Brief Communication

Identification of the Chinese IVS-II-654 (C→T) β-Thalassemia Mutation in an Immigrant Turkish Family: Recurrence or Migration?

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Abstract In this study we describe the Chinese IVS-II-654 (C→T) β-thalassemia mutation for the first time in an immigrant Turkish family living in Istanbul and originating from Xanthe, Greece. Four members of the family, representing 3 generations, are heterozygous for this mutation. A detailed family history demonstrated a Greek origin for members of 5 generations with no records of migration or consanguineous marriages. Analysis of polymorphic nucleotides located at the 5′ end of the β-globin chromosomes bearing the IVS-II-654 mutation in the family described carried the $(AT)_9(T)_5$ type of microsatellite sequence and the ACATCCCCA haplotype. These 2 haplotype components favor a non–Eastern Asian origin for this chromosome, hence suggesting an independent origin for the IVS-II-654 mutation described in this family.

β-Thalassemia is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia and by diminished (β^+) or absent (β^0) β-globin-chain synthesis (Huisman et al. 1997). Molecular analyses of β-globin genes from Turkey revealed the presence of approximately 40 alleles causing the β-thalassemia phenotype (Altay and Başak 1995; Tadmouri, Tüzmen et al. 1998). This number is increasing as previously uncharacterized chromosomes are subjected to DNA sequencing (Tadmouri et al. 1997; Tadmouri, Saglamer et al. 1998; Tadmouri, Bilenoglu et al. 1998). In this communication we

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report the first observation of the Chinese IVS-II-654 (C→T) mutation (Cheng et al. 1984) in an immigrant Turkish family.

Subjects and Methods

Subjects. The proband was a 26-year-old pregnant woman who came in with her husband for prenatal diagnosis. The couple had been diagnosed as β-thalassemia carriers in their hometown, Xanthe, Greece, from where they had migrated to Turkey 3 years ago. Once she was found to carry a rare mutation, members of her family, belonging to 3 generations, underwent hematological and molecular analyses. A detailed family history was also obtained.

DNA Isolation and Molecular Analysis. Blood samples were collected in tubes containing EDTA. Informed consent was obtained, and DNA was extracted from white blood cells according to the method of Poncz et al. (1982). In vitro amplification of genomic DNA was performed using the technique of Saiki et al. (1988). The mutations were investigated by reverse dot-blot analysis (β-Globin StripAssay Kit, Vienna Labs) and by direct sequencing of the polymerase chain reaction (PCR) product. Both manual (Sequenase Version 2.0, Amersham; ^{35}S-dATPαS, Izotop, Hungary) and automatic fluorescent sequencing techniques were implemented (Sanger et al. 1977). Automated sequencing was performed on an ABI Prism 377 DNA Sequencer (Perkin Elmer), using ABI Cycle Sequencing Dye Primer Ready Reaction Kits containing Amplitaq DNA polymerase FS, according to the manufacturer's instructions. The β-globin gene of the proband was amplified by PCR using M13 tailed primers. A 46-mer forward primer (5′-TGTAAAACGACGGCCAGTCTTTACACAGTCTGCCTAGTACATTACT-3′) and a 45-mer reverse primer (5′-CAGGAAACAGCTATGACCTTTTCCCAAGGTTTCAACTAGCTCTTC-3′) were used to amplify a 900-bp fragment of the β-globin gene spanning two-thirds of IVS-II, exon 3, and the untranslated region downstream from the poly-A signal. The PCR product was purified using the Prep-A-Gene DNA purification kit according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, California). Sequencing reactions were subsequently carried out using M13 forward and reverse Dye Primer Ready Reaction Kits and electrophoresed on 36-cm 5% LonRanger (FMC) gels in the 2×A thin run module on the 377 DNA Sequencer (Krishnan and Chaplin 1994).

In addition, putative *cis*-acting determinants modulating HbF levels within the β-globin gene cluster were analyzed. The −158 C→T polymorphism in the Gγ-globin gene promoter was assessed by *Xmn*I restriction of a 351-bp amplified sequence from that region (Tadmouri, Saglamer et al. 1998). The AT-rich region located 530 bp upstream from the β-globin gene was

