Non-S Sickling Haemoglobin Variants: Historical, Genetic, Diagnostic and Clinical Perspectives

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Abstract

Apart from Haemoglobin-S (HbS), there are other Hb variants (non-S sickling Hb variants) that cause sickle cell disease. However, the profiles of these non-S sickling Hb variants have neither been collated nor harmonised. Literature search in Pubmed (and other search engines) revealed 14 non-S sickling Hb variants *viz:* HbC-Harlem, HbC-Ziguinchor, HbS-Travis, HbS-Antilles, HbS-Providence, HbS-Oman, HbS-Cameroon, HbS-South End, Hb Jamaica Plain, HbC-Ndjamena, HbS-Clichy, HbS-San Martin, HbS-Wake and HbS-São Paulo. Generally, the non-S sickling Hb variants are double mutants that have the HbS mutation (GAG>GTG: βGlu6Val) and additional β-chain mutations. Consequently, non-S sickling Hb variants give positive solubility and sickling tests, but they differ from HbS with respect to stability, oxygen affinity and electro-chromatographic characteristics. Similarities and discrepancies between HbS and non-S sickling Hb variants create diagnostic pitfalls that can only be resolved by elaborate electro-chromatographic and/or genetic tests. It is therefore imperative that tropical haematologists should have thorough understanding of these atypical sickling Hb variants. Collated and harmonised appraisal of the non-S sickling Hb variants have not been previously under-taken. Hence, the aim of this paper is to provide comprehensive but concise historical, genetic, comparative, diagnostic and clinical overview of non-S sickling Hb variants. The elaborate techniques that are often required for precise diagnosis of non-S sickling Hb variants are regrettably not readily available in low resource tropical countries, which paradoxically carry the heaviest burden of sickling disorders. In order to avoid misdiagnosis of these atypical Hb mutants it is strongly recommended that tropical countries should upgrade their diagnostic laboratory facilities.

Keywords: Non-S Sickling; Haemoglobin Variants; Sickle Cell Disease

Introduction

Haemoglobin-S (HbS) is a variant of HbA that arose as a result of GAG>GTG base transition at codon-6 of the β-globin gene on chromosome-11, which corresponds to substitution of glutamic acid (polar amino acid) by valine (neutral amino acid) in position-6 of the β-globin chain (βGlu6Val).[1,2] Consequently, HbS has less anionic potential, slower electrophoretic mobility and reduced deoxygenated solubility that leads to polymerization and red cell sickling.[1,2] Prevalence of sickle β-gene in Nigeria and other tropical countries is as high as 25-30% or even higher.[3] This is because the sickle cell trait (SCT) protects against severe malaria[3] and confers survival advantage through natural selection[4], balanced polymorphism[5] and immunological and biochemical protective mechanisms against malaria parasite.[6] There are at least five different sickle β-gene mutation haplotypes. The Arab-Asian and Senegal haplotypes are associated with higher HbF levels and mild sickle cell disease (SCD), while the Benin, Bantu and Cameroon haplotypes are associated with lower HbF levels and severe SCD.[7] Despite these haplotypic variations, the diagnostic presence of HbS in persons with SCT or SCD is uniformly based on two fundamental principles: physicochemical and electrochemical principles. The physicochemical principle is based on insolubility and polymerization of deoxy HbS, which forms the pathophysiologic basis for Hb solubility and red cell sickling tests.[8] The electrochemical principle is based on charge-mobility alterations in HbS, which forms the pathophysiologic basis for Hb electrophoresis, iso-electric focusing and chromatography.[8]

Sickle cell disease arises as a result of homozygous inheritance of HbS (HbSS) or double heterozygosity with another haemoglobinopathy (e.g. HbSC, HbSD, HbSE, HbSO, HbSβthal).[1] Clinical course of SCD is characterised by painless period of relative well-being referred to as 'steady state', which is intermittently interrupted by painful periods referred to as 'vaso-occlusive crisis' (VOC).[9] Clinical transition from steady state to VOC is usually triggered by various factors that range from physiological (eg menstruation) to non-physiological (e.g. infection) factors on the one hand, and from psychological (e.g. mental stress) to physical (e.g. physical exhaustion) factors on the other hand.[9] The red cells of persons with SCT have the HbAS phenotype, thus expressing both HbS (20-40%) and HbA (60-80%).[10] The relative abundance of HbA in SCT red cells prevents sickling and undue haemolysis.[10] Hence, SCT red cells have normal life span and SCT carriers have normal life expectancy.[11] Therefore, HbS gene is genetically recessive and SCT carriers are essentially asymptomatic except for the occasional episodes of renal papillary necrosis[10], splenic infarct at high altitude[12], or bone pain upon exposure to haematopoietic growth factors.[13]

With the largest black population of about 200 million, frequency of SCT of 25-30% and prevalence of SCD of 2-3%, Nigeria is thought to carry the heaviest burden of sickling disorders in the world.[14] Since the official discovery of SCD by James B. Herrick in a black Grenadian dental student studying in the United States in 1910[15], HbS has remained the most thoroughly studied sickling Hb variant. Nonetheless, HbS is not the only Hb variant that sickles. Indeed, many sickling Hb variants other than HbS (i.e. non-S sickling Hb variants) have been discovered and reported. But the profiles of the non-S sickling Hb variants have neither been holistically collated nor chronologically harmonized. Hence, we conducted a literature search for non-S sickling Hb variants by using relevant search terms and sub-terms related to 'red cell sickling and non-S sickling haemoglobin' in Pubmed, Medline, Google Scholar and other search engines.

