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Novel and Mediterranean β thalassemia mutations in the indigenous Northern Ireland population

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Abstract

β thalassemia is one of the most common genetic diseases worldwide resulting from aberrant β-globin chain production. It is highly prevalent in regions with endemic malaria, but it is also present at low frequency in the indigenous populations of non-tropical areas such as Britain. Screening β thalassemia trait individuals from Northern Ireland has detected 2 Mediterranean mutations, 39 (C → T) and IVS-I-110 (G → A); the previously reported IVS-II-850 (G → A) mutation originally described in individuals of Scottish/English ancestry; and 2 novel mutations, initiation codon A → C and 109 delG. Haplotype analysis indicates that the Mediterranean mutations are present on previously described haplotypes, suggesting that they have arisen due to migration. It remains to be established whether the novel mutations have arisen de novo in Northern Ireland.

Keywords: β thalassemia; β thalassemia trait; $\beta^0$39 (C → T); β^+IVS-I-110 (G → A); β^0IVS-II-850 (G → A)

Introduction

During normal hemoglobin production, alpha (α) and beta (β) globin chain synthesis is tightly regulated and any imbalance in this process gives rise to the thalassemia group of genetic disorders. β thalassemia is one of the most common genetic disorders in the world. It has been estimated that globally there are 80 million carriers [1]. Absent or deficient β-globin chain synthesis results in either β^0 or β^+ thalassemia respectively. Heterozygosity provides some resistance against *Plasmodium falciparum* malaria, allowing β thalassemia mutations to be retained in populations by natural selection. Although β thalassemia heterozygotes are symptomless, they possess microcytic, hypochromic red cells and exhibit raised hemoglobin (Hb) A_2 levels, low mean cell volume (MCV), low mean cell Hb (MCH) values and high red blood cell (RBC) counts with mild or no anemia.

Over 200 different mutations have been reported to cause β thalassemia (see Globin Gene Server at website: http://globin.cse.psu.edu/), and each population is characterized by a few common and a number of rare mutations. Intriguingly, β thalassemia also occurs in non-malarial regions. Population studies in countries such as Britain [2], Germany [3] and Belgium [4] have indicated the presence of both novel and common β thalassemia mutations. Consequently, during the differential diagnosis of hypochromic anemia in these non-tropical countries, it is important to eliminate the possibility of β thalassemia mutations playing a role in the development of anemia and to prevent unnecessary iron replacement therapy.

The first reported case of a heterozygous β thalassemia individual living in Ireland was described by Nelson in 1964 [5]. Since the individual was a native born Irish woman living in Belfast with no suggestion of foreign ancestry, the author

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proposed that this defect had arisen de novo in this lady. However, Nelson [5] did indicate the strong possibility that β thalassemia mutations could have been introduced into Ireland as a result "of the centuries old trading association with Spain" and concluded that β thalassemia was more likely to be more common in the south and west of Ireland than in the North. Subsequently, in 1968, McCarthy et al. [6] described an Irish family with β thalassemia trait, whose ancestors could be dated to originating from Co. Kildare or Dublin City around 1850. There was no evidence of family links with southwest Ireland, where the Spanish trade was prevalent, or mainland Europe, though foreign ancestry could not be ruled out completely.

Knowing that β thalassemia mutations occur at low frequencies in the indigenous British population [2] and that it has also been reported in the Irish population [5,6], it is of interest to investigate the molecular basis of β thalassemia trait in the local population. In addition, haplotype analysis was performed to indicate the possible origins of the detected β thalassemia mutations.

