

# Three cases of the hemoglobin G-Chinese variant detected in patients of southern Chinese origin

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**Abstract.** Hemoglobin (Hb) G-Chinese [ $\alpha$ 30 (B11) Glu→Gln], also known as Hb G-Honolulu, Hb G-Hongkong or Hb G-Singapore, was first identified in a Chinese woman in Singapore, and was subsequently observed in several Chinese families. This Hb variant results from a GAG→CAG mutation at codon 30 of the  $\alpha$ -globin gene. The aim of the present study was to identify the Hb G-Chinese mutation in three Cantonese individuals. The presence of the Hb variant was confirmed by cellulose acetate electrophoresis. DNA analysis based on the polymerase chain reaction (PCR) and sequencing were conducted to confirm the presence of the mutation in the  $\alpha$ -globin gene. A G→C substitution at codon 30 of the  $\alpha_2$ -globin gene was observed. According to a previous study, the G→C substitution in Hb G-Chinese creates a *Pst*I restriction site; we amplified the  $\alpha_2$ -globin gene, then digested the PCR products with *Pst*I. The results indicated that only the PCR product of Hb G-Chinese  $\alpha_2$ -globin was cut by *Pst*I. The digestive products were 120 and 730 bp, respectively. Therefore, we determined that the three cases were of the heterozygous Hb G-Chinese variant.

## Introduction

Hemoglobin (Hb) G-Chinese [ $\alpha$ 30 (B11) Glu→Gln], also known as Hb G-Honolulu, Hb G-Hongkong and Hb G-Singapore, was first identified in a Chinese woman in Singapore by Vella *et al* in 1958 (1), and was subsequently observed in several Chinese families (2,3). This Hb variant results from a GAG→CAG mutation at codon 30 of the  $\alpha$ -globin gene. Hb G-Chinese carriers do not present the clinical symptoms or hematological changes often observed in thalassemia screening. Almost all previous reports identified the Hb G-Chinese variant in Chinese families from Singapore, Taiwan, Honolulu and Hong Kong (1-4). To the

best of our knowledge, only two reported cases involved individuals from mainland China (5,6), and in these reports the diagnoses were made using amino acid chemical structural analysis, with no detailed information provided regarding the globin gene. There have been no reports on the Hb G-Chinese variant in mainland China in the English literature.

According to the results of hemoglobin electrophoresis, Hb G-Chinese is a slow-moving hemoglobin variant with a total hemoglobin concentration of 12-15% (1-8) that migrates towards the anode faster than Hb S and more slowly than Hb A upon cellulose acetate electrophoresis (9). While conducting recent screening for thalassemia in the Chaozhou area of Guangdong Province, we observed a hemoglobin mutation in three Chinese male individuals, which proved to be a heterozygous Hb G-Chinese variant.

## Materials and methods

**Subjects and hematological analysis.** We examined three subjects of Han nationality who had an unknown Hb variant with no clinical manifestations. The subjects were unrelated local residents of the Chaozhou area undergoing screening for anemia as medical outpatients at the Chaozhou Central Hospital, Guangdong, China. EDTA blood samples were taken and immediately sent (at 4°C) to the Hematology and Molecular Laboratory for further analysis. Hematological data were collected on an automated blood cell counter (Abbott Cell-Dyn CD3700; Abbott Laboratories, Illinois, USA). Hb analysis was carried out by standard cellulose acetate electrophoresis at pH 8.6. Written informed consent was obtained from all patients prior to their participation in the study.

**DNA analysis.** Genomic DNA was extracted from peripheral blood leukocytes with the genomic DNA Mini-Preparation kit (Decipher Bioscience Shenzhen Ltd.) as previously described (10). The  $\alpha_2$ -globin gene was amplified by PCR in a DNA Thermal Cycler KP-TC48 (Chaozhou HybriBio Biotechnology, China) with previously described primers (11). The 50- $\mu$ l PCR reaction mixture contained 0.1  $\mu$ g DNA, 15 pmol primers, 200  $\mu$ mol dNTPs and 2.5 units Taq DNA polymerase (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China.) in 10 mmol/l

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Table I. Summary of the hematological findings of the patients.

Parameter	Case 1	Case 2	Case 3
Gender	M	M	M
RBC ( $10^{12}$ )	6.3	4.36	5.04
Hb (g/l)	176.0	133.0	152.0
MCV (fl)	82.5	91.8	87.5
HCT (l/l)	0.517	0.4	0.441
MCH (pg)	27.9	30.5	30.2
MCHC (g/l)	340.0	333.0	345.0
RDW	13.8	12.8	13.3
SI ( $\mu\text{mol/l}$ )	22.5	17.2	11.8
Hb A (%)	77.9	78.3	78.9
Hb A <sub>2</sub> (%)	1.9	1.9	2.0
Hb G-Chinese (%)	20.2	19.8	19.1
$\alpha$ -globin gene type	$\alpha\alpha/\alpha\alpha^{\text{Chinese}}$	$\alpha\alpha/\alpha\alpha^{\text{Chinese}}$	$\alpha\alpha/\alpha\alpha^{\text{Chinese}}$

M, male; RBC, red blood cells; Hb, hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; SI, serum iron.

