



# A diabetic patient in whom Hb Weesp was incidentally detected when her HbA1c level was measured

Tomomi Hatayama<sup>1,2</sup> · Fumio Umeda<sup>2</sup> · Teruaki Yamauchi<sup>2</sup> · Hiroshi Ideguchi<sup>3</sup>

Received: 19 March 2019 / Accepted: 25 June 2019 / Published online: 5 July 2019  
© The Japan Diabetes Society 2019

## Abstract

The level of glycated hemoglobin A1c (HbA1c) is widely used to monitor long-term glycemic control in patients with diabetes mellitus. There are more than 30 methods for measuring HbA1c levels. In recent times, high-performance liquid chromatography (HPLC) has become the most commonly used method in Japan. However, HPLC-based HbA1c level measurements do not accurately reflect glycemic control in the presence of Hb variants. We report the case of a patient with type 2 diabetes mellitus, who was incidentally found to having an extremely rare Hb variant. A 69-year-old Japanese female visited our clinic and was diagnosed with diabetes mellitus. Her HbA1c level, which was measured using HPLC at our clinic, could not be determined. DNA sequencing revealed a heterozygous mutation in the  $\alpha 1$  globin gene (HBA1: c.301C>T, p.Leu101Phe). Hb Weesp was detected. Many Hb variants have been reported; however, to the best of our knowledge, this is only the second report about Hb Weesp in the world and the first from Japan. Clinicians should consider the possibility of Hb variants in cases in which abnormal elution patterns are detected during the measurement of HbA1c using HPLC.

**Keywords** HbA1c · Hb Weesp · Hb variant · HPLC

## Introduction

The glycated hemoglobin A1c (HbA1c) level is indispensable for diagnosing, treating, and monitoring patients with diabetes mellitus. It reflects the previous 8–12 weeks of glycemic control [1]. There are several methods for measuring HbA1c levels, including ion-exchange high-performance liquid chromatography (HPLC), boronate-affinity HPLC, immunoassays, and enzyme-based assays. Ion-exchange HPLC is the most commonly used method for measuring HbA1c levels because it is fast, accurate, and reliable [2].

According to the guidelines developed by the American Diabetes Association in 2009, diabetes mellitus should be

diagnosed based on HbA1c level measurements [3]. However, there are several technical issues that can interfere with HbA1c assays. Hemoglobin (Hb) variants are one such factor. About 20% of patients with Hb variants exhibit clinical symptoms, such as polycythemia, hemolytic anemia, or methemoglobinemia. On the other hand, the other 80% display no clinical symptoms [4].

Recently, there have been a number of cases in which Hb variants have been incidentally detected when patients with diabetes mellitus had their HbA1c levels measured. Here, we report the case of a patient with type 2 diabetes mellitus, who was incidentally found to have an extremely rare Hb variant.

## Case

A 69-year-old Japanese female with a 2-year history of type 2 diabetes mellitus visited our clinic. She had been diagnosed with diabetes mellitus by a previous physician, but had not been treated for a few months. She had lost 6 kg of weight (69 kg → 63 kg) during the last 2 months and visited our clinic to achieve glycemic control. She did not have a family history of diabetes. A physical examination

✉ Tomomi Hatayama  
hatayama@med.kyushu-u.ac.jp

<sup>1</sup> Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

<sup>2</sup> Department of Diabetes, Yukuhashi Central Hospital, 5-5-42, Nishimiyaichi, Yukuhashi, Fukuoka 824-0031, Japan

<sup>3</sup> Department of Clinical Laboratory, Fukuoka Sanno Hospital, 3-6-45, Momochihama, Sawara-ku, Fukuoka 814-0001, Japan

showed that her body mass index was 31.1 kg/m<sup>2</sup>, and her blood pressure was 156/100 mmHg. Blood tests revealed the following results: white blood cell count: 5100/mm<sup>3</sup>, Hb: 15.2 mg/dl, platelets: 46.6 × 10<sup>4</sup>/mm<sup>3</sup>, fasting blood glucose: 136 mg/dl, HbA1c: n.d. (ADAMS A1c HA-8160 analyzer, ver. 4.05; Arkray, Kyoto, Japan). An abnormal peak (arrow) was observed before the HbA1c peak, as shown in Fig. 1a. Figure 1b shows the results of high-resolution ion-exchange HPLC separation of the Hb components in the patient's hemolysate. An abnormal peak (arrow), which was co-eluted with normal Hb(A0), was detected as a right "shoulder peak".

