0	0	0	+	0
Mi.VIII	GP.Joh	0	0	0	0	0	0	0	0	+	+	0
Mi.IX	GP.Dane	0	0	0	+	0	0	0	0	0	0	+
Mi.X	GP.HF	+	0	0	0	+	+	0	+	0	0	0
Mi.XI	GP.JL	0	0	0	0	0	0	+	+	0	0	0

Table 2 Distribution of variant MNS hybrid glycoporphins in 1,216 Mi(a+) Thai blood donors

Mi Classes	MNS hybrid glycoporphins	MNS phenotypes	Reactions with antisera							Number	%
			Mi ^a	Mur	MUT	Vw	Hil	Hop	Anek		
GP.Vw	Mi I	MMss	+	0	0	+	0	0	0	1	0.082
GP.Hut	Mi II	MMss	+	0	+	0	0	0	0	8	0.658
GP.Hut		MNss	+	0	+	0	0	0	0		
GP.Mur	Mi III	MMss	+	+	+	0	+	0	0	1,122	92.270
GP.Mur		MNss	+	+	+	0	+	0	0		
GP.Mur		MNSs	+	+	+	0	+	0	0		
GP.Mur		MMSs	+	+	+	0	+	0	0		
GP.Mur		NNss	+	+	+	0	+	0	0		
GP.Mur		NNSs	+	+	+	0	+	0	0		
GP.Bun	Mi VI	MMss	+	+	+	0	+	+	+	74	6.086
GP.Bun		MNss	+	+	+	0	+	+	+		
GP.Bun		MNSs	+	+	+	0	+	+	+		
GP.Bun		MMSs	+	+	+	0	+	+	+		
New phenotype (GP.Nuk)		MMss	+	+	+	0	0	0	0	11	0.905
New phenotype (GP.Nuk)		MNss	+	+	+	0	0	0	0		

Discussion

In this study, the results showed that GP.Mur (Mi. III) was the most common MNS hybrid glycoporphin in Thai blood donors. GP.Vw (Mi.I), GP.Hut (Mi.II), and GP.Bun (Mi.VI) were less common. A novel phenotype, proposed name of GP.Nuk, was identified in this study.

MNS glycoporphin variants in Thai blood donors reported by *Chandanyingyong and Pejchandra* (1975)⁴ were 243 in 2,500 subjects (9.7%) consisting of 1 GP.Vw (Mi.I), 1 GP.Hut (Mi.II) and 241 GP.Mur (Mi.III), indicated that the most common variant MNS glycoporphin was GP.Mur (Mi.III) which is consistent with this study. The prevalence of Mi(a+) consisting of GP.Hut (Mi.II), GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Bun (Mi.VI) and GP.HF (Mi.X) in central Thai blood donors were 7.48%; whereas, it was 23.0% in northern Thai blood donors. The detection of GP.Hut (Mi.II), GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Bun (Mi.VI) and GP.HF (Mi.X) in northern Thai blood donors was significantly different from central Thai blood donors ($p < 0.001$) reported by Kaset, et al (2015), using PCR-SSP method.⁷ The prevalence of Mi(a+) in Japanese was 1 in 3,350 reported by Mohn,

et al in 1963 and the prevalence of Mi(a+) in Caucasians was very rare.⁵ The occurrence rate of GP.Mur (Mi.III) in Taiwan is high (21.2-88.4%).⁸ The prevalence among several indigenous tribes was 7.3%⁸ in the general population. The frequencies are higher in Southeast Asian than in other ethnic group, the frequencies are 9.6% in Thai, 6.28% in Hong Kong Chinese, 4.7% in American Chinese, and 9.5% in Chinese donors. The frequencies of Mi(a+) are 0.22% in Australians and 0.01% in Japanese.⁸ These study results are useful for selecting suitable Mi(a+) red cells to produce antibody screening cells and antibody identification panel cells. GP.Mur (Mi.III) and GP.BUN (Mi.VI) are used to produce antibody screening cells and antibody identification panel cells but GP.Vw (Mi.I), GP.Hut (Mi.II) and the novel phenotype (GP.Nuk) should not be selected for production because GP.Vw (Mi.I), GP.Hut (Mi.II) cannot detect anti-Mur and the novel phenotype (GP.Nuk) can not detect anti-Hil, both antibodies are the most common antibodies found in Thais. This study has two limitations: GP.Nuk red cells were tested with a limited panel of antisera and the gene encoding GP.Nuk is not known. The genetic basis for

the novel phenotype (GP.Nuk) is under investigation in collaboration with the Australian Red Cross Lifeblood in Brisbane, Australia.

Conclusion

The study showed that GP.Mur (Mi.III) was the most common MNS glycoprotein variants in Mi(a+) Thai blood donors. GP.Vw (Mi.I), GP.Hut (Mi.II), and GP.Bun (Mi.VI) were rare. A novel phenotype, proposed name of GP.Nuk, was found in this study. Further studies are required to characterize the structure of the gene that encodes GP.Nuk. This study is useful for antibody screening cells and monoclonal Anti-Mi^a production.

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