Disorders of Erythropoiesis

β-thalassemia in the indigenous population of Brittany: identification of three rare mutations

β-thalassemia mutations were determined in ten Breton probands using a reverse-hybridization method or denaturing gradient gel electrophoresis and sequencing. Six different mutations were found: three from the Mediterranean area and three rare. Mutations responsible for β-thalassemia in Brittany are quite heterogeneous. The mutation in the initiation codon ATG→ACG was found in four families and may result from an ancient founder effect, as suggested by the haplotype analysis.

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The molecular bases of β-thalassemia have been largely elucidated and some 700 different molecular defects in the β-globin have been characterized. β-thalassemia is a very frequent disease in countries in which malaria is endemic.[1] Eight mutations account for more than 90% of the cases of β-thalassemia in the Mediterranean area. On the other hand, β-thalassemia is quite rare in people from northern Europe, with a few cases having been described in Ireland and Britain.[2]

To our knowledge, the presence of thalassemia minor in the indigenous Breton population has never been studied. However, about 20 Breton families with β-thalassemic trait are known at the University Hospital (CHU) of Brest. The probands were ascertained through different sources between 1996 and 2004 (CHU Brest and Blood Bank of Western Brittany). Only subjects with Breton ancestors were included in the study. Informed consent was signed by all probands, in agreement with French regulations, prior to blood sampling. The diagnosis of β-thalassemia minor was verified by the presence of microcytosis, hemoglobin (Hb) levels between 9 and 13 g/dL and a high concentration of HbA2 (>3.5%). The other causes of anemia or high HbA2 concentration were excluded by measurement of C-reactive protein, ferritinemia and haptoglobin. Hb variants were identified and quantified by alkaline electrophoresis on agarose gel. DNA was isolated from leukocytes of whole blood using the *Qiagen FlexiGene DNA Kit* according to manufacturer's instructions (Qiagen S.A., Courtaboeuf, France) and stored at 2°C.

The Viennalab β-Globin Strip Assay (Viennalab labordiagnostika GmbH, Vienna, Austria), which detects 22 of the most frequent β-globin mutations in Mediterranean countries, was used as a first step to identify β-thalassemia mutations in the samples. Seven samples with unidentified defects were screened for other β-thalassemia mutations using a denaturing gradient gel electrophoresis (DGGE) method, as previously described.[3] Fragments displaying an abnormal pattern were then sequenced on both strands using the Big Dye Terminator V3-1 kit and an ABI 3100 Genetic Analyzer (Applied Biosystem, Foster City, USA) for the identification of the molecular defect.

Because several samples displayed the same mutation, we looked for evidence of a founder effect. Several polymorphic markers outside the β-globin cluster (11p15.5) were analyzed. We used four highly polymorphic repeats located on chromosome 11, five single nucleotide polymorphisms (SNP) spreading over the β-globin locus and two polymorphic regions which consist in combinations between SNP resulting in different patterns when studied using DGGE (ie , intragenic β globin fw[3]: β globin gene codon 2 C/T, IVS2 nt 16 C/G, IVS2 nt 74 G/T and IVS2 nt 81 C/T; PGγFw: upstream region of $^{G}\gamma$ globin gene promoter[4] nt - 1225 G/A and - 1280 A/G).

Ten individuals (seven women and three men) from ten independent families among the 70 patients with a thalassemic trait were included in the molecular study.

The biological data and the β-gene mutation of the ten probands are given in Table 1. All individuals, but one (# 4) had HbA2 levels indicative of β thalassemia carriership. Three mutations were identified with the β-globin Strip Assay: IVS-I-5 (G→C) in proband 7, codon 39 (C→T) in proband 8, and codon 5 (-CT) in proband 9.

In the other seven probands, three different β^0 thalassemia mutations were found, leading to genotypes consistent with the phenotype. In one patient (number 3), no

Table 1. Biological data and β-gene mutations of the ten probands included in the study as well as haplotypes carried by four patients with the intitiation codon ATG->ACG mutation.