The Non-S sickling Hb variants

There are presently fourteen clinically significant non-S sickling Hb variants (discovered between 1966 and 2012), which include HbC-Harlem, HbC-Ziguinchor, HbS-Travis, HbS-Antilles, HbS-Providence, HbS-Oman, HbS-Cameroon, HbS-South End, Hb Jamaica Plain, HbC-Ndjamena, HbS-Clichy, HbS-San Martin, HbS-Wake and HbS-São Paulo as collated in this report. Similar to HbS, all non-S sickling Hb variants have normal alpha chains. However, the non-S sickling Hb variants differ from HbS because in addition to the HbS gene mutation, each one of them also has an additional single point mutation in the β-globin chain gene cluster on chromosome-11 (2 point mutations on β-globin gene). Consequently, the non-S sickling Hb variants give positive solubility and sickling tests in similarity with HbS, but they may differ from HbS with respect to stability, oxygen affinity and electro-chromatographic patterns.[16] These discrepancies often produce a perplexing asynchrony between clinical features and laboratory findings thus creating diagnostic pitfalls in the evaluation of patients affected by non-S sickling Hb variants. The diagnostic puzzles that shroud the non-S sickling Hb variants may therefore only be completely resolved by a combination of simple and complex diagnostic techniques including red cell sickling, Hb solubility, stability and oxygen affinity tests, Hb tetramer and globin chain electrophoresis, iso-electric focusing, high performance liquid chromatography (HPLC) and genetic studies. Although the non-S sickling Hb variant mutations are rare, they are nonetheless clinically significant for two reasons. First, some non-S sickling variant mutations (e.g. HbS-Oman, HbS-Antilles, HbS-Jamaica Plain, HbS-São Paulo) can cause SCD even in the heterozygotes and are thus sometimes referred to as 'dominant or super-sickling Hb variants'.[17,18] Second, any non-S sickling variant mutation can cause SCD if it is inherited as homozygous or co-inherited in double heterozygosity with other haemoglobinopathy mutations such as HbS, HbC, HbE, HbD, HbO or β-thalassemia.[19] The non-S sickling Hb variants should be kept in the mind of haematologists practicing in areas with high prevalence of sickling disorders (tropical Africa, Mediterranean and Asian countries) because they may cause diagnostically perplexing SCD. Hence, the need for thorough understanding of these atypical sickling Hb variants. To the best of our knowledge, a collated and holistic appraisal of the various non-S sickling Hb variants has not been previously under taken. Therefore, the aim of this paper is to provide a comprehensive but concise overview of the historical, genetic, comparative, diagnostic and clinical perspectives of the non-S sickling Hb variants as outlined in Table-1.

Table 1: Chronological Overview of Non-S Sickling Haemoglobin Variants.

Serial No.	HAEMOGLOBIN	FIRST REPORT (References)	AMINO ACID SUBSTITUTIONS (CODON MUTATIONS)	GENETIC AND CLINICAL EXPRESSION
1	HbC-Harlem	Bookchin et al, 1966	βGlu6Val (GAG>GTG), βAsp73Asn (GAT>AAT)	Recessive. Causes SCD if coinherited with other abnormal haemoglobins.
2	HbC-Ziguinchor	Goossens et al, 1975	βGlu6Val (GAG>GTG), βPro58Arg (CCT>CGT)	Recessive. Causes SCD if coinherited with other abnormal haemoglobins.
3	HbS-Travis	Moo-Penn et al, 1977	βGlu6Val (GAG>GTG), βAla142Val (GCC>GTC)	Recessive. SCD due to homozygous or double heterozygous inheritance not reported.
4	HbS-Antilles	Monplaisir et al, 1986	βGlu6Val (GAG>GTG), βVal23Ile (GTT>ATT)	Dominant. Also causes severe SCD if coinherited with other abnormal haemoglobins.
5	HbS-Providence	Gale et al, 1988	βGlu6Val (GAG>GTG), βLys82Asn (AAG>AAT)	Recessive. SCD due to homozygous or double heterozygous inheritance not reported.
6	HbS-Oman	Langdown et al, 1989	βGlu6Val (GAG>GTG), βGlu121Lys (GAA>AAA)	Dominant. Also causes severe SCD if coinherited with HbS.
7	HbS-Cameroon	Bundgaard et al, 2004	βGlu6Val (GAG>GTG), βGlu90Lys (GAG>AAG)	Recessive. SCD due to homozygous or double heterozygous inheritance not reported.
8	HbS-South End	Luo et al, 2004	βGlu6Val (GAG>GTG), βLys132Asn (AAA>AAC)	Simple heterozygote not reported. SCD due to double heterozygous HbS/HbS-South End reported in index case.
9	Hb Jamaica Plain	Geva et al, 2004	βGlu6Val (GAG>GTG), βLeu68Phe (CTC>TTC)	Dominant. Would cause severe SCD if coinherited with other haemoglobinopathies.
10	HbC-Ndjamena	Ducrocq et al, 2006	βGlu6Val (GAG>GTG), βTrp37Gly (TGG>GGG)	Recessive. Two cases of SCD due to double heterozygous HbS/HbC-Ndjamena reported.
11	HbS-Clichy	Zanella-Cleon et al, 2009	βGlu6Val (GAG>GTG), βLys8Thr (AAA>ACA)	Recessive. Homozygous or double heterozygous SCD not reported
12	HbS-San Martin	Feliu-Torres et al, 2010	βGlu6Val (GAG>GTG), βLeu105Pro (CTC>CCC)	Dominant for haemolysis but recessive for pain. Homozygous or double heterozygous SCD not reported
13	HbS-Wake	Kutlar et al, 2010	βGlu6Val (GAG>GTG), βAsn139Ser (AAT>AGT)	Recessive. One case of SCD due to double heterozygous HbS/HbS-Wake reported. Homozygous not reported.
14	HbS-São Paulo	Jorge et al, 2012	βGlu6Val (GAG>GTG), βLys65Glu (AAG>GAG)	Dominant. Would also cause severe SCD if coinherited with other abnormal haemoglobins.