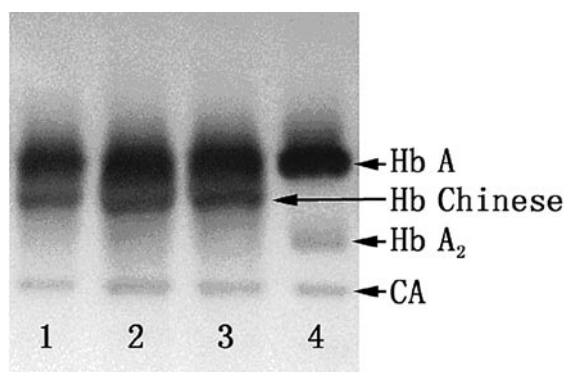


Figure 1. Hemoglobin analysis of the patients with Hb G-Chinese by cellulose acetate electrophoresis at pH 8.6. Lanes 1-3, cases 1-3; lane 4, normal control.

Tris-HCl (pH 8.0), 50 mmol/l KCl and 3 mmol/l  $\text{MgCl}_2$ . The amplification reaction was carried out in the DNA Thermal Cycler KP-TC48. Initial denaturation was performed at  $94^\circ\text{C}$  for 3 min, followed by 35 cycles at  $94^\circ\text{C}$  for 1 min and  $55^\circ\text{C}$  for 30 sec, followed by a final extension at  $72^\circ\text{C}$  for 2 min. Amplified products were separated by 2% agarose gel and detected with UV light following ethidium bromide staining. PCR products were analyzed by DNA sequencing using the ABI 377 DNA Sequencer (Applied Biosystems, CA, USA).

***PstI* restriction enzyme digestion.** According to a previous study, the G→C substitution found in patients with the Hb G-Chinese variant creates a *PstI* restriction site (3). We therefore amplified the  $\alpha_2$ -globin gene, and subsequently digested the PCR products with *PstI*.

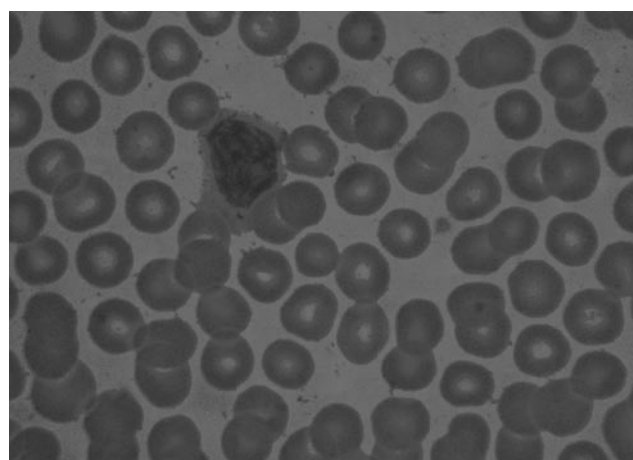


Figure 2. Wright's staining of red blood cells in samples from the patients with the  $\alpha_2$ -globin Hb G-Chinese gene mutation. Red blood cell morphology was normal. Magnification,  $\times 100$ .

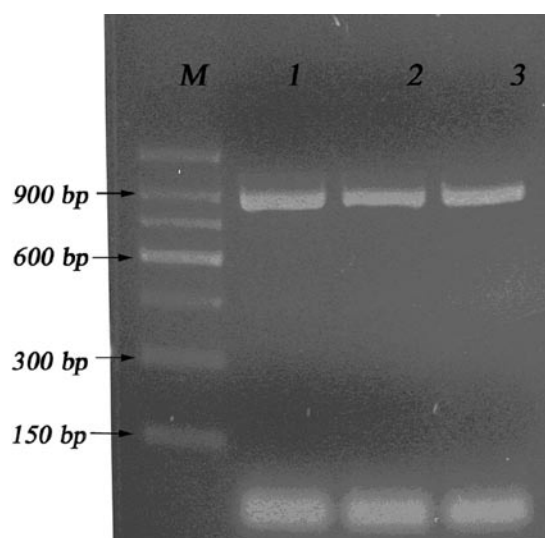


Figure 3. PCR products of  $\alpha_2$ -globin in the patients with the Hb G-Chinese variant. Lane M, maker; lane 1, case 1; lane 2, case 2; lane 3, case 3.

## Results

The hematological data of the patients are summarized in Table I. A serum iron test showed no iron deficiency. The Hb variant patterns were revealed by Hb electrophoresis (Fig. 1). An isopropanol test revealed that the Hb levels were stable ( $>40$  min). The red blood cell morphology of the patients (determined by Wright's staining) was normal (Fig. 2).

