To the Editor

Sickle cell disease (SCD), a group of inherited disorders that affect hemoglobin (Hb), is the most common monogenic disorder and affects millions of people worldwide. In the USA, the number of people with SCD is about 100,000, and additional 3 million people carry sickle cell trait [1]. The impact of hemoglobinopathies on healthcare is considerable and has great social and economic consequences. Washington, District of Columbia (DC), with its southern Maryland and northern Virginia suburbs, is home to a large population of African Americans that include descendants of forced emigration from Africa in the trans-Atlantic slave trade and more recent immigrants. It is estimated that one in 365 American blacks has SCD, and one in 12 carries the HbS gene (sickle cell trait) [1]. Major sickle cell mutations of the β-globin gene are Glu6Val that leads to HbS [2], Glu6Lys that produces HbC [2] and Glu26Lys that generates HbE [3]. Over 1,300 Hb variants are known of which only a few are considered clinically relevant. HbSS is the most serious phenotype.

Here we estimated the frequency of the sickle cell variants in metropolitan Washington, DC, from a retrospective study of individuals who were screened at Howard University Center for Sickle Cell Disease during the period 2009 - 2018. We acquired blood samples from individuals who attended our free screening service that we provided at Howard University or through community-based outreach programs and health fairs. Participation was voluntary and no restriction was placed on age, demography, gender or ethnicity. Hemoglobin screening program was approved by Clinical Laboratory Improvement Amendments (CLIA) and we obtained written consent from participants. Retrospective analysis of the de-identified collected data was approved by the Institutional Review Board of Howard University. Blood samples were collected by minimally invasive finger prick. Testing was completed in the CLIA-certified laboratory at Howard University Center for Sickle Cell Disease. Expression level of Hb variants F, A, A2, S and C were determined by high-performance liquid chromatography (HPLC) using Ultra2 Variant System (Trinity BioTech, Jamestown, NY, USA). Samples were processed using a high-resolution method, which requires about 10 min run time per sample, thus allowing for higher sensitivity and specificity in identifying rare Hb variants. We asked participants for demographic data including age, gender, ethnic grouping and residence and contact information. Individuals whose analysis by HPLC indicated a Hb variant were contacted and offered genetic counseling. They were further given referral to a hematologist for confirmation and follow-up as needed. Inferential statistics was based on an assumption of Poisson distribution.

We analyzed blood samples from 3,677 voluntary participants. Of those participants who responded fully to the questionnaire for demographic information, 67% were female and 33% were male (Supplementary Material 1a, www.thejh.org). The majority of participants (88.8%, 2,283) identified themselves as African American or black, while 4.7% (122) identified themselves as Hispanic Americans and 6.5% (166) responded as other (Supplementary Material 1b, www.thejh.org). Eight hundred thirty-seven participants (26.7%) did not state their race. Our study participants were all adults and uniformly distributed in age from 18 to 66 years and above (Supplementary Material 1c, www.thejh.org). The median (interquartile range) age was 48 (33 - 59) years. About half of the participants (52.8%, 1,785) lived in the DC, and nearly all the rest (48.7%, 1,305) resided in southern Maryland. A small number (6.1%, 206) were from counties in northern Virginia (Supplementary Material 1d, www.thejh.org).

As expected, the predominant majority of the screened...
population had normal adult Hb (HbAA, 86.5%, 3,182, Table 1). This was the case in both men and women (85.2% and 87.4%, respectively) (Supplementary Material 2a, www.thejh.org). The most frequent Hb variant was HbAS (9.8%, 3,182, Table 1). The second most prevalent Hb variant was HbAC (2.7%, 98, Table 1), about one-third of HbAS frequency. We identified 14 cases of SCD: HbSC (6), HbSS (4), HbS β+ thalassemia (2), HbSF (1), and HbS α-thalassemia (1) (Table 1). There were also three cases of HbCC (Table 1). Additional Hb variant cases included HPFH (6), HbA G-Philadelphia (3), HbAE (2), HbAF (2), HbA N-Baltimore (2), β+ thalassemia (2), HbA prime (1), Hb Hekinan (1), Hb Trenton (1) and Hb Osu-Christiansborg (1) (Table 1). The distribution of Hb genotype was even within statistical errors across age and gender group (Supplementary Materials 2a, b, www.thejh.org). By race, there is significant difference in the incidence of HbAS as well as HbAC between blacks, Hispanic Americans and other races (Supplementary Material 2c, www.thejh.org). Among the 2,296 subjects who identify themselves by race, the frequency of HbAS was 10.9% among blacks and 5.5% among Hispanic Americans; the latter is unexpectedly high. The corresponding numbers for HbAC were 3.3% and 1.8% (Supplementary Material 2c, www.thejh.org). Prevalence of the genotypes observed so far is reproduced evenly by geographic subdivision within the metropolis (Supplementary Material 2d, www.thejh.org). The prevalence of HbAS and HbAC traits for all participants was 12.5% (Table 1). Total incidence of abnormal Hb variants in this cohort is 13.5%. A limitation of our study is that the sample cohort was not random, but rather self-select- ed. Nevertheless, it allows us to capture the distribution of Hb variants in Greater Washington, DC area.

