



The First Korean Family with Hemoglobin-M Milwaukee-2 Leading to Hereditary Methemoglobinemia

Dae Sung Kim¹, Hee Jo Baek^{1,2}, Bo Ram Kim¹, Bo Ae Yoon^{1,2}, Jun Hyung Lee³, and Hoon Kook^{1,2}

¹Department of Pediatrics, Chonnam National University Hwasun Hospital, Hwasun;

²Department of Pediatrics, Chonnam National University Medical School, Gwangju;

³Department of Laboratory Medicine, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Gwangju, Korea.

Hemoglobin M (HbM) is a group of abnormal hemoglobin variants that form methemoglobin, which leads to cyanosis and hemolytic anemia. HbM-Milwaukee-2 is a rare variant caused by the point mutation CAC>TAC on codon 93 of the hemoglobin subunit beta (*HBB*) gene, resulting in the replacement of histidine by tyrosine. We here report the first Korean family with HbM-Milwaukee-2, whose diagnosis was confirmed by gene sequencing. A high index of suspicion for this rare Hb variant is necessary in a patient presenting with cyanosis since childhood, along with methemoglobinemia and a family history of cyanosis.

Key Words: Hemoglobin M-Milwaukee-2, methemoglobinemia, cyanosis, congenital hemolytic anemia

INTRODUCTION

Hemoglobinopathy refers to abnormalities in hemoglobin (Hb), and more than 1300 variants thereof have been described.¹ Hemoglobin M (HbM) is a group of abnormal Hb variants that form methemoglobin (metHb), leading to cyanosis and hemolytic anemia.² MetHb is an altered state of Hb in which the heme iron is oxidized from the ferrous (Fe²⁺) to the ferric state (Fe³⁺). Since the ferric heme of MetHb does not bind with O₂ and the remaining ferrous heme groups have an increased affinity to O₂, the release of oxygen to the tissues is reduced.³⁻⁵

In a healthy individual, metHb is spontaneously formed by auto-oxidation of Hb at a slow rate.⁶ However, metHb is maintained at a low level of approximately 1% by the metHb reduc-

tion pathway.⁶ Methemoglobinemia occurs when metHb levels exceed 1.5% in blood.⁷ Hereditary methemoglobinemia is often caused by methemoglobin reductase enzyme deficiency, rare HbM disease, or the rarest cytochrome *b₅* deficiency.⁷

HbM disease is caused by select variants in the α -, β -, or rarely, γ - globin genes.^{1,8} Among the 13 known variants of HbM, HbM-Milwaukee-2 is rare and is caused by a point mutation of CAC>TAC on codon 93 of the hemoglobin subunit beta (*HBB*) gene, resulting in the replacement of histidine by tyrosine.⁷⁻¹⁴ In this study, we describe the first Korean family with HbM-Milwaukee-2, whose diagnosis was confirmed by gene sequencing.

CASE REPORT

A 16-year-old girl was referred to our hospital for cholecystectomy due to gallstones. Her face and lips appeared cyanotic; however, there was no tachypnea or dyspnea. There was no history of cardiopulmonary illness or exposure to drugs and chemicals. Physical examination revealed no clubbing of fingers and toes and no organomegaly. No abnormality was found on chest auscultation.

Her mother and two brothers showed similar cyanosis. Furthermore, there was a history of lifelong cyanosis in many of her maternal family members (Fig. 1A and B). Her younger broth-

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Corresponding author: Hee Jo Baek, MD, PhD, Department of Pediatrics, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, 322 Seoyang-ro, Hwasun 58128, Korea.

Tel: 82-61-379-7695, Fax: 82-61-379-7697, E-mail: swan93@naver.com

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er had a history of stone removal in the common bile duct.

Peripheral arterial oxygen saturation (SpO₂) on pulse oximetry was 35% at room air, which did not improve with administration of oxygen. Her, her mother's, and her older brother's arterial blood was dark chocolate in color, similar to the color of venous blood (Fig. 1C). However, the patient's arterial blood gas analysis showed normal findings: arterial partial pressure of oxygen (PaO₂) of 115 mm Hg, arterial partial pressure of carbon dioxide (PaCO₂) of 34.5 mm Hg, and arterial oxygen saturation (SaO₂) of 92.9%. Laboratory investigations showed findings consistent with hemolytic anemia (Table 1). Peripheral blood smear showed anisocytosis, poikilocytosis, and increased reticulocytes. Chest radiography, electrocardiography, and echocardiography findings were normal. Pulmonary disease was excluded by chest CT and pulmonary function test. Glucose-6-phosphate dehydrogenase activity was normal. The blood MetHb concentration was 13.0% (normal range, 0–1.5%), leading to the suspicion of hereditary methemoglobinemia.