1. Haemoglobin C-Harlem (α2β2: βglu6val, βasp73asn)

HbC-Harlem is historically the first non-S sickling Hb variant to be described. It was reported by Bookchin et al in 1966 among African Americans living in Harlem.[20] The mutant gene contains both the HbS mutation (βGlu6Val) and another mutation in the same β-globin gene (βAsp73Asn).[20] The mutation βAsp73Asn is also associated with Hb Korle-Bu, a well characterized clinically innocuous Hb that electrophoretically migrates like HbS, but does not sickle.[21] Hence, it is highly likely that a chromosomal crossing over between HbS gene and Hb Korle-Bu gene was responsible for the production of HbC-Harlem gene, which consequently contains both βGlu6Val and βAsp73Asn mutations.[20,21] These double mutations were also described in a Hb variant that was previously designated as HbC-Georgetown, which was subsequently identified to be identical to HbC-Harlem.[22] Like other non-S sickling Hb variants, HbC-Harlem gives positive sickling and solubility tests, which are pathophysiologic reflections of the βGlu6Val mutation.[16] Nonetheless, HbC-Harlem has relatively lower thermal and mechanical stability as compared with HbS.[23] Moreover, HbC-Harlem migrates like HbC in alkaline electrophoresis, hence HbSC-Harlem disease can easily be misdiagnosed as HbSC disease as previously reported in the literature.[24] The similarity in alkaline electrophoretic mobility between HbC and HbC-Harlem makes it possible to misdiagnose 'HbCC ' as 'HbCC-Harlem' if alkaline electrophoresis is used as the sole diagnostic technique.[25] But this misdiagnosis and diagnostic pitfall can be clinically clarified by the presence of vaso-occlusive crisis (VOC) in HbCC-Harlem, whereas HbCC disease does not cause VOC.[25] Diagnostic issues for HbC-Harlem can be further clarified by more detailed investigations, which would reveal that HbC-Harlem, unlike HbC, migrates as HbS in acid pH electrophoresis, migrates as HbA2 in iso-electric focusing, and elutes with HbA2 in anion exchange chromatography.[24-26] HbC-Harlem trait is genetically recessive as the heterozygous state is essentially benign, except for the occurrence of hyposthenuria[27], which is similar to the renal manifestation of HbS trait.[10] However, when HbC-Harlem is coinherited in a double heterozygous combination with HbS or other haemoglobinopathies, the affected individuals would present as cases of severe SCD with haemolysis, recurrent VOC and/or haematuria[27-29], which are similar to the haemolytic, vaso-occlusive and renal manifestations of classical SCD.[10] Because of the rarity of HbC-Harlem, its effect on pregnancy has not been adequately studied. Nevertheless, a single case report of a pregnant woman of African descend with double heterozygous combination of HbC and HbC-Harlem suggested that HbCC-Harlem disease had adverse effects on maternal morbidity, and foetal growth and survival[30], a finding that is comparable to the effect of classical SCD on pregnancy.[31]

2. Haemoglobin C-Ziguinchor (α2β2: βGlu6Val, βPro58Arg)

HbC-Ziguinchor is the second non-S sickling Hb variant to be described after HbC-Harlem. HbC-Ziguinchor was first detected in a 40 year old African man in Dakar, Senegal by Goossens et al in 1975.[32] The β-globin gene for HbC-Ziguinchor contains both the HbS substitution mutation (βGlu6Val) and an additional second substitution mutation (βPro58Arg).[32] The second substitution mutation (βPro58Arg) had previously been described in association with Hb-Dhofar (discovered in a South Arabian Veddoid from the Qara mountains of Dhofar), and in association with Hb-Yukuhashi (discovered in a Japanese individual).[33] Both Hb-Dhofar and Hb-Yukuhashi have since been regarded as one and same Hb variant since they are genetically identical and phenotypically similar even though the two index cases were discovered in two different racial settings (i.e. Arab and Japanese races).[33] It is therefore presumed that HbC-Ziguinchor might have arisen as a result of chromosomal crossing-over involving the HbS gene and Hb-Dhofar/Yukahashi gene.[32] HbC-Ziguinchor does not have any abnormalities in either its heat stability or isopropanol solubility, but it retains the fundamental physical abnormalities of HbS (i.e. sickling, gelation and relative insolubility in its deoxy form).[32,34] HbC-Ziguinchor is clearly distinguishable from HbS as it migrates slightly cathodal to both HbA and HbC in alkaline cellulose acetate electrophoresis.[32,34] However, in acidic agar gel electrophoresis, HbC-Ziguinchor is indistinguishable from HbS, and it elutes after HbA2 in anion exchange chromatography.[32,34] Diagnostic issues regarding this abnormal Hb variant can be further resolved by globin chain electrophoresis, which consistently reveals that the β-globin chain of HbC-Ziguinchor moves more anodally than that of HbC in both alkaline and acidic media.[32,34] The clinical features of HbC-Ziguinchor has not been adequately evaluated because of the rarity of the variant. However, a limited clinical experience had shown that HbC-Ziguinchor trait is genetically recessive as the heterozygote does not present any haematological or clinical manifestation and is completely asymptomatic.[35] Conversely, when HbC-Ziguinchor is coinherited with HbS or other haemoglobinopathies such as HbC or β-thalassemic trait, the affected patients present with severe haemolysis and VOC that are indistinguishable from classical forms of SCD.[35] Moreover, a single case report of coinherited HbC-Ziguinchor and hereditary persistence of foetal Hb foetal (HPFH) revealed an elevated level of HbF with commensurate reduction in the frequency of VOC.[36]