The frequency of the sickle cell trait (HbAS, 9.8%) was higher than expected from previous study conducted at Howard University in mid-1980s where the prevalence of HbAS was 6.7% [4].

This is an increase in 46% which may reflect an increase in the number of immigrants from Africa in the Washington DC area. This is also much higher than recently reported 7% in black Americans, 0.5% in Hispanics and 0.2% in whites among USA military recruits [5]. Presence of two individuals with HbAE and one individual with rare Hb Hekinan suggests the increased immigration of Asians to DC or admixture.

Taken together, the increased prevalence of sickle cell trait and appearance of rare trait variants underscores the need to increase the availability of hemoglobinopathy screening in the general population, as well as provide resources for a growing population of adults with SCD and other hemoglobinopathies.

### Supplementary Material

**Suppl 1.** Demographic Description of Respondents.

**Suppl 2.** Genotype Distribution by Gender, Race, Age, and Geographic Subdivision.

### Table 1. Hemoglobin Variants

| Hb type                  | Description                                                                 | Total (%)            |
|-------------------------|----------------------------------------------------------------------------|----------------------|
| HbAA                    | Normal Hb                                                                  | 3,182 (86.538%)     |
| HbAS                    | Sickle cell trait                                                          | 360 (9.791%)         |
| HbAC                    | HbC carrier                                                                | 98 (2.665%)          |
| HbSC                    | Hb S-C disease                                                             | 6 (0.163%)           |
| HbAHPFH                 | High HbF                                                                   | 5 (0.135%)           |
| HbSS                    | SCD                                                                        | 4 (0.109%)           |
| HbCC                    | Hb C disease                                                               | 3 (0.082%)           |
| HbA G-Philadelphia α-thalassemia | α-chain variant and α-globin gene deletion                              | 3 (0.082%)           |
| HbAE                    | HbE carrier                                                                | 2 (0.054%)           |
| HbAF                    | Normal HbA with high HbF                                                  | 2 (0.054%)           |
| HbA N-Baltimore          | β-chain variant                                                            | 2 (0.054%)           |
| HbA β+ thalassemia      | β-globin gene deletion carrier                                            | 2 (0.054%)           |
| HbS β+ thalassemia      | β-globin gene deletion and HbS                                              | 2 (0.054%)           |
| HbSF                    | HbS with high HbF                                                         | 1 (0.027%)           |
| HbA2' (A2 prime)        | δ-chain variant                                                            | 1 (0.027%)           |
| Hb Hekinan              | α-chain variant                                                            | 1 (0.027%)           |
| Hb Trenton              | α-chain variant                                                            | 1 (0.027%)           |
| Hb A Hb Osu-Christiansborg | β-chain variant                                            | 1 (0.027%)           |
| HbS α-thalassemia       | SCA and α-globin gene deletion                                            | 1 (0.027%)           |
| Total                   |                                                                           | 3,677 (100%)         |

Hb: hemoglobin; SCD: sickle cell disease; SCA: sickle cell anemia.
Acknowledgments

None to declare.

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Conflict of Interest

None to declare.

Informed Consent

We obtained written consent from participants of CLIA-approved hemoglobin screening program at Howard University Center for Sickle Cell Disease. Retrospective analysis of the de-identified collected data was approved by the Institutional Review Board of Howard University.

Author Contributions

XN, TA, and JSA conducted HPLC analysis and maintained CLIA certification. SMN and CSP analyzed the data and CSP wrote the original draft of the manuscript. XM, AM, PAO, and SN obtained informed consents and collected blood samples. BH reported the results back to patients. PAO, VGR, JGT, and SN supervised the hemoglobin testing laboratory, helped to maintain CLIA certification, and revised the manuscript. SN conducted the overall supervision of the study and finalized the manuscript.

Data Availability

The authors declare that data supporting the findings of this study are available within the article and its supplemental materials. Any additional data are available upon the request to the corresponding author.

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