American Journal of Hematology 80:303–305 (2005)

*Xmn*I $^{G}\gamma$-Polymorphism in Six Unrelated Pakistani Families With Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$ δβ-Thalassemia

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The *Xmn*I $^{G}\gamma$-polymorphism (C–T polymorphism at position –158 to the $^{G}\gamma$-globin gene) was studied in 13 individuals from six unrelated Pakistani families with δβ-thalassemia. All of the subjects had the Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$ that included six heterozygotes, six homozygotes, and one compound heterozygote of δβ- and β-thalassemia. All seven δβ-thalassemia heterozygotes (including one compound heterozygote) had the –/+ genotype, whereas all six of the homozygotes had the +/+ genotype. The results strongly suggest a tight linkage between the *Xmn*I $^{G}\gamma$-polymorphism and the Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$. The finding could explain the unusually well-preserved capacity to produce fetal hemoglobin in δβ-thalassemia. Am. J. Hematol. 80:303–305, 2005. © 2005 Wiley-Liss, Inc.

Key words: δβ-thalassemia; *Xmn*I polymorphism; Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$; Pakistan

INTRODUCTION

The δβ-thalassemias are mostly caused by gene deletions that can be divided into $(\delta\beta)^{\circ}$ and $(^{A}\gamma\delta\beta)^{\circ}$, depending on whether the $^{G}\gamma$ gene is preserved or not [1]. In spite of the deletion of the entire δ- and the β-globin genes, these patients suffer from a very mild disorder, attributed primarily to the production of a relatively large amount of γ-chains after the neonatal period. The study of deletions causing the disorder has not identified any specific reason for the continued production of γ-globin chains. The loss of regulatory regions for the γ-genes in the deletions, rearrangement of the sequences within the β-gene complex bringing enhancer sequences close to the $^{G}\gamma$-globin gene promoter, and the loss of competition for a common locus control region (LCR) between the γ-, δ-, and the β-gene promoters may be involved in the continued $^{G}\gamma$-gene activity in δβ-thalassemia [2].

The presence of a C–T polymorphism at position −158 to the $^{G}\gamma$ globin gene, recognized by *Xmn*I, is known to be associated with elevated $^{G}\gamma$-globin production [3]. The high Hb-F production in δβ-thalassemia and the linkage between the *Xmn*I polymorphism and δβ-thalassemia were studied in six unrelated families from three diverse ethnic groups in Pakistan.

PATIENTS AND METHODS

Thirteen individuals with a hematological diagnosis of δβ-thalassemia from six unrelated Pakistani families were studied. The DNA samples were screened for the β-thalassemia mutations found in the Pakistani population [4] and the Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$ mutation [1].

*Xmn*I Polymorphism

A 641-bp fragment of DNA flanking the C–T polymorphism at −158 to the $^{G}\gamma$-gene was amplified with primers 5′-GAACTTAAGAGATAATGGCCTAA and 5′-ATGACCCATGGCGTCTGGACTAG (Invitrogen, Carlsbad, CA). The amplified fragments were

Contract grant sponsor: Pakistan Council for Science and Technology (Research Productivity Allowance [RPA])

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Received for publication 26 December 2004; Accepted 3 March 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20376

digested overnight at 37°C with 10 units of *Pdm*I (*Xmn*I) restriction enzyme (Fermentas Life Sciences, Vilnius, Lithuania). The results were read after electrophoresis on 6% mini-polyacrylamide gel electrophoresis (PAGE) and staining by silver nitrate.

RESULTS AND DISCUSSION

All 13 individuals from the six unrelated families with a hematological diagnosis of δβ-thalassemia had the Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$ mutation. They included six heterozygotes, six homozygotes, and one compound heterozygote of δβ- and β-thalassemia (IVSI-5 (G–C) mutation).

All seven δβ-thalassemia heterozygotes (including one compound heterozygote) and the six homozygotes had *Xmn*I $^{G}\gamma$ −/+ and +/+ genotypes, respectively (Fig. 1). Three of the six families were Punjabi, one was Pathan, and two were from the Bohra community. The three ethnic groups have very different racial as well as geographical backgrounds. This gives a clear evidence for the tight linkage between the C–T polymorphism at −158 to the Cap site of the $^{G}\gamma$-globin gene and the Asian-Indian Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$.

The switch from fetal to adult hemoglobin is incomplete, and in normal adults, a slight amount of Hb-F is continuously produced. In β-thalassemia, however, the ability to produce Hb-F is preserved to a variable extent, which also determines the phenotypic severity of the disorder [2]. The C–T polymorphism at position −158 to the $^{G}\gamma$-gene is known to be associated with enhanced Hb-F production [3]. The *Xmn*I site is thought to be involved in the expression of the $^{G}\gamma$-gene through interaction with the transcription factors that may be encoded by a quantitative trait locus on chromosome 8 [5,6]. The association of $^{G}\gamma$-polymorphism and increased production of Hb-F, causing lessening of the severity of the disorder is well documented in sickling disorders from Saudi Arabia and India [7,8]. Several β-thalassemia mutations also show a milder phenotype when linked to the *Xmn*I polymorphism [9]. A linkage between the *Xmn*I $^{G}\gamma$-polymorphism and any of the δβ-thalassemia mutations has not yet been described. Our results provide yet another example of an association between the polymorphism and a disorder characterized by high levels of Hb-F and a very mild phenotype.

All reports of the association between the *Xmn*I $^{G}\gamma$-polymorphism and the enhanced production of Hb-F represent the β-gene mutations that leave its promoter region intact. Ours is the first example in which the polymorphism is associated with a completely deleted β-gene. Rare β-thalassemia deletions that involve the promoter region of the gene have a mild phenotype possibly because of the lack of competition between the β- and the γ-gene promoters for the common LCR [10]. What contribution the *Xmn*I polymorphism makes in the high Hb-F levels in δβ-thalassemia is not known. A relatively more severe phenotype and the level of Hb-F in β-thalassemia associated with *Xmn*I $^{G}\gamma$-polymorphism compared to the δβ-thalassemia [2] suggest that *Xmn*I $^{G}\gamma$-polymorphism may be significant but not the only factor in causing the high Hb-F levels in δβ-thalassemia.

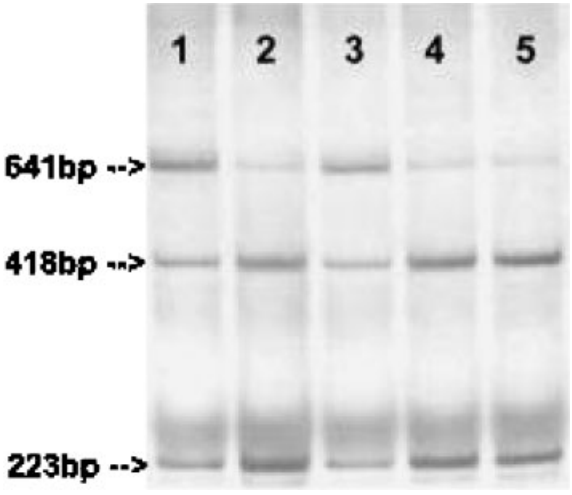

Fig. 1. Silver-stained PAGE of the *Pdm*I (*Xmn*I)-digested PCR products from patients with the Inv/Del $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$ mutation. The 641-bp fragment represents the uncut (–) site, while the 448- and 223-bp fragments represent the cut (+) site. Samples 1, 3, and 6 are –/+, whereas samples 2, 4, and 5 are +/+ genotypes.

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