3. Haemoglobin S-Travis (α2β2: βglu6val, βala142val)

HbS-Travis is the third non-S sickling Hb variant to be described after HbC-Harlem and HbC-Ziguinchor. HbS-Travis was first discovered in five members of a Black family in Travis, USA as reported by Moo-Penn et al in 1977.[37] All of the five studied family members were heterozygotes, and genetic analysis revealed that HbS-Travis has two amino acid substitutions in the β-globin chain that include βGlu6Val and βAla142Val.[37], which most probably arose as a result of genetic crossing over between HbS gene (βGlu6Val) on chromosome-11 and another chromosome carrying the βAla142Val mutation.[37] Electrophoretically, HbS-Travis moves to a position between HbS and HbF at alkaline pH, but at acidic pH it moves between HbA and HbS.[37] Nonetheless, HbS-Travis and HbA can be separated by anion exchange chromatography as it elutes immediately before HbA.[37] Functional studies on HbS-Travis revealed that in addition to insolubility, polymerization and sicklability of the deoxy form, HbS-Travis has significantly decreased affinity for 2,3-diphosphoglycerate (2,3-DPG) with a commensurate increase in oxygen affinity.[37] HbS-Travis is therefore functionally a high oxygen affinity Hb variant.[37] Moreover, HbS-Travis tends to undergo auto-oxidation and is therefore relatively unstable.[37] The mean gelling concentration of HbS-Travis is similar to that of HbS, but the quantity of HbS-Travis in the heterozygote (HbS-Travis trait) is considerably low at about 14% (cf. about 40% HbS in HbS trait), hence HbS-Travis heterozygotes are essentially asymptomatic and the trait is thus genetically recessive.[37] Sickle cell disease due to HbS-Travis homozygosity or its coinheritance (double heterozygosity) with HbS or other haemoglobinopathies have not been seen or reported in the literature to date. It may nevertheless be presumed that HbS-Travis SCD (if found in the future) would be generally mild to moderate in severity because HbS-Travis has high oxygen affinity[37] and would predictably simulate the anti-sickling effect of HbF[38] by 'auto-protecting' itself from excessive desaturation, polymerization and sickling. However, this prediction can only be ascertained if and when HbS-Travis SCD is eventually discovered in any patient in the future.

4. Haemoglobin S-Antilles (α2β2: βGlu6Val, βVal23Ile)

HbS-Antilles is the fourth non-S sickling Hb variant to be described in the literature, and it was first found in members of a family from Martinique in French West Indies as reported by Monplaisir etal in 1986.[39] HbS-Antilles is a double mutant with two amino acid substitutions in the β-globin chain, which includes βGlu6Val and βVal23Ile that most probably arose as a result of genetic crossing over between HbS gene (βGlu6Val) on chromosome-11 and another chromosome carrying the βVal23Ile mutation.[39] It was later revealed by chromosomal analysis that the βVal23Ile mutation initially occurred on another chromosome-11 bearing β-globin gene of the Benin type haplotype that was subsequently justa-positioned to the HbS gene (βGlu6Val), thus generating the double mutant HbS-Antilles.[40] The electrophoretic mobility of HbS-Antilles is identical to that of HbS in standard alkaline medium, but slightly slower than HbS in iso-electric focusing.[39] HbS-Antilles and HbA can be readily separate by chromatography.[39] Functional studies on HbS-Antilles shows normal Bohr effect, but the oxygen affinity of HbS-Antilles is significantly decreased and the solubility of deoxy HbS-Antilles is considerably less than that of deoxy HbS.[39] Consequently, HbS-Antilles gives stronger positive sickling test.[39] The quantity of HbS-Antilles in the heterozygotes was between 40% and 50%.[39] In comparison with HbS, HbS-Antilles has higher quantity in heterozygotes, lower oxygen affinity, greater insolubility in the deoxy form, higher polymerization tendency and faster sickling rate.[39] Consequently, unlike HbS trait, HbS-Antilles trait is associated with symptoms of haemolysis and vaso-occlusive pain consistent with mild to moderate SCD, which makes HbS-Antilles a genetically dominant trait.[39] Homozygous HbS-Antilles SCD has not been reported in the literature to date. But severe SCD can occur if HbS-Antilles is coinherited with HbS or other haemoglobinopathies. For example severe SCD had occurred when HbS-Antilles was coinherited in combination with HbC and HbS.[39] The haematological and pathological effects of HbS-Antilles were later studied in detail by using transgenic mice models produced by genomic insertions of HbS-Antilles gene to produce a simple heterozygote (HbAS-Antilles)[41] or in combination with HbS gene to produce a double heterozygote (HbSS-Antilles).[42] Both transgenic models exhibited haematological parameters and multiple organ damage pathology that were quite similar to the haematological and pathological features of classical SCD as reported in humans.[41,42] The haematological anomalies and painful symptoms that were documented in the simple heterozygous index patients (HbS-Antilles traits)[39], simple heterozygous transgenic mice[41] and double heterozygous (HbSS-Antilles) mice[42] have confirmed the dominant nature of HbS-Antilles trait and its ability to cause SCD in both heterozygous and double heterozygous individuals.

