Variants in genetic modifiers of β-thalassemia can help to predict the major or intermedia type of the disease

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ABSTRACT

A cohort of 106 patients included in the French National Registry for Thalassemia were genotyped for 5 genetic modifiers of severity: i) β-thalassemia mutations; (ii) the XmnI SNP; (iii) the -3.7 kb α-thal deletion; (iv) the tag-SNP rs 11886868 in *BCL11A* exon 2; and (v) the tag-SNP rs9399137 in the *HBSB1L-cMYB* inter-region.

Multivariate analysis was performed to study the risk of thalassemia Intermedia phenotype associated with the different combinations of alleles. The presence or absence of the favorable alleles could accurately predict the type of thalassemia in 83.2% of the cases. The percentage of correct predictions made from the β-thalassemia mutations and the *Xmn*I SNP alone were significantly improved by the adjustment with the 3 other modifiers; from 73.6% to 83.2% ($P<0.001$).

In this study, we showed that predictions based on genetic modifiers can foresee the Major or Intermedia type of β-thalassemia, even in cohorts of patients with various β-globin genotypes.

Key words: β-thalassemia, Major, Intermedia, genetic modifiers.

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Introduction

Disease severity in patients with β-thalassemia varies greatly and patients are usually classified into Thalassemia Major (TM) or Intermedia (TI) according to clinical criteria. The requirement for at least 8 transfusions a year before the age of four years is often used to distinguish the 2 types of the disease.[1,2] The major determinant of disease severity is the degree of β-globin chain deficit (complete absence or variable reduction), resulting from the nature of the β-thalassemia alleles. Other genetic modifiers affecting the degree of α and non-α globin chain imbalance also impact the phenotypic severity.[3] An associated α-thalassemia which minimizes the excess of α-globin chains tends to produce a less severe β-thalassemia condition.[4] An increased residual level of HbF in adult life which compensates the decreased β-globin chain is also a major determinant of less severe disease.[5] Three major HbF Quantitative Trait Loci (QTL), accounting for 20-50% of HbF variation, have been identified so far. The first, the so-called -158 C>T *Xmn*I SNP (rs7482144), is located in the fetal Ggamma-globin gene promoter.[6] The other two are located in the *BCL11A* gene and in the *HBSB1L-cMYB* inter-region, and are either involved directly in fetal gene silencing in adult life or in cell proliferation and differentiation[7-10] Some particular tag-SNPs in these regions are associated with high HbF levels in healthy adults, as well as in thalassemia and sickle cell disease (SCD) patients.[9,11,12] Besides these favorable determinants, aggravating factors such as the coexistence of α-gene duplications may produce severe TI when associated with β-thalassemia traits.[13]

In this study, we investigated the effect that the combination of SNPs and β and α-thalassemia mutations might have on β-thalassemia severity in a cohort of 106 affected patients.

Design and Methods

A cohort of 106 thalassemia patients initially evaluated for molecular diagnosis of β-thalassemia mutations and included in the French National Registry for Thalassemia patients were genotyped for the *Xmn*I SNP, the -3.7 kb α-thalassemia deletion in *HBA* locus, the tag-SNPs rs 11886868 and rs4671393 in *BCL11A* exon 2, and the tag-SNPs rs9399137 and rs28384513 in the *HBSB1L-cMYB* inter-region. The

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choice of the tag-SNP was based on previously published association studies.[7-9] The clinical data collection in the registry has been approved by the Commission Nationale Informatique et Libertés (CNIL) and written consent for genetic studies was obtained from all patients prior to sampling. All patients were classified into TM (n=71) or TI (n=35) according to the registry criteria, i.e. TM: requirement for at least 8 transfusions a year before the age of four years. They represented 20% of the entire registry cohort and the ratio TM/TI was the same as recorded in the registry. Clinical characteristics of the TI patients at the time of the study are given in Table 1. β-thalassemia mutation spectrum of the entire study cohort was as follow: β39 (26%), IVS1-110 G>A (19%), HbE (6.5%), IVS1-6T>C (6%), IVS2-1G>A (5.5%), FsCd6-A (5%), IVS1-1G>A/T (4.5%), IVS1-2 T>A/C/G (3.5%), others (22 different mutations) (29%).