Materials and methods

Twenty-three Caucasian patients from the County Down region of Northern Ireland were selected from a larger group of individuals with heterozygous β thalassemia, with one individual being selected from each family. These cases were first recognized during routine laboratory screening from 1975 onwards. Most of the patients had a long history of mild anemia (Hb 10.0–14.7 g/dl) and microcytosis (MCV 54–73 fl), with the most common indication being tiredness in the absence of any other notable anemia-related symptoms. Many of these individuals had prolonged courses of iron therapy, and several had gastro-intestinal investigations before the diagnosis of β thalassemia trait was made, especially cases pre-1975. On entry to the study, all 23 individuals had the diagnosis of β thalassemia confirmed through hemoglobin electrophoresis and Hb A_2 levels. Diagnostic tests were performed immediately for UPN15 and UPN21 because of a family history of β thalassemia. None of the individuals in this study had a therapeutic intervention such as a blood transfusion, but they were carefully followed up by their family physicians to monitor and subsequently treat any other precipitating cause of anemia.

Peripheral blood samples were collected from each of the study participants after signed informed consent according to the Declaration of Helsinki. Genomic DNA was extracted from isolated lymphocytes using the Nucleon BACC 1 DNA extraction kit (Nucleon Biosciences, Manchester, UK). The β-globin gene was amplified in 3 fragments using standard protocols with the following primers: Exon 1 + 2 plus Upstream forward primer—5′-ACTCCTAAGCCAGTGCCAGA-3′; Exon 1 + 2 plus Upstream reverse primer—5′-AACGATCCT-GAGACTTCCACA-3′; Intron 2 forward primer—5′-GCACGTGGATCCTGAGAACT-3′; Intron 2 reverse primer —5′-CACACAGACCAGCACGTTG-3′; Exon 3 plus 3′UTR forward primer—5′-TATCATGCCTCTTTGCACCA-3′; Exon 3 plus 3′UTR reverse primer—5′-GACCTCCCACATTCC-CTTTT-3′. The DNA amplification was performed with 35 cycles of denaturing at 94°C (1 min), annealing at 55°C (1 min) and extension at 72°C (1 min). PCR products were purified using the GENECLEAN® SPIN KIT (Qbiogene, Livingstone, Scotland), sequenced using BigDye ® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) and analyzed on the ABI 3100 Genetic Analyser (Applied Biosystems). Haplotype analysis was performed using the seven polymorphic sites of the β-globin gene cluster on chromosome 11 characterized by Orkin et al. [7] and amplified by PCR using the primers described by Weatherall and Clegg [8]. All PCR products were analyzed by restriction digest, and haplotypes were constructed according to Orkin et al. [7].

Results and discussion

Sequencing 23 β thalassemia trait individuals revealed that 7 possessed the $\beta^0$39 (C → T) and another 7 the β^+IVS-I-110 (G → A) mutations in the heterozygous state (Table 1). Both mutations are commonly present in the Mediterranean population and have arisen on several different haplotypes (see [2] and [9]). Although it is possible that the 39 (C → T) and the IVS-I-110 (G → A) mutations have arisen independently in Northern Ireland, it is more likely that they have arrived by migration as haplotype analysis indicated that all individuals possessed the same haplotype known as I (+−−−−++; see Table 1). This haplotype group has been previously reported in the Mediterranean population to be associated with both these mutations ([2,10] and [11]). In a previous study, the 39 (C → T) and the IVS-I-110 (G → A) mutations were detected in British Anglo-Saxon individuals and were also found to be associated with Haplotype I suggesting a foreign origin of these mutations [2].

A further 7 individuals were found to be heterozygous for the β^0IVS-II-850 (G → A) mutation, and this mutation was associated with Haplotype II (−++−+++; Table 1) in all individuals. The IVS-II-850 (G → A) mutation has been described previously in a Japanese family [12] and a North European family of Scottish/English ancestry [13], but no haplotype analysis was performed in either study. In the case of the latter family, the mutation was associated with the Gγ promoter polymorphism of C → T at −158. Screening these 7 individuals indicated that they all possessed this same polymorphism, suggesting a common ancestor. The Gγ promoter polymorphism increases γ chain production resulting in elevated Hb F levels providing an advantage in times of hematopoietic stress. The presence of the T polymorphism was compared with Hb F levels in all β thalassemia trait individuals (Table 1), and no correlation between the level of Hb F and the presence of the T base at −158 in the Gγ promoter was evident.

