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Letter to the Editor

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Hemoglobin variants found in relation to HbA_{1c} testing: high occurrence of Hb Athens-Georgia in the Northern Jutland, Denmark

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To the Editor,

Hemoglobinopathies are usually divided into those caused by an impaired synthesis of one or more hemoglobin chains (α-, β- and γ-thalassemia) and those caused by inherited structural alteration of one of the globin chains (hemoglobin variants). Heterozygotes of a mutation related to the hemoglobin synthesis in most cases will be asymptomatic, whereas homozygosity – especially for α-thalassemia, β-thalassemia, and certain hemoglobin variants – will result in serious clinical conditions. Mutations related to the hemoglobin synthesis are very common. Some mutations are endemic in certain geographic regions (i.e. Hb E and α-thalassemia in the Southeastern Asia, Hb S and Hb C in Central and Western Africa, and β-thalassemia in the Eastern Mediterranean countries), whereas the more uncommon mutations are found all over the world [1]. More than 450 mutations causing α- or β-thalassemia and more than 1300 mutations causing hemoglobin variants have so far been reported in the Database of Human Hemoglobin Variants and Thalassemias [2]. Probably, around 270 million people carry variant β-globin genes [1]. Nevertheless, hemoglobinopathies have been considered rare in Caucasians.

Depending on the method used for HbA_{1c} testing, the presence of a hemoglobin variant may cause analytical interference [3–5] leading to a misinterpretation of the diabetes status of the patient concerned. Moreover, hemoglobin variants cause an increased erythrocyte turn over which by itself will result in a low HbA_{1c} value [5]. Capillary electrophoresis provides a good separation of the different hemoglobin fractions in normal blood samples [6]. It also provides a good separation in blood samples from individuals being heterozygous for HbS, HbC, HbD and HbE, with analytically valid HbA_{1c} results as a consequence [4]. Furthermore, capillary electrophoresis enables the detection of a great number of other hemoglobinopathies, including persistent HbF [7].

During an 18-month period (February 2016–July 2017) 139,000 individuals in Northern Jutland, Denmark, were routine tested by capillary electrophoresis using a Capillarys 3 Tera and the CAPI 3 HbA_{1c} kit (Sebia, France) according to the protocol provided by the manufacturer. Curves with a profile indicating the presence of a hemoglobin variant were registered in 163 of the 139,000 tested individuals of these, a complementary test for hemoglobinopathy was performed in 91 randomly selected cases (56% corresponding to a background population of 77,600). The procedure for detecting hemoglobinopathy included fractionation by ultra-performance liquid chromatography on a Waters Acquity ultra-performance liquid chromatography system (Waters Corporation, Milford, MA, USA) using a PolyCAT A column (3 µm, 1500 Å) (PolyLC Inc., Columbia, MD, USA). When either fractionation (HbA_{1c} or UPLC) was suspect for a variant hemoglobin, sequencing of the β (HBB) and/or α globin genes (*HBA1* and *HBA2*) were performed on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using BigDye v. 3.1 sequencing chemistry (Applied Biosystems). Table 1 shows the results obtained.

In total, we identified 22 different hemoglobin variants, including persistent Hb F and Hb H disease. Only 33 of the 91 individuals carried the variants Hb E, Hb S, or Hb C. In the remaining 58 cases, we identified 19 different hemoglobin variants. Thirty-three of these, all with Danish names, carried the globally uncommon variant

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Table 1: Hemoglobin variants accidentally found in relation to routine HbA_{1c} testing.

