Hb Aalesund – An unstable α-globin variant found in a Norwegian patient causing moderate hemolytic anemia and falsely high Hb A_{1c} using ion-exchange HPLC.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Abstract

A new unstable hemoglobin (Hb) variant, named Hb Aalesund, was detected during Hb A_{1c} measurement in a patient with a nearly compensated hemolytic anemia. Sequencing of the α-globin genes revealed a seven base pair deletion in exon three of the *HBA2* gene (*HBA2*:c.400_406delAGCACCG) (NM_000517.4) causing a frameshift and a premature termination codon two positions downstream. Apparently, the transcript bypassed nonsense-mediated decay and a truncated protein was translated. The unstable Hb variant presumably underwent rapid denaturation, as heterozygosity of Hb Aalesund was associated with mild hemolytic anemia. In addition, the Hb variant interfered with Hb A_{1c} measurement by cation exchange high performance liquid chromatography (HPLC), causing a falsely high Hb A_{1c} result when using the Bio-Rad D10™ Hemoglobin Analyzer fast Hb A_{1c} Program.

Key words: Unstable hemoglobin (Hb), α-globin gene, hemolytic anemia, hemoglobinopathy, Hb A_{1c}

Inherited hemoglobin (Hb) disorders are among the most common monogenic diseases in the world and more than 1300 Hb variants have been reported [1]. Most Hb variants result from single nucleotide changes, but occasionally small insertions or deletions cause truncated or elongated protein chains [2]. Sequence variants may alter Hb biochemical properties with physiological effects ranging from insignificant to severe [3]. Changes in certain regions important for protein structure that affect size or charge of an amino acid side chain or deletion of amino acids may result in unstable Hb variants [4]. Structural hemoglobinopathies may also interfere with Hb A_{1c} measurement, and result in erroneous Hb A_{1c} values [5]. Here, we describe a novel unstable Hb variant, named Hb Aalesund, discovered during Hb A_{1c} measurement in a woman of Norwegian origin with a nearly compensated hemolytic anemia. Regional Committees for Medical and Health Research Ethics (ethical agreement REK 2015/2352) approved the study and written informed consent was obtained from the patient.

An unexpectedly high Hb A_{1c} concentration was observed in a 35-year-old woman with fatigue whose blood sample was sent to the Department of Medical Biochemistry at Aalesund Hospital for a routine health check. Hb A_{1c} was measured using cation-exchange high-pressure liquid chromatography (HPLC) on a Bio-Rad D10™ Hemoglobin Analyzer (Bio-Rad Laboratories, Hercules, CA, USA) with the fast Hb A_{1c} Program and her Hb A_{1c} value was measured to 72 mmol/mol (8.7%). The chromatogram showed no additional peaks and no flags were generated (Figure 1A). Complete blood count (CBC) performed on a Sysmex XN-2000 (Sysmex Corporation, Kobe, Japan) and biochemical parameters analyzed on a Roche Cobas 8000 (Roche Diagnostics, Mannheim, Germany) showed a nearly compensated hemolytic anemia (Table 1). The patient was referred to the Department of Endocrinology at Aalesund Hospital for assessment of new-onset diabetes mellitus. There, Hb A_{1c} control measurement was performed with an immunological method on a DCA 2000 Analyzer (Siemens AG, München, Germany). In contrast to the Bio-Rad D10™ instrument, the DCA 2000 Analyzer showed a Hb A_{1c} value in the lower range of the reference interval, 22 mmol/mol (4.2%) (reference interval 20-42 mmol/mol, 4.0%-6.0%). Hemoglobin variants are known to interfere with different Hb A_{1c} analysis methods; hence, a blood sample was sent to the Department of Medical Biochemistry at Oslo University Hospital for hemoglobinopathy evaluation. Hemoglobin HPLC performed with the β-Thalassemia Short Program (VARIANT-II Bio-Rad Laboratories, Hercules, CA, USA) showed a Hb pattern with normal Hb A and Hb A_2. Importantly, the chromatogram showed a broadened P2 peak, suggesting the presence of a Hb variant (Figure 1C). Multiplex gap-PCR detecting the seven most common deletions causing α-thalassemia [6] was negative (data not shown) and hematological data analyzed with XN-9000 Analyzer (Sysmex Corporation) confirmed a mild anemia and a moderately increased number of reticulocytes (data not shown). Measurement of Hb A_{1c} by affinity chromatography on Premier Hb9210 (Trinity Biotech Plc, Co Wicklow, Ireland) showed the same Hb A_{1c} value as for DCA 2000 Analyzer, 22

mmol/mol (4.2%), strongly suggesting a falsely high Hb A_{1c} measurement by the Bio-Rad D10™ instrument.