The  $\alpha_2$ -globin gene was amplified by PCR, the products of which are shown in Fig. 3. A G→C substitution at codon 30 of the  $\alpha_2$ -globin gene was observed (Fig. 4). The three patients were heterozygous for the Hb G-Chinese variant. The results of *PstI* digestion indicated that only the PCR product of Hb G-Chinese  $\alpha_2$ -globin could be cut by *PstI*. The digestive products were 120 and 730 bp, respectively. The PCR product of the wild-type normal control could not be cut, and remained at 850 bp (Fig. 5).

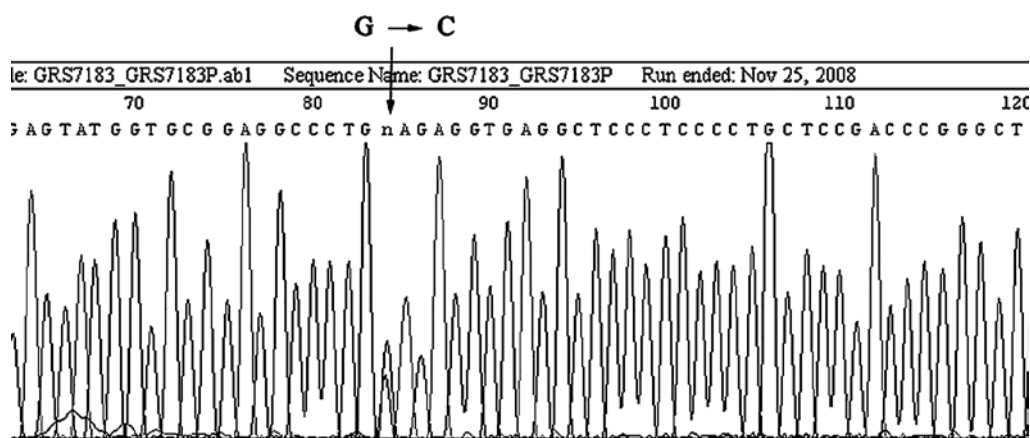


Figure 4. Sequence analysis of the amplified  $\alpha_2$ -globin gene in the patients with the Hb G-Chinese variant. The downward arrow indicates the G-C substitution at codon 30 of the  $\alpha_2$ -globin gene.

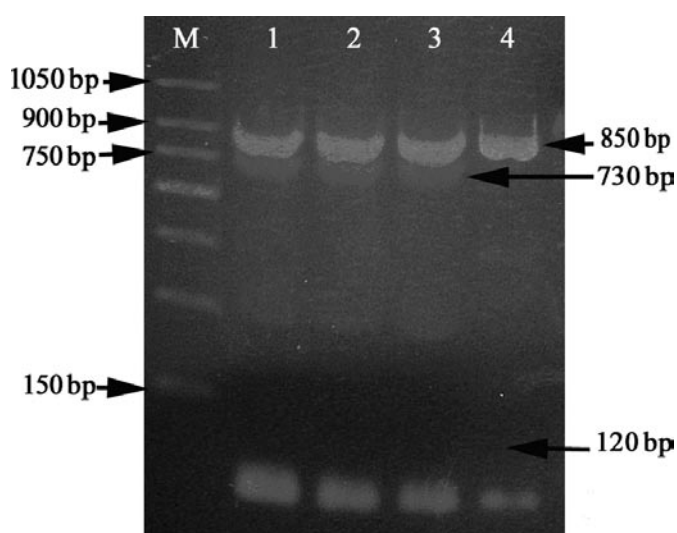


Figure 5. Results of *Pst*I restriction enzyme digestion. The Hb G-Chinese  $\alpha_2$ -globin PCR products have a *Pst*I cutting site (lanes 1, 2 and 3), while the wild-type normal control (lane 4) does not. M, 150-bp ladder marker; lanes 1-3, cases 1-3; lane 4, normal control. Due to long-term electrophoresis and DNA diffusion, the 120-bp products were difficult to discern.

## Discussion

A hemoglobin screening carried out 20 years ago in the Chaozhou area of China identified 10 cases of the Hb G and D variants among 5,946 individuals studied, a prevalence of 0.168%. Several of these cases may have been of the Hb G-Chinese variant; however, since nucleic acid determination was not yet available, a positive identification of the variant was not possible (9).

In the present study, we used  $\alpha_2$ -globin gene amplification and *Pst*I restriction enzyme digestion to identify three cases of the Hb G-Chinese variant, which results from a GAG→CAG mutation at codon 30 of the  $\alpha$ -globin gene. All three subjects carrying the mutation were of Han nationality from the Chaozhou area.

Chaozhou is located in the east of Guangdong Province, which is near the southern border of Fujian Province. Residents of Chaozhou trace their ancestry to Fujian. The

majority of residents of Taiwan and of other Chinese living overseas are descended from emigrants from southern China, including the Fujian and Canton Provinces. It is therefore likely that the Hb G-Chinese variant found in Taiwan (3,7,8) is the same as the variant we detected in the individuals from Chaozhou.

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