In addition, two novel mutations were detected: one A to C change at the initiation codon resulting in the loss of the ATG, which codes for methionine, and replacement with CTG. Consequently, the initiation of translation would now occur downstream at codons 21 to 22 and termination of translation at codons 60 to 61. Since no β chains would be synthesized from this allele, the mutation would cause β^0 thalassemia. There are nine possible single base mutations of the β-globin gene

Table 1
Hematological data for the β thalassemia patients and the Gγ promoter polymorphism

Patient	Age/Sex	Hb (g/dl)	RBC ($\times 10^{12}$/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A_2	Hb F	Genotype	Gγ −158	Haplotype analysis (I–IX)
UPN1	66 M	10.0	3.6	63	20.6	32.9	5.2	1.3	39 (C → T)	CT	+−−−−++ (I)
UPN2	57 M	11.7	6.0	62	19.4	31.3	4.9	<1.0	39 (C → T)	CT	+−−−−++ (I)
UPN3	58 F	10.3	5.0	66	20.6	31.3	4.6	2.5	39 (C → T)	CC	+−−−−++ (I)
UPN4	67 M	NA	NA	NA	NA	NA	5.2	4.0	39 (C → T)	CT	+−−−−++ (I)
UPN5	39 M	NA	NA	NA	NA	NA	6.0	4.0	39 (C → T)	CT	+−−−−++ (I)
UPN6	30 M	12.0	NA	63	NA	NA	NA	NA	39 (C → T)	CC	+−−−−++ (I)
UPN7	20 F	10.8	4.67	73	23.1	31.9	NA	NA	39 (C → T)	CC	+−−−−++ (I)
UPN8	40 F	11.6	5.6	65	20.7	31.8	NA	NA	IVS-I-110 (G → A)	CT	+−−−−++ (I)
UPN9	32 F	10.1	4.7	67	21.4	32.1	NA	NA	IVS-I-110 (G → A)	CT	+−−−−++ (I)
UPN10	71 F	12.0	5.9	65	20.4	31.4	4.8	<1.0	IVS-I-110 (G → A)	CT	+−−−−++ (I)
UPN11	66 M	14.7	6.9	66	21.4	32.2	4.0	<1.0	IVS-I-110 (G → A)	CT	+−−−−++ (I)
UPN12	78 M	11.1	5.0	68	22.0	32.5	NA	NA	IVS-I-110 (G → A)	CT	+−−−−++ (I)
UPN13	64 F	11.6	5.7	66	20.5	31.4	4.1	NA	IVS-I-110 (G → A)	CC	+−−−−++ (I)
UPN14	60 F	10.0	5.0	64	19.9	31.3	5.8	0.5	IVS-I-110 (G → A)	CC	+−−−−++ (I)
UPN15	16 M	NA	NA	NA	NA	NA	5.0	4.0	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN16	38 F	11.0	5.6	61	19.6	32.1	5.8	2.2	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN17	63 M	12.5	6.3	64	20.0	31.1	6.0	2.4	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN18	25 M	NA	NA	NA	NA	NA	6.1	2.0	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN19	60 F	10.2	5.3	59	19.1	32.4	4.4	1.9	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN20	30 F	10.5	4.8	68	22.1	32.7	5.2	2.8	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN21	11 F	NA	NA	NA	NA	NA	4.7	<1.0	IVS-II-850 (G → A)	CT	−++−+++ (II)
UPN22	38 F	10.1	5.8	54	17.6	32.4	4.8	5.0	initiation codon ATG to CTG	TT	ND
UPN23	71 F	11.4	5.2	68	21.9	32.1	4.0	6.4	109 Del G	CT	ND

Abbreviation: UPN—unique patient number; M—male; F—female; Hb—hemoglobin; RBC—red blood cells; MCV—mean cell volume; MCH—mean cell hemoglobin, MCHC—mean cell hemoglobin concentration; ND—not determined; NA—not available.

initiation codon, seven of which have been previously described and are listed in the Hemoglobin Variant data base of the Globin Gene Server at website http://globin.cse.psu.edu/ and [14]. The haplotypes associated with this mutation could not be determined as other family members were unavailable for the study.

