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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) beta-thalassemia mutations, (ii) the $XmnI$ SNP, (iii) the -3.7 kb alpha-thal deletion, (iv) the tag-SNP rs11886868 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 favourable alleles could accurately predict the type of thalassemia in 83.2% of the cases. The percentage of correct predictions made from the beta-thalassemia mutations and the $XmnI$ SNP alone were significantly improved by the adjustment with the 3 other modifiers, moving 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 beta-thalassemia, even in cohorts of patients with various beta-globin genotypes.
Introduction

Patients with beta-thalassemia display a large variability in disease severity and are usually classified into thalassemia major (TM) or intermedia (TI) according to clinical criteria. The requirement for at least 8 transfusions a year before 4 years of age is often used to distinguish the 2 types of the disease. The major determinant of the severity is the degree of beta-globin chain deficit (complete absence or variable reduction), resulting from the nature of the beta-thalassemia alleles. Other genetic modifiers affecting the degree of alpha and non alpha-globin chain imbalance, also impact the phenotypic severity. An associated alpha-thalassemia which minimizes the excess of alpha-globin chains, tends to produce a less severe beta-thalassemia condition. An increased residual level of HbF in adult life which compensates the decreased beta-globin chain, is also a major amelioration determinant. Three major HbF Quantitative Trait Loci (QTL), accounting for 20-50% of HbF variation, have been identified so far. The first one, the so-called -158 C>T XmnI SNP (rs7482144), is located in the fetal Ggamma-globin gene promoter. The two others are located in the BCL11A gene and in the HBSB1L-cMYB inter-region and are either involved directly in fetal genes silencing in adult life or in cell proliferation and differentiation. Some particular tag-SNPs in these regions are associated with high HbF levels in healthy adults as well as in Thalassemia and SCD patients. Aside these ameliorating determinants, aggravating factors such as the coexistence of alpha-gene duplications may produce severe TI when associated with beta-thalassemia traits.

In this study, we investigated the effect that these SNPs and beta and alpha-thalassemia mutations might exert in combination on beta-thalassemia severity, in a cohort of 106 affected patients.
Design and Methods

A cohort of 106 thalassemia patients initially addressed for molecular diagnosis of beta-thalassemia mutations and included in the French National Registry for thalassemia patients, were genotyped for the XmnI SNP, the -3.7 kb alpha-thalassemia deletion in HBA locus, the tag-SNPs rs 11886868 and rs4671393 in BCL11A exons 2 and (v) the tag-SNPs rs9399137 and rs28384513 in the HBSB1L-cMYB inter-region. The choice of the tag-SNP was based on previously published association studies. The clinical data collection in the registry has been approved by the Commission Nationale Informatique et Libertés (CNIL) and written consents for genetic studies were obtained prior to sampling. All patients were classified into TM (n=71) or TI (n=35) according to the registry criteria (requirement for at least 8 transfusions a year before 4 years of age). They represented 20% of the whole cohort included in the registry and the ratio TM/TI was the same as in the registry. Clinical characteristics of the TI patients at the time of the study are given in Table 1. Beta-thalassemia mutations spectrum of the all cohort was as follow: Beta39 (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 conducted 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 favourable 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**

Alleles 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 case of $\beta^+$-thalassemia mutations (OR: 5.644 [2.343-13.595], presence of the $XmnI$ 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 alpha-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 $HBSIL-MYB$ inter-region.

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

In order to settle 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. This variable can be
evaluated by the clinicians without the need of 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 beta° mutations, the scores ranged between 0 and 5 and were even more discriminant: 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).

Galanello et al showed previously that scoring based on genetic modifiers can predict the type of thalassemia. However their study only concerned a group of highly homogeneous beta-thalassemia patients, homozygous for the beta39 mutation and negative for the XmnI SNP. Conversely, our present study concerns patients with various geographic origins and different beta-globin gene genotypes, as it is often the case in non-endemic countries 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 thus, often necessitates at least 4 years of follow up before classification. Variant genotyping of genetic modifiers may possibly help to predict early in life, the type of thalassemia developed later on by a patient. If further validated, this prediction tool of severity may have implications for genetic counselling but also for decisions regarding therapeutic options such as Hematopoietic Stem Cell transplantation.
Acknowledgments

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Authorship and Disclosures

CB took primary responsibility for the paper and wrote it; IA, KG and SF performed experimental study; CB, SP and PJ collected and analysed the data, MCS and AL performed statistical analysis. IT and AF provided administrative support and access to study material. All authors contributed to critical revision and final approval of the version to be published. The authors reported no potential conflict of interest.

References


### TI Patients characteristics

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<table>
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<tbody>
<tr>
<td>Median age (range)</td>
<td>26y (7-57)</td>
</tr>
<tr>
<td>Transfusions: n (%)</td>
<td></td>
</tr>
<tr>
<td>None:</td>
<td>12 (34%)</td>
</tr>
<tr>
<td>Occasional:</td>
<td>15 (43%)</td>
</tr>
<tr>
<td>Systematic</td>
<td>8 (23%)</td>
</tr>
<tr>
<td>Splenectomy: n (%)</td>
<td>20 (48%)</td>
</tr>
<tr>
<td>Hydroxyurea: n (%)</td>
<td>11 (31%)</td>
</tr>
</tbody>
</table>

**Table 1.** Clinical characteristics of TI patients (n=35).
<table>
<thead>
<tr>
<th>Ameliorating alleles</th>
<th>Alleles frequencies</th>
<th>Multivariate analysis</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TM (n=71)</td>
<td>TI (n=35)</td>
<td>OR</td>
<td>95% CI (p)</td>
<td>AUC</td>
<td>95% CI</td>
</tr>
<tr>
<td>Beta+-thalassemia</td>
<td>0.13</td>
<td>0.40</td>
<td>5.354</td>
<td>1.762-16.270 (0.003)</td>
<td>0.702</td>
<td>(0.593-0.811)</td>
</tr>
<tr>
<td>Alpha-thalassemia α⁻³⁻⁷kb</td>
<td>0.01</td>
<td>0.09</td>
<td>13.355</td>
<td>1.758-101.437 (0.012)</td>
<td>0.556</td>
<td>(0.435-0.678)</td>
</tr>
<tr>
<td>-158XmnI (A)</td>
<td>0.13</td>
<td>0.43</td>
<td>9.182</td>
<td>2.849-29.594 (&lt;0.001)</td>
<td>0.702</td>
<td>(0.592-0.812)</td>
</tr>
<tr>
<td>BCL11A rs11886868 (C)</td>
<td>0.25</td>
<td>0.48</td>
<td>6.370</td>
<td>1.841-22.034 (0.003)</td>
<td>0.681</td>
<td>(0.575-0.788)</td>
</tr>
<tr>
<td>HBS1L rs9399137 (C)</td>
<td>0.20</td>
<td>0.26</td>
<td>3.895</td>
<td>1.192-12.726 (0.024)</td>
<td>0.538</td>
<td>(0.421-0.656)</td>
</tr>
</tbody>
</table>

**Table 2.** Association of ameliorating alleles with TI.

Beta-thalassemia mutations classification into beta⁺ or beta⁻ type was done 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 beta⁻-thalassemia mutations; Hb E was considered as a beta⁺ mutation.

OR: Odd Ratio; AUC: Area Under the Curve.
Figure 1. Distribution of the patients in the 2 groups, Thalassemia Major (blue) and Thalassemia Intermedia (purple) 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 beta° mutations.