5. Haemoglobin S-Providence (α2β2: βGlu6Val, βLys82ASn/Asp)

HbS-Providence is the fifth non-S sickling Hb variant to be described. It was discovered in a woman of west African descent by Gale et al in 1988.[43] HbS-Providence is a double mutant as genetic analysis of the affected chromosome-11 revealed the presence of HbS mutation (βGlu6Val) and a second β-chain mutation (Hb-Providence mutation: βLys82Asn/Asp).[43] The Hb-Providence mutation primarily synthesizes 'βLys82Asn' globin chain but the 'Asn' is post-synthetically de-amidated to 'Asp' to produce 'βLys82Asp' globin chain.[44] The lysine residue at position-82 is important in 2,3-diphosphoglycerate binding, hence Hb-Providence is a high oxygen affinity variant that exists in two phenotypic forms and the mutation is thus conventionally designated as 'βLys82ASn/Asp'.[44] Although relatively rare, Hb-Providence mutation has been described across racial barriers (probably due to inter-racial marriages) as the initially index cases were found in persons of African descent[44], and subsequent cases were found in persons of apparently pure European descent.[45] On the basis of genetic permutations and probabilities, HbS-Providence might have arisen as a result of crossing over between chromosomes that carry the HbS and Hb-Providence genes.[43] Although HbS-Providence exhibits positive sickling and solubility tests, it is nonetheless indistinguishable from HbA by routine electrophoresis at either alkaline or acidic pH.[43] Nevertheless, the β-globin chains of HbA, HbS and HbS-Providence can be separated and identified by further testing with chromatography.[43] Therefore any case of positive sickling or solubility test that presents as HbAA on electrophoresis must be considered as a possible case of HbS-Providence and be subjected to further investigations. The heterozygous index case of HbS-Providence as described by Gale et al had less than 50% HbS-Providence and was haematologically and clinically normal, which suggested that HbS-Providence mutation is a recessive trait.[43] Sickle cell disease due to homozygous or double heterozygous inheritance of HbS-Providence with HbS have not been reported. However, it can be presumed that HbS-Providence SCD (if seen in the future) would be generally mild to moderate in severity because HbS-Providence has functional impairment of 2,3-DPG binding with resultant high oxygen affinity.[46] In similarity to other high affinity non-S sickling Hb variants such as HbS-Travis (see earlier sections), HbS-Providence would be expected to ‘auto-protect’ itself from excessive desaturation, polymerization and sickling if and when inherited as homozygous or double heterozygous SCD in the future.

6. Haemoglobin S-Oman (α2β2: βGlu6Val, βGlu121Lys)

HbS-Oman is the sixth non-S sickling Hb variant to be described in the literature, and it was first found in an Omani Arab as reported by Langdown et al in 1989.[47] Genetic analysis revealed that HbS-Oman is a double mutant that carries two mutations (βGlu6Val and βGlu121Lys) in the β-globin chain.[47] Because both valine and lysine are non-polar amino acids, HbS-Oman incurs an aggregate loss of four negative charges per Hb molecule and is thus electrophoretically slower than HbS, which has an aggregate loss of only two charges per Hb molecule.[47] The βGlu121Lys mutation had previously been described in HbO-Arab.[48] Hence, HbS-Oman most probably arose as a result of genetic crossing over and gene translocation between two chromosomes: one chromosome carrying the HbS gene (βGlu6Val) and the other chromosome carrying the HbO-Arab gene (βGlu121Lys), resulting in the production of HbS-Oman chromosome, which carries both genes (HbS [βGlu6Val] and HbO-Arab [βGlu121Lys]).[47] The quantity and proportion of HbS-Oman in the heterozygote red cell is affected by the coinheritance of alpha thalassemia trait.[17,18] Hence, the proportion of HbS-Oman in the heterozygote red cells is 14-20%, and heterozygotes with concurrent alpha thalassemia trait tend to have lower quantity of HbS-Oman in their red cells.[17,18] In similarity with HbS-Antilles[39], the solubility of the deoxy HbS-Oman is much lower than that of deoxy HbS[47], which makes both haemoglobin variants (HbS-Antilles and HbS-Oman) to have stronger (as compared with HbS) sickling tendencies and behave as ‘super-sickling’ variants with ‘dominant’ haemolytic and vaso-occlusive clinical manifestations even in the heterozygotes.[17,18,39,47] Moreover, HbS-Oman is particularly highly sicklable even in the heterozygote because the effect of the sickle mutation (βGlu6Val) is enhanced by the adjacent HbO-Arab mutation (βGlu121Lys), which is known for its capability to cause red cell dehydration and raise mean corpuscular haemoglobin concentration.[48] Despite having positive sickling and solubility tests, HbS-Oman is slower and separates distinctly from HbS, HbA and HbF in both alkali and acid gel electrophoresis.[49,50] Nonetheless, HbS-Oman moves as HbC in alkaline and acid electrophoresis, and elutes in the same position as HbC in HPLC.[49,50] The sickled red cells seen in the peripheral blood of patients with heterozygous or double heterozygous HbS-Oman SCD have a peculiar centrally bulbous configuration that is reminiscent of the 'yarn-knitting needle' or 'Napoleon Hat', which are morphologically pathognomonic and microscopically diagnostic of HbS-Oman disease.[18,49,50] Sickle cell disease due to homozygous HbS-Oman disease has not been previously reported. Nonetheless, HbS-Oman has been known to cause severe SCD with haemolytic and vaso-occlusive clinical features if it is coinherited in double heterozygosity with HbS as previously reported.[49,50, 51]

7. Haemoglobin S-Cameroon (α2β2: βGlu6Val, βGlu90Lys)
HbS-Cameroon is the seventh non-S sickling Hb variant to be described, and it was first reported by Bundgaard et al in a Cameroonian in 2004.[52] HbS-Cameroon is a double mutant that carries two mutations (βGlu6Val and βGlu90Lys) in the β-globin chain.[52] HbS-Cameroon has only been described in the heterozygous state as reported in the index case.[52] Absence of abnormal haematological features and vaso-occlusive symptoms in the index case would suggest that HbS-Cameroon is a genetically recessive trait.[52] Nonetheless, because it bears the βGlu6Val substitution, HbS-Cameroon is electrophoretically slow and is positive by solubility and sickling tests.[52] However, HbS-Cameroon can be distinguished from HbS by chromatography.[52] Moreover, HbS-Cameroon has reduced oxygen affinity because the second mutation (βGlu90Lys) is identical to the mutation that was previously described in Hb-Agenogi (a rare variant reported in Japanese, Africans, Hungarians and Argentineans), which is associated with low oxygen affinity.[53] Hence, HbS-Cameroon is functionally a low oxygen affinity variant (as is the case with HbS-Antilles: see earlier sections), and would therefore be vulnerable to excessive desaturation and polymerization, both of which would lead to severe SCD if and when HbS-Cameroon is inherited as homozygous (HbS-Cameroon/HbS-Cameroon) or double heterozygous with HbS (HbS-Cameroon/HbS) in the future.