							β-globin locus (β>δ>Ψβ>aγ>gγ>ε, total length # 50Kb)							*11p1411p12,13*	*11p12*	
Nr.	*Hb (g/dL)*	*MCV (fL)*	*Hb A2 (%)*	*Hb F (%)*	*Ferritinemia (mg/L)*	*Mutation*	*11p15-5 d11s4046 3.9c M**	*Fw (intra gene)*	*5'β Hinc II*	*AγIVSII Hind III*	*GγIVS2 Hind III*	*5'Gγ XmnI*	*FpGγ*	*5'ε Hinc II*	*d11s902 24.7 cM*	*d11s935 40.6 cM*	*d11s905 55.7 cM*
1	10.4	63.8	6.3	0	402	IVS-II-849 (A→G)											
2	9.3	52.3	6	0	246	Initiation codon ATG-→ACG	112/124	1/2	+/+	–/–	+/+	+/+	1/1	–/–	157/159	203/212	277/288
3	12	75.1	5.5	0	30	No mutation identified											
4	7	75.6	2.5	8.6	1410	Initiation codon ATG→ACG	112/122	1/1	+/+	–/–	+/–	–/–	1/ 4	–/–	153/159	203	277/288
5	9.5	52.2	6.4	0	21.9	Initiation codon ATG-→ACG	112/108	1/1	+/+	–/–	–/–	–/–	4/4	–/–	153/167	203/205	275/288
6	10.6	62.8	4.6	0	68.8	IVS-I-1 (G→C)											
7	12.6	59.4	5.8	0	113	IVS-1-5 (G→C)											
8	11.7	66.1	4.4	7.6	42.5	Codon 39 (C→T)											
9	10.7	61.4	6.5	0	155	Codon 5 (-CT)											
10	10.2	54.4	4.7	5.6	41.8	Initiation codon ATG->ACG	110/124	1/2	+/+	–/–	–/+	–/–	2/4	+/–	153/?	196/212	277/292

**Genetic distance from the short arm telomere are indicated below each microsatellite. These values refer to the "Genethon" genetic maps, "0" being the telomere of the short arm of chromosome 11. Normal values for ferritinemia (individuals older than 30 years): males 49-421 mg/L; females 14-200 mg/. Proband 4 also has another hemoglobin anomaly for which she refused to be investigated. Other siblings have thalassemia minor.*

mutation was found using the DGGE method. Genotyping of several polymorphic sites along the short arm of chromosome 11 of the four probands with the initiation codon ATG→ACG identified a common restricted haplotype extending from intragenic Fw to the $^{A}\gamma$ globin gene (1/+/-) in all four patients (Table 1).

Of the six mutations found, three are common mutations in the Mediterranean area. They could have been introduced in the population by chance migration from southern Europe.

The other three mutations are rare, but already characterized in Balkan, Black and Japanese populations: (i) the IVS-I-1(G→C), β^0 5'splice site mutation, was found in two families in Japan[5] and Tadjikistan.[6] It is not referenced in the Hbvar database[1,2] (*http://globin.cse.psu.edu/hbvar/ menu.html, last accessed May 2006*); (ii) the IVS-II-849 (A→G), β^0 3'splice consensus has been identified in a few Black-American families;[7] the initiation codon ATG→ACG mutation (β^0 thal), already described in one Yugoslavian,[8] one Swiss[9] and one noble Russian family,[10] was found in four patients (probands 2, 4, 5 and 10).

Genotyping some polymorphic sites around the initiation codon ATG→ACG mutation provided evidence for a common haplotype in all four probands (1/+/-). Therefore, a founder effect, although quite ancient, is likely. Actually, it is known that the Breton population was quite autarchic up to the Second World War. Furthermore, the four families with this mutation were clustered in a small area in the North of Finistère, within a 30 kilometers radius (between Coat-Meal and Landivisiau). Genealogical reconstruction would possibly prove the linkage of all four families in the probably recent past, according to geographical data and Brittany demographics. However, these large chromosomal rearrangements indicate that it would not be possible to go far enough back in genealogy to identify the founder(s), as was done in the French-Canadian population in Portneuf County in Quebec.[11]

In conclusion, the molecular basis of β-thalassemia in the indigenous population of Finistère is quite heterogeneous, with the initiation codon mutation being present in four of the ten families carrying a thalassemia trait.

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