Table 1. Hematological Data for the Proband and Her Family

Hematological Characteristic	*Grandmother (Age 68)*	*Father (Age 48)*	*Proband (Age 26)*	*Brother (Age 25)*	*Mother (Age 50)*
White blood cells (10^3/ml)	5.91	5.63	5.69	6.66	5.72
Red blood cells (10^6/ml)	5.34	5.72	4.47	6.42	4.10
Hemoglobin (g/dl)	10.9	11.9	9.72	12.5	11.9
Hematocrit (%)	34.6	38.1	29.7	39.6	34.8
Mean corpuscular volume (fl)	64.8	66.6	66.3	61.6	84.7
Mean corpuscular hemoglobin (pg)	20.4	20.8	21.7	19.5	29.1
Mean corpuscular hemoglobin concentration (g/dl)	31.5	31.3	32.7	31.6	34.3
PLT (10^3/ml)	201	193	318	258	235
HbA_2 (%)	5.50	5.20	5.40	5.40	2.90
HbF (%)	–	1.1	2.2	2.0	–
HbA (%)	93.40	92.60	91.2	91.50	96.60
Genotype	*IVS-II-654/N*	*IVS-II-654/N*	*IVS-II-654/N*	*IVS-II-654/N*	*N/N*

analyzed by amplifying and directly sequencing a 790-bp DNA fragment (Trabuchet et al. 1991a,b; Bernantchez et al. 1992; Perrin et al. 1998).

Results

Hematological Data. The hematological data of the proband and her family are given in Table 1. All family members except her mother show typical features of heterozygous β-thalassemia with decreased mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration levels and increased HbA_2 levels.

Analysis of Mutations. The initial investigation of the β-globin genes of the couple using the β-Globin StripAssay revealed the presence of the IVS-I-110 (G→A) mutation in the husband. The DNA of the proband was negative for all the common β-thalassemia mutations present on the strip (−87, IVS-I-1, IVS-I-6, IVS-I-110, Cd39, IVS-II-1, IVS-II-745). Consequently, the proband DNA was subjected to sequencing. Because her pregnancy was near term, an aliquot of blood was sent to Augusta, Georgia, for parallel automatic sequencing. This sequencing revealed the presence of the IVS-II-654 (C→T) mutation. This mutation was confirmed in Istanbul by manual sequencing of samples from the proband, her father, her grandmother, and her brother (Table 1).

Haplotype Analysis. Analysis of the −158 C→T polymorphism in the Gγ-globin gene promoter showed no change from the reference sequence of

Poncz et al. (1983). Sequence analysis of the polymorphic nucleotides −1069, −989, −780, −710, −703, −551, −543, −521, and −491 and the hypervariable microsatellite of composite sequences $(AT)_xT_y$ located near the 5′ end of the IVS-II-654 β-globin genes of the proband and members of her family showed the occurrence of ACATCCCCA and AT_9T_5 at these positions, respectively.

Discussion

Analysis of a great number of β-globin genes from different countries of the world has demonstrated that nearly 180 different alleles are responsible for the occurrence of β-thalassemia worldwide (Huisman et al. 1997). Yet population studies indicate that probably only 13 alleles account for more than 80% of the β-thalassemic chromosomes in the world (unpublished data). The most common mutations tend to be the most widespread geographically and presumably also the oldest. Of these mutations the substitution of (C→T) at position IVS-II-654 of the β-globin gene (Cheng et al. 1984) is described as one of the most common molecular lesions leading to β-thalassemia in Chinese populations (Figure 1). In this study we report the occurrence of the IVS-II-654 mutation in 3 generations of a family that recently migrated from Xanthe, Greece (western Thrace), to Turkey.

To identify the origin and the chromosomal background of the mutation described in this family, a detailed family history was collected and analysis of polymorphic nucleotides located near the 5′ end of the β-globin gene was undertaken. The Balkan (western Thrace) origin of the family was confirmed in at least 5 generations (about 200–250 years) with no known record of migration or consanguinity. Thus, if migration were to explain the occurrence of this mutation, this event would have taken place a long time ago (>250 years ago) and would be expected to result in a wider distribution of the allele in at least several other families in western Thrace. However, this is not the case because extensive analyses of β-thalassemia genes from Balkan countries have not revealed the presence of the IVS-II-654 mutation (Huisman 1990). Moreover, this mutation has not been reported in the region between the Mediterranean and China; in fact, a strict barrier seems to exist between China and India (M.T. Akbari, personal communication, 1997; S.Q. Mehdi, personal communication, 1997; Varawalla et al. 1991; Huisman et al. 1997).