8. Haemoglobin S-South End (α2β2: βGlu6Val, βLys132Asn)
HbS-South End is the eighth non-S sickling Hb variant to be described in the literature. It was found in African American patient of Ugandan extraction as reported by Lou et al in 2004.[54] In similarity with other non-S sickling mutants, HbS-South End is a double mutant that carries two mutations (βGlu6Val and βLys132Asn) in the β-globin chain.[54] Because HbS-South End bears the βGlu6Val, it produces positive results by solubility and sickling tests.[54] In addition, HbS-South End mimics HbA on alkaline and acid electrophoresis (as is the case with HbS-Providence: see earlier sections) and by HPLC, but it migrates as HbF on capillary zone electrophoresis and iso-electric focusing, a situation that creates major diagnostic pitfalls unless further testing by Hb tetramer and globin chain electrophoresis or DNA and genomic analyses are undertaken.[54] The index patient presented as a case of SCD with double heterozygous coinheritance of HbS/HbS-South End.[54] Unfortunately other family members of the index case were not available for study.[54] Consequently, simple heterozygotes for HbS-South End have not been described, hence no categorical statement can be made on whether the HbS-South End mutation is a recessive or dominant trait. Moreover, patients with homozygous HbS-South End SCD have also not been reported in the literature. So the only case of HbS-South End that was reported in the literature is the compound heterozygous (HbS/HbS-South End) index case.[54] Interestingly, the index case presented as a severe case of SCD that was associated with severe haemolytic and vaso-occlusive symptoms because the second mutation (βLys132Asn) in HbS-South End is identical to the mutation in Hb Yamakata, a low oxygen affinity Hb variant that

was discovered in a Japanese family in 1990.[54,55] The amino acid substitution resulting from the βLys132Asn mutation in Hb Yamakata facilitates 2,3-DPG binding, which functionally lowers oxygen affinity, increases the rate of oxygen release and enhance desaturation.[55] Therefore, HbS-South End is functionally a low oxygen affinity variant (as is the case with HbS-Antilles and HbS-Cameroon: see earlier sections) and would thus be vulnerable to excessive desaturation and polymerization, both of which would increase red cell sickling and ultimately produce a clinically severe SCD as reported in the index patient.[54]

9. Haemoglobin Jamaica Plain (α2β2: βGlu6Val, βLeu68Phe)

Hb Jamaica Plain was the ninth non-S sickling Hb variant to described in the literature. It was found in a baby girl of Puerto Rican descent as reported by Geva et al in 2004.[56] The index patient presented with symptomatic SCD exacerbated by mild hypoxemia, despite a newborn screening that suggested the diagnosis of 'HbS trait'.[56] This is because Hb Jamaica Plain masquerades as HbS in its physico-chemical characteristics, which include positive sickling and solubility tests, slow electrophoretic mobility in alkaline and acid pH, coupled with its similarity with HbS by isoelectric focusing and HPLC.[56] However, Hb Jamaica Plain was identified in the index case by DNA sequencing of the patient's β-globin gene, which revealed that her maternal β-globin allele was normal, while her paternal allele had not only the expected HbS trait mutation (βGlu6Val), but also a second, charge-neutral mutation, (βLeu68Phe) , which the baby must have acquired through spontaneous germ-line mutation.[56] Hb Jamaica Plain is therefore a charge-neutral double-mutant (βGlu6Val and βLeu68Phe) Hb variant that is difficult to detect without DNA sequencing since it cannot be distinguished from HbS by isoelectric focusing or HPLC.[56] Hb Jamaica Plain may thus be responsible for sporadic and inexplicable cases of clinically severe vaso-occlusive symptoms in persons with presumed 'HbS trait' if only basic electrophoretic and chromatographic techniques are used for diagnosis.[56] It is noteworthy that the second mutation (βLeu68Phe) was previously reported as an isolated finding in Hb Rockford[57], now known as Hb Loves Park[58], which is associated with low oxygen affinity. Consequently, Hb Jamaica Plain is functionally a low oxygen affinity variant (as is the case with Antilles, HbS-Cameroon, HbS-South End: see earlier sections) characterised by marked decrease in oxygen affinity that is even lower than that of HbS Antilles.[39,56] This functional characteristic makes Hb Jamaica Plain to be particularly susceptible to deoxygenation, desaturation and polymerization with resultant severe and dominant clinical phenotype that manifests as severe SCD even in the heterozygous state as seen in the index case.[56] Hb Jamaica plain is therefore a member of the of 'the so-called dominant/super-sickling non-S Hb variants' with the other members being HbS-Antilles[39] and HbS-Oman[17,18,47] [see previous sections] and HbS-São Paulo [see subsequent sections].