Hb electrophoresis at alkaline pH revealed increased HbF (5.7%) and an abnormal band (10.5%) between HbF and HbA₂ (Fig. 2A). For further evaluation of this unknown band, we analyzed the *HBB* gene sequence by polymerase chain reaction

and direct sequencing. This revealed a heterozygous point mutation of *HBB* gene (c277C>T, p.His93Tyr) confirming HbM-Milwaukee-2 (Fig. 2B).

Laboratory findings in her family members were similar (Table 1). The patient's mother (II-5 in Fig. 1A) and brother (III-8 in Fig. 1A) had unknown bands on Hb electrophoresis, which were 13.6% and 16.8%, respectively (Figures not shown). These unknown fractions were later attributed to HbM-Milwaukee-2 (Figures not shown).

The patient successfully underwent laparoscopic cholecystectomy for gallstones under monitoring of arterial oxygenation by co-oximetry.

Informed consent was obtained from the patients regarding the reporting and publication of this case report.

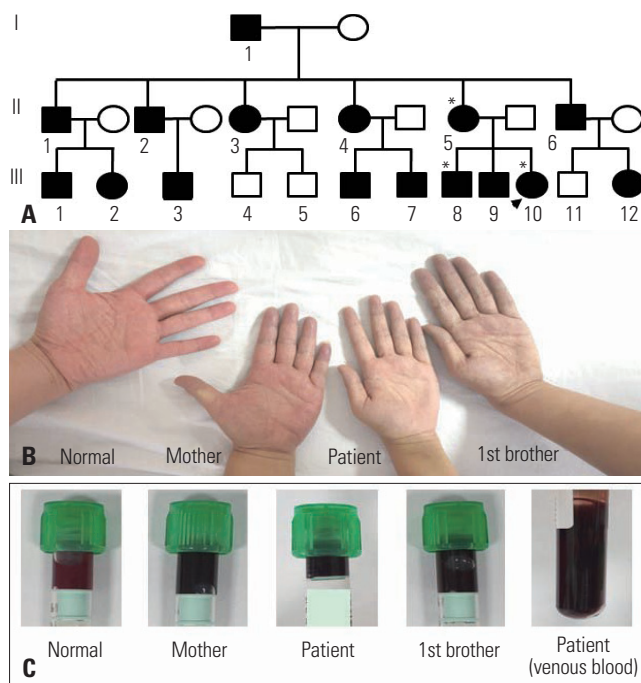


Fig. 1. Family tree of the patient's maternal family with a history of lifelong cyanosis and skin color and arterial blood color in the patient's family. (A) Black symbols indicate clinically affected family members. The arrowhead indicates the proband with the *HBB* variant and asterisk (*) indicates family members who were genetically confirmed. (B) Cyanotic skin color of the patient, her mother, and 1st brother compared to that of a normal healthy person. (C) Patient, her mother, and 1st brother's arterial blood had dark brown color compared to the bright red color in a normal healthy person. Of note, the patient's arterial blood color is almost the same as the color of her venous blood. *HBB*, hemoglobin subunit beta.

Table 1. Laboratory Findings and Oxygen Saturation on Pulse Oximetry in the Family Members with HbM-Milwaukee-2

	Normal	Patient Mother	1st brother	2nd brother	
Laboratory findings					
Hemoglobin (g/dL)	12–18	10.9	11.4	12.9	12.5
MCV (fL)	80–99	94.9	101.9	97.9	99.0
RDW (%)	11.5–14.5	15.3	15.4	14.9	14.4
Reticulocyte (%)	0.5–1.5	4.15	5.25	3.52	2.45
Indirect bilirubin (mg/dL)	0.3–1.0	2.8	1.3	2.4	12.82
Haptoglobin (mg/dL)	30–200	<7.75	<7.75	<7.75	<7.75
Fetal hemoglobin (%)	0–2	4.2	-	-	-
Methemoglobin (%)	0.2–0.6	13.0	6.0	6.3	13.1
Pulse oximetry					
SpO ₂ (%)	96–100	35	46	65	51
Arterial blood gas analysis					
SaO ₂ (%)	92–98.5	92.9	92.3	91.4	89.7
PaO ₂ (mm Hg)	75–100	115	117	113	97.3

MCV, mean corpuscular volume; RDW, red blood cell distribution width; SpO₂, peripheral arterial oxygen saturation; SaO₂, arterial oxygen saturation; PaO₂, partial arterial pressure of oxygen.