α Chain cluster	
Hb H (α thalassemia, deletion)	2
Unnamed (Thr 67 Ile; *HBA1*:c.203C>T)	1
Unnamed (Val 93 Leu; *HBA1*:c.280G>T)	1
Hb Cibeles (Gly 25 Asp; *HBA2*:c.77G>A)	1
Hb St. Tuiden (Asn 68 His; *HBA2*:c.205A>C)	1
β Chain cluster	
Hb Lepore (Δ-β thalassemia, deletion)	1
Hb F (HPFH; deletion)	2
Hb Doha (Val 1 Glu; HBB:c.5T>A)	1
Hb C (Glu 6 Lys; HBB:c.19G>A)	3
Hb S (Glu 6 Val; HBB:c.20A>T)	8
Hb J-Lens (Ala 13 Asp; HBB:c.41C>A)	1
Hb E (Glu 26 Lys; HBB:c.79G>A)	22
Hb Tacoma (Arg 30 Ser; HBB:c.93G>T)	1
Hb Athens-GA (Arg 40 Lys; HBB:c.122G>A)	33
Hb Niteroi (del CD43/45; HBB:c.130_138delGAGTCCTTT)	1
Hb Aalborg (Gly 74 Arg; HBB:c.223G>C)	2
Hb J-Iran (His 77 Asp; HBB:c.232C>G)	1
Hb Helsinki (Lys 82 Met; HBB:c.248A>T)	2
Hb Köln (Val 98 Met; HBB:c.295G>A)	1
Unnamed (Leu 105 Val; HBB:c.316C>G)	1
Hb Villejuif (Tre 123 Ile; HBB:c.371C>T)	3
Hb Hope (Gly 136 Asp; HBB:c.410G>A	2
Total	91

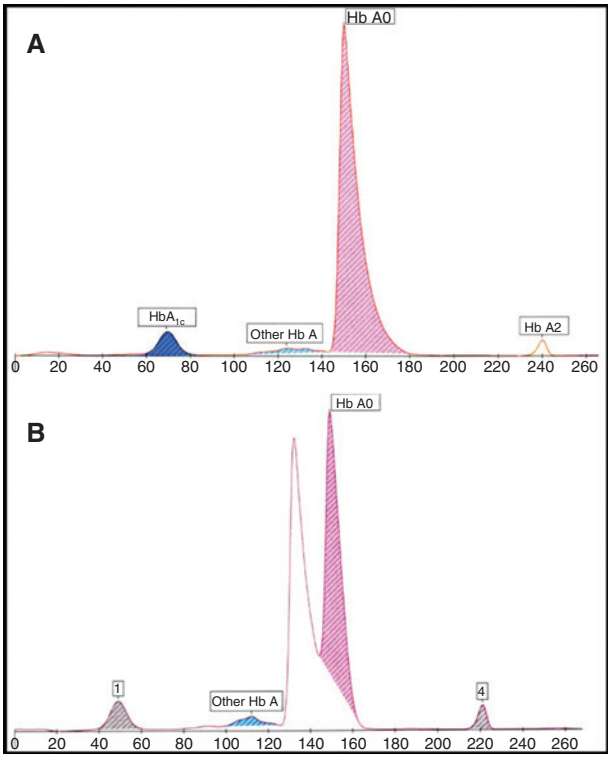

Figure 1: Electropherograms from the Capillarys 3 automated multicapillary zone electrophoresis system.
Normal HbA_{1c} electropherogram (A), and a HbA_{1c} electropherogram from a patient with the Hb variant, Hb Athens-Georgia (B).

Hb Athens-Georgia (Figure 1). Three of the detected variants (α1-chain: Thr 67 Ile, Val 93 Leu; β-chain: Leu 105 Val) were not registered in the Database of Human Hemoglobin Variants and Thalassemias [2].

Hb Athens-Georgia is considered a rare variant found in a few Caucasian families in the south-eastern USA and Belgium [2, 8], but in 1988 the variant was also found in a Danish family [9]. Hb Athens-Georgia is not detected by cation-exchange high-performance liquid chromatography, nor by boronate affinity chromatography, which are commonly used techniques for measuring HbA_{1c} [8]. The variant is clinically silent [2]. The high occurrence of Hb Athens-Georgia in Northern Jutland, corresponding to an estimated prevalence of 43:100,000 (equivalent to 33/77,600), is unexplained. The high occurrence may be due to a local founder effect or to a general underreporting due to the asymptomatic phenotype and the widespread use of cation-exchange high-performance liquid chromatography or boronate affinity chromatography for HbA_{1c} testing. The occurrence of Hb Athens-Georgia in other parts of Denmark is unknown.

Our findings confirm that the prevalence of clinically silent Hb-variants may be higher than expected so far [10]. HbA_{1c} is defined as the fraction of HbA carrying a covalently bound glucose molecule to the N-terminal valine of the β-chains. Depending on the method used, some of the hemoglobin variants do not interfere with the actual measurement of HbA_{1c} [4, 7], but some of them will, i.e. due to an increased erythrocyte turn over, interfere with the interpretation of the result obtained.

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