10. Haemoglobin C-Ndjamena (α2β2: βGlu6Val, βTrp37Gly)

HbC-Ndjamena is chronologically the tenth non-S sickling Hb variant to be described. It was discovered in an Chadian patient with double heterozygous SCD due to coinheritance of HbS/HbC-Ndjamena as described by Ducrocq et al in 2006.[59] Another case of double heterozygous HbS/HbC-Ndjamena SCD was also reported in 2011 by Bouzid et al.[60] HbC-Ndjamena is a double mutant as genetic analysis of the affected chromosome-11 revealed the presence of HbS mutation (βGlu6Val) and a second β-chain mutation (βTrp37Gly).[59,60] The βTrp37Gly has earlier been reported by Owen et al in 1993 as an isolated non-sickling mutant allele in a Hb variant called Hb Howick, which is functionally characterised by impairment of

2,3 DPG binding and high oxygen affinity.[61] In similarity with HbS, HbC-Ndjamena gives positive solubility and sickling tests.[59,60] However, HbC-Ndjamena is clearly distinguishable from HbS as it migrates close to (but a little faster than) HbC in alkaline cellulose acetate electrophoresis and iso-electric focusing, and it elutes faster than HbS in HPLC.[59,60] The HbS/HbC-Ndjamena patient reported by Bouzid et al[60] inherited the HbS allele from the mother and the HbC-Ndjamena allele from the father; both parents were clinically asymptomatic, thus HbC-Ndjamena allele is a genetically recessive trait.[60] Patients with homozygous HbC-Ndjamena SCD have not been reported in the literature. Nonetheless, the two aforementioned cases of SCD due to coinheritance of HbS/HbC-Ndjamena[59,60] were associated with mild to moderate haemolytic and vaso-occlusive symptoms because the second mutation (βTrp37Gly) in HbC-Ndjamena is identical to the mutation in Hb Howick, which is associated with high oxygen affinity.[61] Hence, HbC-Ndjamena is essentially a high oxygen affinity variant (as is the case with HbS-Travis and HbS-Providence: see earlier sections) and would therefore pathophysiologically 'auto-protect' itself from excessive desaturation and polymerization, both of which would reduce red cell sickling and ultimately produce a clinically non-severe SCD as reported in the two index patients.[59,60]

11. Haemoglobin S-Clichy (α2β2: βGlu6Val, βLys8Thr)

HbS-Clichy is the eleventh non-S sickling Hb variant that was discovered in an African European, and it was first reported by Zanella-Cleon et al in 2009.[62] In similarity with other non-S sickling mutants, HbS-Clichy is a double mutant that carries two mutant substitutions (βGlu6Val and βLys8Thr) in the β-globin chain.[62] It was detected as a slow variant by routine electrophoretic techniques and HPLC in the asymptomatic heterozygous index case.[62] In consistency with the presence of βGlu6Val, HbS-Clichy produces positive solubility and sickling tests.[62] However, DNA and genomic analyses showed that in addition to the glutamic acid substitution by valine at codon 6, the lysine at codon 8 was replaced by a threonine.[62] The absence of haemolytic and vaso-occlusive symptoms in the index heterozygote suggests that HbS-Clichy allele is genetically recessive trait.[62] Nonetheless, HbS-Clichy would most probably cause clinically symptomatic SCD if and when it is inherited as homozygous or coinherited as double heterozygous with HbS or other haemoglobinopathies in the future.

12. Haemoglobin S-San Martin (α2β2: βGlu6Val, βLeu105Pro)

HbS-San Martin is the twelfth non-S sickling Hb to be described. It was discovered in a ten year old boy in an Argentinean family from San Martin, Buenos Aires, Argentina as described by Feliu-Torres et al in 2010.[63] HbS-San Martin is a double mutant characterised by the presence of one mutation on exon-1 [GAG>GTG] that corresponds to HbS [βGlu6Val] substitution, and the second mutation on exon-3 [CTC>CCC], which corresponds to βLeu105Pro substitution.[63] In addition to giving positive solubility and sickling tests, HbS-San Martin migrated in similarity with HbS as depicted by alkaline cellulose and acid pH agar gel electrophoresis.[63] Moreover, heat stability and isopropanol tests were positive indicating that HbS-San Martin is an unstable Hb variant.[63] The index case was a simple heterozygote for the HbS-San Martin mutation, which he inherited from his mother in whom the mutation might have occurred spontaneously.[63] Both the index case and his mother revealed the presence of pallor and jaundice, but neither had any history of pain or VOC.[63] These findings suggest that HbS-San Martin is recessive with respect to pain and vaso-occlusive symptoms, but it is dominant with respect to haemolytic manifestations.[63] It can easily be deduced that the dominant haemolytic manifestation of HbS-

San Martin is a reflection of the configurational attributes of its second mutation (βLeu105Pro) in which the substitution of leucine by proline interferes with the structural stability of the Hb molecule resulting in haemolytic clinical manifestations that are typical of unstable Hb variants.[63] However, HbS-San Martin may possibly cause typical SCD (with both haemolytic and vaso-occlusive manifestations) if it is inherited as homozygous or coinherited as double heterozygous with HbS or other haemoglobinopathies in the future. Haematologists should therefore raise their index of suspicion for HbS-San Martin on any patient that presents as a case of haemolytic anaemia (devoid of pain) with Hb instability, positive sickling test and electrophoretic pattern suggestive of HbS trait.

13. Haemoglobin S-Wake ($\alpha 2\beta 2$: βGlu6Val, βAsn139Ser)

HbS-Wake is the thirteenth non-S sickling Hb variant to be described. It was discovered in a 14 year old African-American boy who was diagnosed with SCD as reported by Kutlar et al in 2010.[64] Like other non-S sickling Hb variants, HbS-Wake is a double mutant that carries two mutant substitutions (βGlu6Val and βAsn139Ser) in the β-globin chain.[64] The presence of the βGlu6Val substitution confers upon HbS-Wake positive solubility and sickling tests.[64] Moreover, HbS-Wake depicts slow electrophoretic mobility in alkaline and acid media but can be differentiated from HbS by iso-electric focusing and HPLC.[64] Laboratory tests showed that the index patient was a compound heterozygote for HbS-Wake with a double mutation (βGlu6Val and βAsn139Ser) on one of his chromosome-11 and a single HbS mutation (βGlu6Val) on the other chromosome-11, leading to HbSS-Wake SCD.[64] The index patient with HbSS-Wake SCD was found to have coinherited homozygous α(+) thalassemia trait, which resulted in lower mean corpuscular haemoglobin concentration (MCHC).[64] Consequently, the patient had a relatively mild haemolytic and vaso-occlusive features of SCD[64], which is consistent with the fact that low MCHC reduces the intensity of HbS polymerisation and red cell sickling in SCD.[65] The absence of haematological and clinical manifestations in the HbS-Wake heterozygous parent of the index patient suggested that HbS-Wake trait is genetically recessive.[64]