To identify the Hb variant, sequencing of the α- and β-globin genes was conducted as described elsewhere [7]. Sequence data was analyzed using SeqScape Software Version 2.7 (Thermo Fisher Scientific, Waltham, MA, USA) and Alamut Visual version 2.4 (Interactive Biosoftware, Rouen, France). Sequencing of the α-globin genes revealed a heterozygous deletion of seven nucleotides in the HBA2 gene, creating a frameshift in codon 133 (HbVar nomenclature) with the new reading frame ending in a premature termination codon (PTC) two positions downstream (Figure 2). The novel variant was designated NM_000517.4(*HBA2*):c.400_406del, p.(Ser134Cysfs*2) by the HGVS recommendations and *HBA2*:c.400_406delAGCACCG, alpha2 133(H16) modified terminal sequence: (133)Cys(134)COOH by the HbVar nomenclature. Hb Aalesund was named after the location of the local hospital where it was first suspected and it was entered into the HbVar database [1] (HbVar ID 3179). Sequencing of the β-globin gene showed no sequence variants that could contribute to the phenotype of the patient.

Normally, transcripts that contain a PTC undergo nonsense-mediated decay (NMD) and rapidly degrade, thus eliminating abnormal transcripts that could have a dominant negative effect [8, 9]. If the PTC is located in the last exon or in the 3' end of the penultimate exon, less than 50-55 base pairs from the final intron, the PTC will escape the surveillance system and a truncated protein will be translated [9]. The PTC in Hb Aalesund occurred in the last exon of HBA2, thus most likely would bypass NMD and a shortened α-globin chain was synthesized. The interaction between heme and the amino acids inside the heme pocket are of paramount importance both for the stability and for the function of the Hb molecule [10]. The truncated α-chain in Hb Aalesund was missing leucine in position 136 (H19), which is one of the amino acids lining the interior of the heme pocket [11]. This might affect the stability of the protein. Unfortunately, we were not able to perform isopropanol stability test. The patient showed a rather well balanced hemolytic anemia with a moderately increased number of reticulocytes and low concentration of haptoglobin. There was no sign of thalassemia (Table1). Any condition that shortens the lifespan of the erythrocytes will tend to reduce Hb A_{1c} concentration, since Hb A_{1c} generation is a slow and irreversible process [12]. This is consistent with the relatively low Hb A_{1c} result obtained by affinity chromatography and immunological method in samples from the patient. In cases of altered erythrocyte turnover, an alternative, non-Hb-based method, such as fructosamine assay or glycated albumin, may be useful for assessing long-term glycemic control [13]. There are numerous reports in the literature of Hb variants that interfere with different Hb A_{1c} methods [5, 14-17]. Thus, it is important to be aware of the potential limitations of each method, especially in cases of discrepancy between Hb A_{1c} results and other findings. Unlike inherited Hb disorders like thalassemia and the common Hb variants, a significant proportion of the unstable Hb variants are de novo variants and

restricted to a single pedigree [2]. Approximately 150 unstable Hb variants have been described in patients from all parts of the world [1]. Previously, two unstable β-globin variants, Hb Sogn (*HBB*:c.44T>G) [18] and Hb Oslo (*HBB*:c.127T>A) [7], were found in patients of Norwegian origin. The latter, affecting the heme pocket, was associated with marked hemolytic anemia and low oxygen saturation [7]. The present study is the first report of an unstable α-globin variant in a Norwegian patient causing mild hemolytic anemia in the heterozygous state. Unstable Hb variants, although uncommon, should always be a consideration in cases of undefined congenital hemolytic anemia.

Table 1. Hematological and biochemical data retrieved from Department of Medical Biochemistry at Aalesund Hospital

Parameter	Proband	Reference interval
Sex-Age (Years)	F-33	
Hb (g/dL)	11.4	11.7-15.3
RBC (10^{12}/L)	3.9	3.9-5.2
MCH (pg)	29	27-33
MCV (fL)	89	82-98
RDW (%)	13.6	0-14.8
Reticulocytes (10^9/L)	140	21-82
Ferritin (µg/L)	12	11-164
LD (U/L)	190	105-205
Bilirubin (µmol/L)	14	0-21
Haptoglobin (g/L)	<0.1	0.3-2.0

Figure legends

Figure 1. Chromatograms of the patient's sample and a normal control sample analyzed with different HPLC methods. Shown are the Bio-Rad D10™ Hemoglobin Analyzer chromatograms of the patient´s sample and a normal control sample (A and B, respectively) and the Bio-Rad Variant II chromatogram using the β-Thalassemia Short Program of the patient´s sample and a normal control sample (C and D, respectively). In (A) Bio-Rad D10™ Hemoglobin Analyzer, the patient sample shows a normal chromatogram, with no sign of an abnormal Hb or its derivatives. In (C), Hb Aalesund is seen as a broadened P2 peak, indicated with red arrows.

Figure 2. DNA sequencing of the HBA2 gene. (A) Sequencing revealed a heterozygous deletion of seven nucleotides, AGCACCG, in exon three of the *HBA2* gene (NM_000517.4). (B) The deletion creates a frameshift in codon 133 and the new reading frame ends in a termination codon two positions downstream.

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