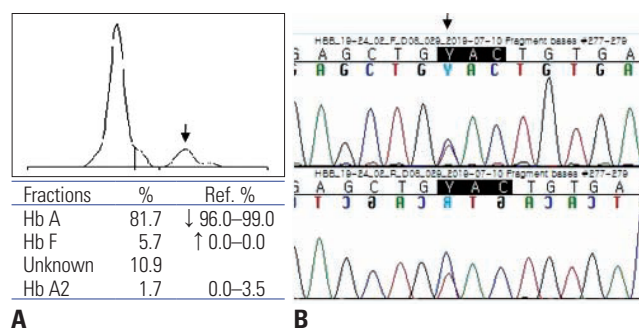


Fig. 2. Hb electrophoresis and β -globin gene sequencing of the patient's family. (A) The patient's Hb electrophoresis showed an unknown band (arrow) between HbF and HbA₂, which was later revealed as HbM-Milwaukee-2. (B) The patient's *HBB* gene sequencing revealed a heterozygous point mutation of CAC>TAC on codon 93, named as HbM-Milwaukee-2. Hb, Hemoglobin; *HBB*, hemoglobin subunit beta.

DISCUSSION

Hemoglobinopathies are rare in Korea. However, the prevalence of thalassemia in young Koreans is recently increasing with the influx of immigrants from South-East Asia.¹⁵ A recent nationwide retrospective cohort study of hereditary hemolytic anemia (HHA) on the Korean pediatric population during 1997–2016 identified 369 children with HHA.¹⁶ Among them, hemoglobinopathies were seen in only 59 patients (16%). Moreover,¹⁶ variants of *HBB* gene mutations were identified in 28 Korean families; however, HbM-Milwaukee-2 was not reported.¹⁶

To date, this is the first report of Korean family with HbM-Milwaukee-2, which was diagnosed by gene sequencing. HbM-Milwaukee-2 was first identified as HbM-Hyde Park in an African-American in 1966.⁹ The same mutation was found in a Japanese patient with congenital cyanosis, and was named as HbM-Akita.¹⁰ Later, DNA sequence analysis revealed that the mutations in HbM-Milwaukee-2, HbM-Hyde Park, and HbM-Akita were all the same.¹⁴

In this report, the affected members of the patient's maternal side had cyanosis without any other symptoms. Cyanosis did not improve with administration of oxygen. Their MetHb levels were between 6.0% and 13.1%. The SpO₂ on pulse oximetry was 35–65%; however, SaO₂ and PaO₂ on arterial blood gas analysis were normal (Table 1).

Cyanosis with low SpO₂ that does not improve with oxygen administration despite normal PaO₂ and SaO₂ are clues for the diagnosis of methemoglobinemia. The diagnosis of HbM disorders is challenging because of their low incidence and the absence of symptoms, except chronic cyanosis.¹³ However, the diagnostic method is relatively simple. Hb analysis by gel electrophoresis, as in our case, or high-performance liquid chromatography provides critical information with which to determine the presence of Hb variants. Furthermore, gene sequencing can confirm the Hb variants.

To date, all reported cases of HbM-Milwaukee-2 had heterogeneous trait.^{7–14} Homogeneity of the variant is thought to be fatal due to high levels of MetHb in the blood.¹³ The heterozygous variant is benign, and individuals with HbM disease have normal life expectancy and pregnancy.⁸ However, *HBB* gene mutation, as seen in our case, can lead to acute hemolytic anemia, which can aggravate during stressful medical conditions, such as gastrointestinal infection or sepsis.⁸ Although there is no specific treatment, avoidance of oxidant drugs and toxins, such as aniline derivatives, nitrates, and other agents that form metHb, is recommended.

A high index of suspicion for this rare Hb variant is necessary in patients who present with chronic cyanosis since childhood, along with methemoglobinemia and a family history of cyanosis. Early genetic diagnosis can help in preventing unnecessary examinations and invasive procedures, in implementing preventive measures, and in providing genetic counseling to patients.

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AUTHOR CONTRIBUTIONS

Conceptualization: Hee Jo Baek. **Investigation:** Dae Sung Kim and Hee Jo Baek. **Supervision:** Bo Ram Kim, Bo Ae Yoon, and Jun Hyung Lee. **Writing—original draft:** Dae Sung Kim. **Writing—review & editing:** Hee Jo Baek and Hoon Kook. **Approval of final manuscript:** all authors.

ORCID iDs

Dae Sung Kim	https://orcid.org/0000-0003-4060-3802
Hee Jo Baek	https://orcid.org/0000-0003-3830-8134
Bo Ram Kim	https://orcid.org/0000-0003-1262-0031
Bo Ae Yoon	https://orcid.org/0000-0002-3028-7335
Jun Hyung Lee	https://orcid.org/0000-0002-8682-3694
Hoon Kook	https://orcid.org/0000-0002-9135-5821

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