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# BAT2 and BAT3 polymorphisms as novel genetic risk factors for rejection after HLA-related stem cell transplantation

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## Abstract

The genetic background of donor and recipient is an important factor determining the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT). We applied a whole genome

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analysis to investigate genetic variants - other than HLA class I and II - associated with negative outcome after HLA-identical sibling allo-HSCT in a cohort of 110 β-Thalassemic patients. We identified two single nucleotide polymorphisms in *BAT2* (A/G) and *BAT3* (T/C) genes, SNP rs11538264 and SNP rs10484558, both located in the HLA class III region, in strong Linkage Disequilibrium between each other ($R^2$=0.92). When considered as single SNP, none of them reached a significant association with graft rejection (nominal $P < 0.00001$ for BAT2 SNP rs11538264, and $P < 0.0001$ for BAT3 SNP rs10484558). Whereas, the BAT2/BAT3 A/C haplotype was present at significantly higher frequency in patients who rejected as compared to those with functional graft (30.0% *vs.* 2.6%, nominal $P = 1.15\times10^{-8}$; and adjusted P = 0.0071).

The BAT2/BAT3 polymorphisms and specifically the A/C haplotype may represent novel immunogenetic factor associated with graft rejection in patients undergoing allo-HSCT.

### Keywords

Beta-thalassemia; Allogeneic hematopoietic stem transplantation; whole genome analysis; BAT2; BAT3

## Introduction

Graft versus Host Disease (GvHD) and graft rejection are the main limiting factors for successful allogeneic hematopoietic stem cell transplantation (allo-HSCT) to cure malignancy or inborn genetic disorders of the hematopoietic system. The improvement of HLA molecular typing has allowed the definition of better criteria for compatibility and donor selection. A more accurate HLA class I and II matching together with improved graft preparations, patient conditioning, and targeted prophylaxis have been essential to decrease the incidence of GvHD, especially in the context of malignant diseases. In addition, recent studies in large cohorts of non-malignant patients transplanted with unrelated allo-HSCT revealed the relevance of allelic and antigenic HLA matching for post-transplant graft failure and rejection, nonetheless a high rate of both complications persists especially in the non-malignant setting [1-5].

In the last decade, the potential role of polymorphisms in genes encoding for non-HLA antigens in predicting HSCT complications has been widely investigated in the attempt to identify novel independent risk factors that may permit a more accurate prediction of transplant-related complications and help in designing individualized prophylaxis. Single nucleotide polymorphisms (SNPs) of cytokines, such as Tumour Necrosis Factor (TNF)-α or Interleukin (IL)-10, cytokines receptors, or genes associated with innate immunity (i.e. Killer-cell immunoglobulin-like receptors) have been associated with allo-HSCT outcomes in patients transplanted with hematopoietic stem cells from related or unrelated HLA-identical donors [5-9].

To date, allo-HSCT represents the only curative treatment for β-Thalassemic patients [10], who still have a lower median life expectancy compared to healthy individuals, despite regular blood transfusions and supportive care. In these patients the incidence of GvHD after transplant is typically low thanks to the optimization of transplant composition,

conditioning, and prophylaxis both in related and unrelated transplants [11]. However, the success of allo-HSCT for the cure of β-Thalassemia is still hampered by the rate of graft rejection that is significantly higher, as compared to patients transplanted for malignancies. Several factors have been evoked to explain this difference. Recipient allo-immunization due to massive pre-transplant exposure to blood products and absence of chemotherapy treatments prior to conditioning contribute to better preserve or even enhance immune reactivity in β-Thalassemic patients [12]. However, a comprehensive characterization of the immunogenetic factors that influence graft rejection in this patient population, as in other non-malignant hematologic diseases, is still missing.

In this study, we performed a whole genome association analysis using Affymetrix gene chip array technology, which allows to genotype more than 900,000 SNPs. We investigated whether specific genetic variants are associated with graft rejection after HLA-related HSCT in a cohort of β-Thalassemic patients treated with fairly homogenous transplant protocols.