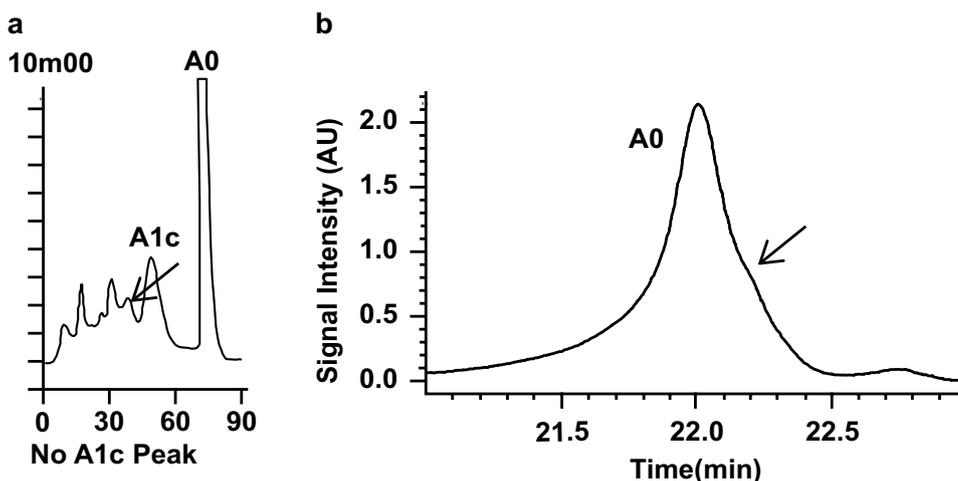
Next, DNA sequencing of the abnormal Hb gene was performed. Nucleotide sequencing of exon 3 of the α1 globin gene revealed a heterozygous mutation (from CTC to TTC, arrow) at codon 100, as shown in Fig. 2. This mutation resulted in the replacement of leucine by phenylalanine, and this form of Hb is known as Hb Weesp [5, 6].

### Discussion

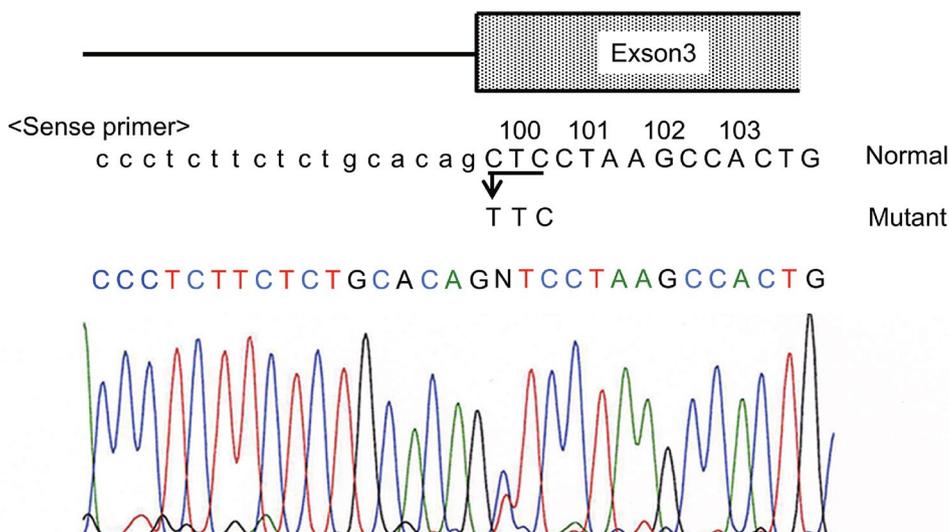
Herein, we report the case of a Japanese patient with type 2 diabetes mellitus, in whom the Hb variant Hb Weesp was incidentally detected when we measured her HbA1c levels using the HA-8160 (ver. 4.05) analyzer. There are more than 30 methods for measuring HbA1c levels [7]. HPLC is the most commonly used method in Japan. HbA1c is used as a marker of long-term glycemic control in patients with diabetes mellitus. We sometimes encounter discrepancies between HbA1c and blood glucose values. Hb variants can interfere with HbA1c assays. Therefore, clinicians should always be aware of the possibility of abnormal Hb variants.

Hb comprises four polypeptide chains, two α chains and two β chains. The major form of Hb found in healthy adults is HbA. Many Hb variants exhibit different elution

**Fig. 1** a Elution patterns obtained with ion-exchange HPLC using the HA-8160 analyzer (ver. 4.05). An abnormal peak was observed before the HbA1c peak (arrow). b Elution patterns obtained with high-resolution ion-exchange HPLC. An abnormal peak, which was co-eluted with normal Hb(A0), was detected as a right shoulder peak (arrow)



**Fig. 2** Identification of the heterozygotic point mutation. At codon 100 of exon 3 of the α1-globin gene, double peaks for C and T were detected in the sense chain



patterns on HPLC. For this reason, HbA1c level measurements are sometimes inaccurate.

