

Compound heterozygosity for hemoglobin variant Hb-Broomhill and the Southeast Asian α -thalassemia deletion does not worsen outcome: a case report of two unrelated patients

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Abstract

We report two unrelated cases of compound heterozygosity for hemoglobin (Hb) variant Broomhill and the Southeast Asian (- -^{SEA}) α -thalassemia deletion, whose clinical features and laboratory findings have never been reported. Hematological analyses revealed abnormal values for both cases as α -thalassemia traits, and capillary electrophoresis suggested an abnormal peak that was incompletely separated from the Hb A peak. A suspension array system and Sanger sequencing were used to characterize the genotypes. Sanger sequencing confirmed the presence of Hb Broomhill [α 114(GH2)Pro→Ala; *HBA1*: c.343C>G]. Eventually, both cases were accurately diagnosed as compound heterozygotes for Hb Broomhill and the (- -^{SEA}) α -thalassemia deletion, which is the first known report of these variants. This information will be useful when providing appropriate genetic counselling and prenatal diagnosis.

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Keywords

Compound heterozygosity, hemoglobin variant, hemoglobin Broomhill, Southeast Asian α -thalassemia deletion, Sanger sequencing, capillary electrophoresis

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Introduction

Hemoglobinopathies, including thalassemia and hemoglobin (Hb) variants, are widespread in Asia, especially in Southeast Asia and southern China. Hb variants consist of manifold types with varied clinical manifestations. To date, over 1300 Hb variants have been listed in the HbVar database.¹ A rare Hb variant, Hb Broomhill, caused by a missense mutation (CCC>GCC) at codon 114 of the α 1-globin gene (*HBA1*), has been infrequently reported and has not been documented as a compound heterozygote combined with α -thalassemia thus far.^{1,2} Here, we report two unrelated Chinese cases of compound heterozygosity for Hb Broomhill and the Southeast Asian (- -^{SEA}) α -thalassemia deletion for the first time.

Case report

Case 1 is a 32-year-old woman originating from Yunfu City in Guangdong Province, China. She was referred to our hospital for a routine prenatal examination at the 18th week of gestation and then underwent thalassemia screening. Case 2 is a 51-year-old man from Guangzhou City in Guangdong Province. He attended our hospital for routine partner testing for thalassemia. These two cases were from unrelated families. Both agreed to participate in our study and signed informed consent forms. All studies were approved by the Ethics Committee of Guangdong Women and Children Hospital.

Blood samples were collected and hematological parameters were determined using a Sysmex XN5000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Hb analysis was conducted with an automated capillary 2 electrophoresis system (Sebia, Lisses, France). Genomic DNA was extracted from peripheral blood leukocytes using the Lab-Aid 820 automation system (Zee San Biotech Company, Fujian, China). Twenty-three mutations common in individuals from southern China were routinely measured in our laboratory by a suspension array system, as previously described.³ α 1-, α 2-, and β -globin genes were independently amplified by PCR using previously described primer pairs,^{4,5} then PCR products were purified and sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were compared with National Centre for Biotechnology Information reference sequences (*HBA1*: NC_000016.10: 176680-177522; *HBA2*: NC_000016.10: 172876-173710; *HBB*: NC_000011.10: 5225464-5227071) using Sequence Scanner software (Applied Biosystems).

Hematological indices and genotypes are summarized in Table 1. Both samples showed characteristics of microcytic and hypochromic erythrocytes. Iron deficiency was excluded (data not shown). In both cases, capillary electrophoresis (CE) revealed an abnormal Hb X fraction which was close to the Hb A peak and easily identified, even if partial overlap persisted (Figure 1). The suspension array

Table 1. Hematological and genotypic data of the two patients.

Parameters	Case 1	Case 2
Sex	Female	Male
Age (years)	32	51
Hb (g/L)	102	131
RBC ($10^{12}/L$)	4.85	6.21
MCV (fL)	73.3	70.8
MCH (pg)	21	21.1
Hb X+Hb A (%)	97.8	98.6
Hb A ₂ (%)	2.2	1.4
HBA genotype	- - ^{SEA} /Hb	- - ^{SEA} /Hb
HBB genotype	Broomhill β^N/β^N	Broomhill β^N/β^N

Hb, hemoglobin; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Hb X, abnormal hemoglobin; β^N , normal HBB allele.

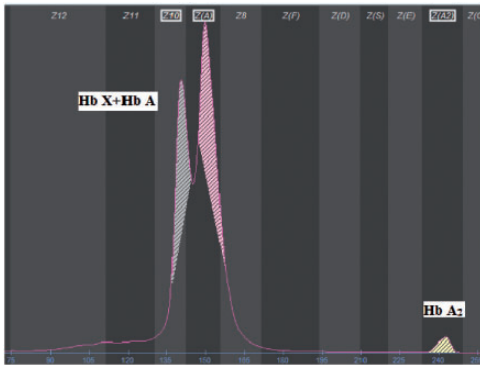


Figure 1. CE result of Hb Broomhill with the characteristic Hb X fraction incompletely separated from the Hb A peak. CE, capillary electrophoresis; Hb, hemoglobin; Hb X, abnormal hemoglobin.

system determined that both cases carried heterozygous (- -^{SEA}) deletions for α -thalassemia and were negative for β -thalassemia (data not shown). DNA sequencing detected a substitution (*HBA1*: c.343C>G) at codon 114 of *HBA1* that was previously reported as Hb Broomhill (Figure 2), which was consistent with CE results. No other mutation with clinical significance was observed in either of the cases.