14. Haemoglobin S-São Paulo ($\alpha 2\beta 2$: βGlu6Val, βLys65Glu)

HbS-São Paulo is the fourteenth, and the latest and most recently described non-S sickling Hb variant. It was discovered in an 18 month old Brazilian male as described by Jorge et al in 2012.[66] Chromosomal and genetic analysis of chromosome-11 revealed that HbS-São Paulo is a double mutant characterised by dual occurrence of GAG>GTG transition at codon-6 that corresponds to HbS [βGlu6Val] substitution and an AAG>GAG transition at codon-65, which corresponds to βLys65Glu substitution at the 65th position of the β-globin chain.[66] The index case was heterozygous for HbS-São Paulo, and most probably inherited the βGlu6Val allele from his mother (who was heterozygous for HbS) in addition to the new mutation (βLys65Glu), which most probably occurred later on the same chromosome-11 via spontaneous germ cell mutation as proposed by the discoverers.[66] HbS-São Paulo gives positive solubility and sickling tests in consistency with the presence of HbS [βGlu6Val] substitution.[66] However, HbS-São Paulo was faster than HbA in alkaline electrophoresis, while acid electrophoresis revealed similar electrophoretic mobility to that of Hb S, but it is distinguishable from both HbA and HbS by iso-electric focusing and HPLC.[66] The βLys65Glu substitution conferred upon HbS-São Paulo structural instability with significant reduction in oxygen affinity.[66] HbS-São Paulo is therefore functionally comparable to other low oxygen affinity variants including HbS-Antilles[39], HbS-Cameroon[52], HbS-South End[54] and Hb Jamaica Plain[56] as previously described. Despite being a simple heterozygote, the index case presented with moderate haemolytic and vaso-occlusive features of SCD that was attributed to the low oxygen affinity of HbS-São Paulo[66], which enhances desaturation, polymerisation, red cell sickling and occurrence of clinical symptoms even in the heterozygotes.[66] Hence, HbS-São Paulo is a dominant genetic trait.[66] HbS-São Paulo is therefore a member of the of 'the so-called dominant/super-sickling non-S Hb variants' with the other members being HbS-Antilles[39], HbS-Oman[17,18,47], and Hb Jamaica Plain[56] [see previous sections]. Because of its low affinity for oxygen, it can be predicted that HbS-São Paulo would produce clinically severe SCD if and when it is inherited as homozygous (HbS-São Paulo/HbS-São Paulo) or double heterozygous with HbS (HbS-São Paulo /HbS) or β-thalassemia (HbS-São Paulo/β-thal) in the future.

Conclusion and Recommendations

To date, there are fourteen non-S sickling Hb variants that have been identified, characterised and documented in the literature within a time span of forty-six years between 1966 and 2012. These atypical sickling haemoglobins arose as a result of additional mutations that were superimposed on pre-existing HbS mutations (βGlu6Val) located on chromosome-11. Hence, the non-S sickling Hb variants are often described as double genetic mutants, some of which probably emanated from chromosomal crossing-over and translocation of a second (additional) gene onto chromosome-11 that carries a pre-existing HbS mutation. It is therefore not coincidental that the index cases of these atypical sickling variants were discovered in people of tropical, African, Mediterranean and Asian regions within which pre-existing HbS mutation is very prevalent. Although the atypical sickling Hb variants are relatively rare, they are nonetheless clinically significant because they may produce haematological, vaso-occlusive and other clinical manifestations in heterozygotes, double heterozygotes or homozygotes depending on whether or not they are genetically recessive or dominant. These relatively rare non-S sickling Hb variants pose diagnostic difficulties because they quite often mimic the electrophoretic and chromatographic characteristics of the normal Hb (i.e. HbA) or one of the well characterized abnormal Hb variants (such as HbS or HbC). Furthermore, some of the non-S sickling Hb variants may even exhibit alterations (low or high) in their oxygen affinity and dissociation profiles. Consequently, the probability of misdiagnosis is very high if only basic investigative techniques (e.g. sickling and solubility test, alkaline cellulose electrophoresis) are used for diagnosis, which is usually the case in low resource tropical laboratories. Therefore, the precise diagnosis of these mutant non-S sickling Hb variants may require more advance and elaborate diagnostic techniques such as acid agar gel Hb tetramer and globin chain electrophoreses, iso-electric focusing, HPLC, DNA and gene analysis, and determination of oxygen affinity and dissociation indices, most of which are regrettably not readily available in low resource laboratories of tropical countries. Paradoxically the tropical countries carry the highest prevalence and heaviest burden of sickle cell disorders in the world. In order to avoid misdiagnosis of these atypical Hb mutants, it is imperative that tropical countries should upgrade their investigative tools and techniques for the diagnosis of haemoglobinopathies. Suffices to say that random mutations and generation of new mutant Hb variants are continuous natural processes, hence many more new non-S sickling Hb variants may emerge in the near or distant future. So the onus lies on tropical, African, Mediterranean and Asian clinicians, scientists and haematologists to maintain a high clinical index of suspicion, sustain diligent scientific and haematological evaluations, and offer proactive genetic analysis and surveillance for prompt detection and diagnosis of any new non-S sickling Hb variants as soon as they emerge.

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