Statistical analyses were performed using PASW Statistics version 17.0. Continuous variables are reported as means and standard deviation or as medians and range (according to their distribution), and categorical variables are reported as count and percentages. Univariate and multivariate analyses were performed using a logistic regression model to estimate the risk of TI associated with the presence of favorable alleles. Odds ratios (OR) were estimated with a 95% confidence interval. Calibration of the logistic model was assessed using the Hosmer-Lemeshow goodness-of-fit test. A classification table was used to evaluate the predictive accuracy of the logistic regression model. Discrimination was assessed using the area under the receiver operating characteristic (ROC) curve: the greater the area under the curve (on a scale of 0.5 to 1), the better the ability of the model to discriminate. For all tests, statistical significance was defined as $P<0.05$.

Results and Discussion

Allele frequencies and OR are summarized in Table 2. Univariate analysis confirmed the links between the predictors and severity, and showed a significant increased risk for TI in the case of β^+-thalassemia mutations (OR: 5.644 [2.343-13.595]), presence of the *Xmn*I SNP (OR 5.817 [2.405-14.070]), presence of allele C at rs11886868 in *BCL11A* exons 2 (OR 4.888 [1.948-12.265]). No significant association was observed with α-thalassemia due to the small number of carriers in our cohort (allele frequency: 8/202) or for the presence of allele C at rs9399137 or allele C at rs28384513 in the *HBS1L-MYB* inter-region.

To assess the cumulative effect of these loci, a multivariate regression analysis was performed. Although α-thalassemia deletion and SNPs in the *HBS1L-MYB* inter-region were not significantly linked to TI in our study, they were included in the multivariate analysis due to the previously reported effects of these 2 modifiers on β-thalassemia.[4,9,14] Two SNPs were tested for each QTL in the *BCL11A* and in the *HBS1L-MYB* inter-region but only one, with the higher OR, was maintained for multivariate analysis. Logistic regression showed that all 5 types of favorable allele are significantly associated with TI phenotype (Table 2) and that the presence or absence of these favorable alleles can predict the Major type of the disease in 90.9% of the cases and the Intermedia type in 68.6% (overall prediction in 83.2%). The accuracy of predictions made only from the 2 major determinants, i.e. β-thalassemia mutations and the presence of the *Xmn*I SNP, was 73.6% and was significantly improved by the adjustment with SNPs in the *BCL11A* (79.2%, $P<0.001$) or in the *HBS1L-cMYB* inter-region (78.3%, $P<0.001$).

In order to determine an easy-to-use prediction tool, we used a variable defined as the number of ameliorating alleles carried by each patient, thus ranging from 0 to 10. Clinicians can evaluate this variable without the need for an algorithm. Following this simple scoring, all patients with score 0 were TM (97% with score 0 or 1) whereas all patients with 5 or 6 were TI (Figure 1A). When considering only patients with 2 β0 mutations, the scores ranged between 0 and 5 and were even more discriminating: 96% of the patients with a score between 0 and 2 were TM and 90% of the patients with a score between 3 and 5 were TI (Figure 1B).

Table 1. Clinical characteristics of TI patients (n=35).

TI patients' characteristics	
Median age (range)	26 years (7-57)
Transfusions n. (%)	
None	12 (34%)
Occasional	15 (43%)
Systematic	8 (23%)
Splenectomy n. (%)	20 (57%)
Hydroxyurea n. (%)	11 (31%)