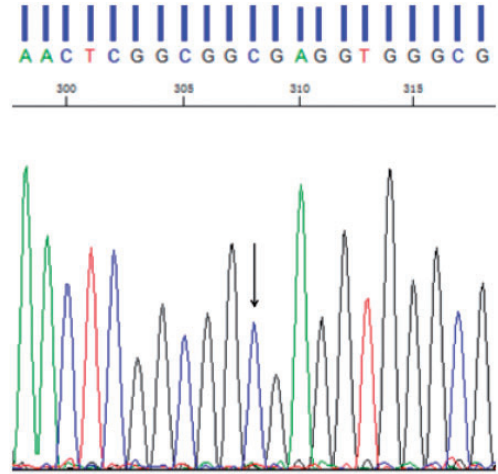


Figure 2. Result of reverse sequencing. Arrow indicates the G>C homozygous substitution (as a result of coexisting with the α -thalassemia deletion) at codon 114 of *HBA1* that was previously reported as Hb Broomhill.

Hb, hemoglobin.

Eventually, the two patients were diagnosed with compound heterozygosity for Hb Broomhill and the (- -^{SEA}) α -thalassemia deletion.

Discussion

Hb variants are caused by mutations in globin genes, which lead to structural changes in the Hb molecule. The phenotype of Hb variants ranges from asymptomatic to severe clinical manifestations.⁶ The sensitive detection of Hb fractions and accurate diagnosis of Hb variants are vital, especially in regions with a high prevalence of hemoglobinopathies.

Hb Broomhill was previously described as a neutral variation in *HBA1* or *HBA2*, which led to an alanine substitution of the original amino acid proline at position 114. It is more specific to the Chinese population and was first recorded in 2010¹ in a heterozygous patient with an abnormal Hb comprising 19% of the total Hb and no clinical

symptoms. The second heterozygote case discovered by Ling *et al.*² confirmed that Hb Broomhill can be detected by CE and that only a small shoulder peak appeared before the Hb A peak. In our study, because Hb Broomhill coexisted with the (- -^{SEA}/) α -thalassemia deletion, CE detected an increased proportion of the abnormal Hb fraction; therefore, obvious incomplete separation between the abnormal Hb peak and Hb A peak was apparent.

Hb Broomhill carriers have seldom been reported and seemed to be rare, but the carrier rate was actually higher than previously believed according to previous molecular epidemiology surveys of hemoglobinopathies in the Chinese population.^{7,8} As previously shown, we found that the Hb Broomhill fraction was small and unseparated from the Hb A fraction in CE analysis; similarly, it was reported to be difficult to detect using high-performance liquid chromatography.² Thus, it is likely that this Hb variant is overlooked during laboratory analysis.

The first recorded case heterozygous for Hb Broomhill was asymptomatic with an Hb level of 116 g/L; the mean corpuscular volume (MCV; 89.7 fL) and mean corpuscular hemoglobin (MCH; 30.1 pg) were normal.¹ The second reported case had mild anemia with an Hb level of 96 g/L; in accordance with the first case, the MCV (95.7 fL) and MCH (29.6 pg) were within reference ranges.² Additionally, this variant had been detected in two molecular epidemiology surveys that lacked further information about clinical presentation or laboratory testing.^{7,8}

The α 114 location appears to be involved in α 1 β 1 interactions, and several other Hb variants exist in the same location as Hb Broomhill, including Hb Chiapas [α 114 (GH2)Pro \rightarrow Arg; *HBAI*: c.344C>G], Hb Melusine [α 114(GH2)Pro \rightarrow Ser; *HBAI*: c.343C>T], Hb Nouakchott [α 114(GH2)Pro \rightarrow Leu; *HBAI*: c.344C>T], and Hb

Hubei [α 114(GH2)Pro \rightarrow His; *HBAI*: c.344C>A].⁹⁻¹¹ The pathogenesis of these variants is poorly understood. Although they may change the GH2 corner of the α -globin chain and the hydrophobicity of this part of the chain,¹² most heterozygotes showed no sign of anemia or α -thalassemia hematological phenotypes. Moreover, the clinical manifestations of individuals with compound heterozygosity for Hb Broomhill and the (- -^{SEA}/) α -thalassemia deletion have never been described to our knowledge. In our report, case 1 presented with mild anemia and case 2 had no anemia, and the hematological features were similar to those of (- -^{SEA}/) α -thalassemia heterozygotes. This indicates that the clinical severity of (- -^{SEA}/) α -thalassemia heterozygotes did not worsen when compounded with Hb Broomhill.

Conclusions

In regions where hemoglobinopathies are prevalent and heterogeneous, interactions of Hb variants with thalassemia are commonly encountered. These interactions may lead to complex syndromes with complicated laboratory diagnostics. In our study, although the interaction of Hb Broomhill with (- -^{SEA}/) α -thalassemia did not lead to Hb H disease, the data assembled from these two unrelated patients will provide more insights into the clinical features and laboratory findings of Hb Broomhill and will be crucial for genetic counselling and prenatal diagnosis in this population.

Declaration of conflicting interest


The authors declare that there is no conflict of interest.

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