Another individual possessed a novel one base pair deletion of G from the third base at codon 109, causing a frameshift mutation (109 Del G). Although valine is retained at codon 109, the frameshift mutation would result in the formation of an elongated β chain of 156 amino acids as the termination codon is displaced to position 157. A previously characterized deletion of G from the first base of codon 109 [15] results in an unstable β-globin chain, thus causing β^0 thalassemia. Therefore, it can be assumed that the deletion of the third base at codon 109 would cause a similar displacement of the termination codon and an elongated β chain. Thalassemia mutations in exon 3 of the β-globin gene often cause a dominant form of thalassemia and show no predilection for malarial-endemic regions of the world [16], however, the individual in this study does not appear to have a hemolytic anemia. Again, the haplotype associated with the 109 Del G mutation could not be determined as other family members were not available for study.

It is interesting to speculate how the Mediterranean mutations may have arrived in the Irish population. Throughout history, Ireland has always played an important role in the Atlantic world in terms of its geographical position in both strategic and economic terms [17]. Contact and trade with the Atlantic Fringes of Europe can be dated back to the Bronze Age, while invasion attempts on Ireland include those from the Vikings and the Anglo-Normans. In addition, merchants from France, Iberia and Italy were trading in Irish ports in the twelfth century, and trade expanded across Europe as agriculture output increased.

Oral tradition in Northern Ireland suggests that β thalassemia mutations may have been introduced into the population by survivors of the Spanish Armada in 1588. This is probably not accurate as the number of survivors was probably too low to have any real impact on the Northern Ireland gene pool. Those Spaniards who survived the wreckage probably either died on landing or were killed by locals on the shores. The remaining survivors probably escaped as quickly as possible to Scotland or the continent. If local folklore about the Spanish Armada were true, i.e. the sailors made it ashore to the Ards Peninsula in County Down and settled there, it would be expected that β thalassemia trait would be more prevalent on the Peninsula than inland. This was not found to be the case as no apparent difference between the incidence of β thalassemia trait on the Peninsula and further inland was evident (FGC Jones, personal communication). Therefore, invasions along with trade and commerce, dating back to the twelfth century, offer the most likely explanation as to how the Mediterranean mutations were introduced into the Northern Irish population.

There has also been extensive association between the Northern Ireland population with both English and Scottish populations, especially during the Ulster Plantation period of 1610–1685. The close proximity of counties Antrim and Down with Scotland attracted predominantly Scottish settlers. During

the 1600s migration to Ireland exceeded emigration and approximately 250,000 English, Welsh and Scottish Protestants settled in Ireland. The IVS-II-850 (G → A) mutation was first described in a family of Scottish/English descent [13], and it is possible that it may have been dispersed among these populations during this period.

In summary, analyzing the β-globin gene sequence in β thalassemia trait individuals from the indigenous Northern Ireland population has detected five different mutations, two of which are novel. Haplotype analysis indicates that the two common Mediterranean mutations, 39 (C → T) and IVS-I-110 (G → A), have arisen on Haplotype I, as have been reported previously. Thus, it is most likely that both mutations have been introduced into the population by migration. However, an independent origin for the IVS-II-850 (G → A) from the family described by Çürük et al. [13] cannot be eliminated without haplotype analysis. Finally, it remains to be established whether the two novel mutations, initiation codon ATG to CTG and 109 Del G, have arisen de novo. In conclusion, this study indicates that β thalassemia mutations can occur at a low frequency in non-tropical populations and should be considered in the differential diagnosis of hypochromic anemia.

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