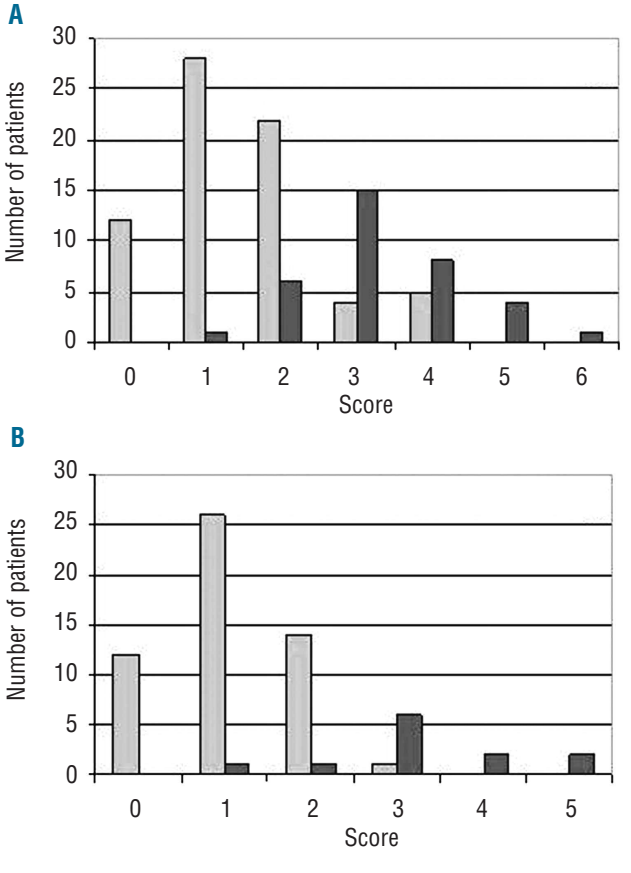

Figure 1. Distribution of the patients in the two groups: thalassemia Major (light gray) and thalassemia Intermedia (dark gray) according to the number of ameliorating alleles. (A) Distribution among all the patients scored for the 5 determinants. (B) Distribution among the 65 patients with 2 beta0 mutations.

Table 2. Association of ameliorating alleles with TI.

Ameliorating alleles	Alleles frequencies		Multivariate analysis			
	TM (n=71)	TI (n=35)	OR	95% CI (P)	AUC	95% CI
Beta+-thalassemia mutations	0.13	0.40	5.354	1.762-16.270 (0.003)	0.702	(0.593-0.811)
Alpha-thalassemia $\alpha^{-3.7kb}$	0.01	0.09	13.355	1.758-101.437 (0.012)	0.556	(0.435-0.678)
-158XmnI (A)	0.13	0.43	9.182	2.849-29.594 (<0.001)	0.702	(0.592-0.812)
BCL11A rs11886868 (C)	0.25	0.48	6.370	1.841-22.034 (0.003)	0.681	(0.575-0.788)
HBS1L rs9399137 (C)	0.20	0.26	3.895	1.192-12.726 (0.024)	0.538	(0.421-0.656)

Beta-thalassemia mutation classification into beta+ or beta0 type was made according to globin gene server (http://globin.cse.psu.edu/) except for IVS1-110 and IVS 2-745 which are severe beta+ and were considered as beta0-thalassemia mutations; Hb E was considered as a beta+ mutation. OR: Odds Ratio; AUC: area under the curve.

Galanello *et al.* has previously shown that scoring based on genetic modifiers can predict the type of thalassemia.[14] However, their study only concerned a group of highly homogeneous β-thalassemia patients; homozygous for the β39 mutation and negative for the *Xmn*I SNP. Conversely, our present study includes patients of various geographical origins and different β-globin gene genotypes, as is often the case in countries non-endemic for hemoglobinopathies. Indeed, up to 30 different mutations found in various combinations have been characterized in our series and are a mix of Mediterranean (3/4) and Asian (1/4) mutations. Despite this large genetic background, the predictions based on variant determination of genetic modifiers can correctly foresee the type of thalassemia in 83.2% of the cases using logistic regression.

Distinction between TM and TI is currently based on clinical criteria and it often, therefore, takes at least four years of follow up before classification can be confirmed. Variant genotyping of genetic modifiers may possibly help in the early prediction of the type of thalassemia the patient will develop later. If further validated, this prediction tool of severity may have implications not only for genetic counseling but also for therapeutic decision making concerning, for example, hematopoietic stem cell transplantation.

Authorship and Disclosures

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