In addition to geographic distribution, haplotype analysis enables the construction of the history of a particular mutation, especially how often its frequency has been independently elevated and in which population it first occurred. Seventeen sequence polymorphisms detectable with restriction endonucleases [restriction fragment length polymorphism (RFLP) haplotypes] have been found in the β-globin cluster, but only 7 of those are commonly reported and form the basis of the β-globin haplotype (Orkin et al. 1982;

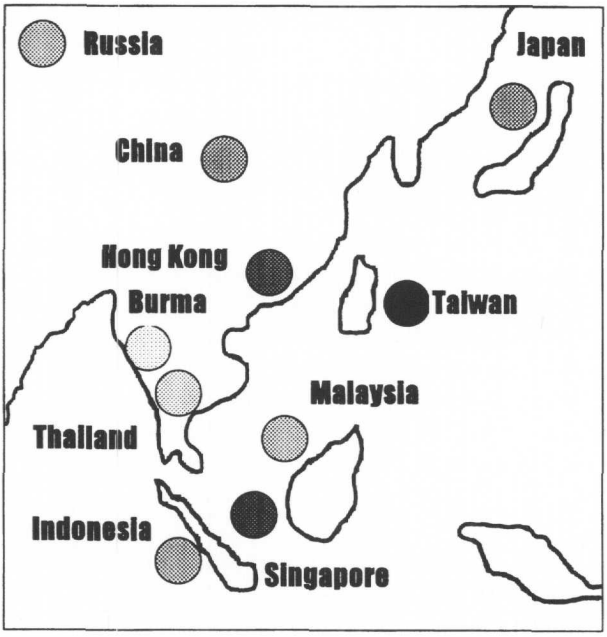

Figure 1. Schematic representation of the distribution of the IVS-II-654 (C→T) β-thalassemia allele in East Asian countries in decreasing order of frequency: Taiwan (46.15%) [composite data from Lin et al. (1991) and Chiou et al. (1993)], Singapore (27.13%) [composite data from Tan et al. (1993) and Ng et al. (1994)], Hong Kong (21.50%) (Chan et al. 1987), Japan (13.91%) (Hattori et al. 1989), China (13.91%) [composite data from Cheng et al. (1984), Zhang et al. (1988), Liu et al. (1989), Huang et al. (1990), and Liang et al. (1994)], Indonesia (11.86%) (Lie-Injo et al. 1989), Russia (8.33%) (Curuk et al. 1994), Malaysia (7.92%) [composite data from Yang et al. (1989) and George et al. (1992)], Thailand (6.28%) [composite data from Laig et al. (1989) and Thein et al. (1990)], and Burma (2.02%) (Brown et al. 1992). The intensity of the shading of the circles represents the mutation density.

Antonarakis et al. 1985). On the other hand, a new sequence polymorphism upstream from the β-globin gene has gained great importance in recent years (Trabuchet et al. 1991a,b). This new system contains an array of 9 highly mutable nucleotides and an alternating purine/pyrimidine track ending with a run of thymines (Trabuchet et al. 1991a). Because approximately 50% of Turkish β-thalassemia chromosomes are expected to occur on RFLP haplotype I and because the Chinese IVS-II-654 mutation is also linked to this same haplotype [reviewed by Flint et al. (1993)], the investigation of the RFLP system seemed unsatisfactory and analysis of the sequence haplotype was preferred. Furthermore, the close proximity of the $(AT)_xT_y$ polymorphism to the β-globin gene (-530 bp from the 5′ end) makes this sequence more informative in terms of determining the chromosomal origin of a particular mutation.

Chromosomes bearing the IVS-II-654 mutation in the studied Turkish family were shown to carry the $(AT)_9(T)_5$ type of microsatellite and the ACA-TCCCCA sequence. Chinese IVS-II-654 chromosomes, however, are strongly linked to the $(AT)_8(T)_5$ type of arrangement; the remaining elements of the haplotype were not reported (Zhou et al. 1995).

To the best of our knowledge, the ACATCCCCA $(AT)_9(T)_5$ compound motif has thus far been described in only 3 IVS-I-110 (G→A) β-thalassemia patients from the Oran region of Algeria (Perrin et al. 1998), in 1 Cd39 (C→T) heterozygote from western Thrace, and in several β-globin genes from the United Kingdom (Fullerton et al. 1994; Harding et al. 1997) and France (P. Perrin, personal communication, 1998). This line of evidence suggests a western Mediterranean and thus an independent origin for the IVS-II-654 (C→T) mutation occurring in our study family. Confirmation and refinement of this finding would need a screening of β-globin chromosomal backgrounds in countries extending from the Balkan region to East Asia.

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