Hb Weesp was first reported in the Netherlands in 2014 [5]. To the best of our knowledge, this is only the second report of Hb Weesp in the world and the first from Japan. Patients with Hb Weesp exhibit a heterozygous mutation in the  $\alpha$ 1-globin gene (HBA1: c.301C > T, p.Leu101Phe). This mutation was demonstrated to be clinically silent [6], and our patient showed no clinical symptoms. Thus, this  $\alpha$ 1-globin mutation does not result in major changes in the functionality of the Hb protein.

We analyzed our patient's blood using the HA-8160 analyzer (ver. 4.05). However, her HbA1c level could not be determined due to the presence of abnormal elution patterns. Therefore, we measured her HbA1c level using a different machine, an HA-8160 (ver. 4.22), which was reprogrammed to exclude the abnormal peak, as shown in Fig. 1a. Using this machine, her HbA1c value was estimated to be 6.0% (according to the Japan Diabetes Society method). HbA1c values are calculated as the ratio of the area of the HbA1c peak to the total area of all Hb peaks. Inaccurate HbA1c values can be obtained when Hb variants or their glycosylated derivatives cannot be separated from HbA or HbA1c. In such cases, the Hb variants can be co-eluted with HbA, as shown in Fig. 1b. If the peak indicated by the arrow in Fig. 1a was the peak for the glycosylated Hb variant, the patient's HbA1c value might have been underestimated. On the other hand, if it was not, it is possible that the patient's HbA1c value was accurate.

More than 1000 Hb variants have been reported worldwide [8], and about 200 Hb variants have been reported in Japan. It is suspected that one in 3000 Japanese have an Hb variant. The most common Hb variants found in Japan are Hb J Cape Town (an  $\alpha$  chain variant, which accounts for 6.6% of all Hb variants in Japan) and Hb Riyadh (a  $\beta$  chain variant, 9.2%) [9]. HbA1c levels are now measured by HPLC in most cases. The HbA1c level has become the most useful and common marker of glycemic control in patients without Hb variants. In cases involving patients with Hb variants, HbA1c levels should be determined using different methods, or other indicators, such as the glycosylated albumin level, should be used.

**Acknowledgements** We thank Dr. Takashi Miyazawa for making valuable suggestions and Arkray Co. for their help with the data analysis.

## Compliance with ethical standards

**Conflict of interest** The authors state that they have no conflicts of interest.

**Human rights statement** All the procedures were performed in accordance with the Helsinki Declaration of 1964 and later versions.

**Informed consent** Informed consent was obtained for the publication of this case report.

## References

1. Inoue K, Nakashima M, Masunaga S, Tanigaki N, Hatoyama Y, Nagashio H, Baba T, Nohara M. A case of HbE-Saskatoon and false-low HbA1c value determined by ion-exchange high-performance liquid chromatography (HPLC). *Igakukensa*. 2018;67(4):575–9.
2. Iizuka K, Mizuno M, Niwa H, Takeda J. A rare case of variant hemoglobin (Hb Yahata) suspected based on inconsistent plasma glucose and HbA1c levels. *Intern Med*. 2015;54(14):1771–5.
3. International Expert Committee. International Expert Committee report on the role of A1C assay in the diagnosis of diabetes. *Diabetes Care*. 2009;32(7):1327–34.
4. Bry L, Chen PC, Sack DB. Effect of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem*. 2001;47:153–63.
5. Van Zweiten R, Veldthuis M, Delzenne B, Berfhuys J, Groen J, Ait Ichou F, Clifford E, Hartevelde CL, Stroobants AK. Hemoglobin analyses in the Netherlands reveal more than 80 different variants including six novel ones. *Hemoglobin*. 2014;38(1):1–7.
6. Bots M, Stroobants AK, Delzenne B, Soeters MR, de Vries JE, Weykamp CW, Norg RJ, Veldthuis M, van Zwieten R. Two novel haemoglobin variants that affect haemoglobin A1c measurement by ion-exchange chromatography. *Clin Chem Lab Med*. 2015;53(9):1465–71.
7. John WG. Glycated haemoglobin analysis. *Ann Clin Biochem*. 1997;34(Pt1):17–31.
8. Server GG: Hb Var: a database of human hemoglobin variants and thalassemias. <http://globin.bx.psu.edu/hbvar/menu.html>. Accessed 30 Oct 2018
9. Yamashiro Y, Hattori Y. Hemoglobinopathies in Japan: characteristics and comparison with those of other ethnic groups. *Rinsho Ketsueki*. 2015;56(7):752–9.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.