## Materials and Methods

### Patients

The present study includes 110 patients who underwent HSCT between 2004 and 2010 from a genotypically HLA-identical 12/12 allele-level matched sibling. All patients were affected by β-Thalassemia Major and received a myeloablative-conditioning regimen followed by the infusion of un-manipulated bone marrow cells. Patients with β-Thalassemia in class 1 or 2 (according to the Pesaro classification) were given protocol (PC)6, a conditioning regimen based on 14 mg/kg Busulfan (Bu) and 200 mg/kg Cyclophosphamide (Cy), or PC6.1, 14 mg/kg Bu, 200 mg/kg Cy and 10 mg/kg Thiothepa (TT). β-Thalassemic patients in class 3 (according to the Pesaro classification) were conditioned with PC26 consisting of a pre-transplant treatment starting at day −45 with 3 mg/Kg Azatioprine (Az), at day −17 with 30 mg/Kg of Hydroxyurea (HU) and from day −16 to day −12 with 30 mg/m$^2$ Fludarabine (Flu), followed by 14 mg/kg Bu and a reduced dose of Cy (160 mg/kg). PC26 MOD was equal to PC26, with the addition of 10 mg/kg TT (Table 1). Intravenous Cyclosporin A was started at 5 mg/kg from day −2 to day +5, and later reduced to 3 mg/kg until post-transplant day 60 when it was tapered off 5% per week and discontinued at 1 year. The desired plasma range was 150-250 ng/mL. Intravenous methylprednisolone (MP) was started at 0.5 mg/kg at day −1 and stopped 30 days post-transplant. Short methotrexate (MTX), 10 mg/m$^2$ was, given intravenously at days 1, 3 and 6 post-transplant with folinic rescue. The study was approved by the Ethical Committee of the Policlinico Tor Vergata, Rome and by the Ethical Committee of San Raffaele Scientific Institute, Milan. We obtained informed consent from patients according to institutional guidelines and to the Helsinki Declaration.

### Analysis of donor chimerism after HSCT

Recipient and donor DNA samples, extracted by QIAamp DNA Blood mini Kit (Qiagen, Valencia, CA, USA) were typed by short tandem repeats (STR) and amelogenin locus using the AmpFISTR Profiler Plus kit (Applera, Foster City, CA, USA). Amplification reactions were carried out using 1-2 ng of input DNA following the manufacturer's recommendations. PCR products were run on an ABI Prism 3130xl Genetic Analyzer (Applera, Foster City,

CA, USA). Informative loci for post-transplant samples were screened for quantification of the donor cell percentage in mixed chimerism (MC). Quantitative determination of engraftment was performed using fluorescent PCR primers for human identity markers based on the ratio between peak areas of donor and recipient alleles. The mean value obtained after performing calculations for each informative STR was considered as the percentage of MC.

### SNP Genotyping

PBMC from β-Thalassemic patients transplanted with HLA-related HSCT and donors were genotyped with the Affymetrix Genome-Wide Human SNP 6.0 Array (Affymetrix, Inc. Santa Clara, CA, USA), including probes for more than 906,000 SNPs and for the detection of copy number variants (CNVs) [13], according to manufacture's instructions. Briefly, total genomic DNA (500 ng) was digested with Nsp I and Sty I enzymes, ligated to adaptors and amplified using a primer that recognizes the adaptor sequence. The amplified DNA was then fragmented, labelled and hybridized to oligonucleotide probes attached to the surface of an array in a GeneChip Hybridization Oven 640 (Affymetrix, Inc.), followed by washing and staining procedures performed on a GeneChip Fluidics Station 450 (Affymetrix, Inc.). Arrays were scanned using the GeneChip Scanner 3000 7G (Affymetrix, Inc.).

### Sequencing

Two different pairs of primers that amplify the DNA fragments containing the rs11538264 and the rs10484558 variants were designed to provide 222 bp and 263 bp fragments, respectively. The template DNA was amplified in a total volume of 25 μl using standard PCR procedures using an annealing primer-specific temperature of 56° C for rs11538264 and 58° C for rs10484558. The two specific amplification primer pairs were a) CTGAGTTGGGTGGAGAGAAG and AGCAATCTTCCCCAGAAATC, b) CTCCACTTCTTCTGCCTCCA and CCATGCCTTCAGGGAGTCTA, respectively. PCR products were purified using the Agencourt Ampure XP system following the manufacturer's instructions. Sequencing reactions were performed using 10 μl of purified PCR products, forward or reverse primers used in PCR reaction and ABI Bigdye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified using a standard ethanol precipitation and the fluorescent labelled extension products were loaded into an ABI 3730 DNA analyzer (Applied Biosystems). To avoid errors or artefacts in DNA sequencing, each sample was sequenced on both DNA strands.

### Data analysis

The obtained intensity files were analysed with the *Affymetrix Power Tools* package (APT version 1.12.0). Quality Control was performed using the Contrast QC algorithm (CQC), carried out with the executable APT-GENO-QC, using the default settings. Eight individuals showing CQC values lower than 0.4 were removed from the dataset. Genotypes used for association analysis were called using the Birdseed v2 algorithm implemented in the executable APT-PROBESET-GENOTYPE, using defaults settings. All samples with a call rate > 90.00 were retained. The all rate of remaining samples was 98.88±1.031, with a maximum of 99.804 and a minimum of 94.277. Finally, the adopted algorithm successfully

called 905,556 SNPs, located on autosomes and sexual chromosomes. Output files were converted in PLINK format [14], and annotated with Affymetrix release 31, corresponding to Build GCRhv37. In order to create an informative dataset of common autosomal markers, to avoid detection of false positive signals of association and to remove SNPs incorrectly genotyped, we excluded from 905,556 called markers, SNPs without map references, SNPs with call rate < 95%, MAF < 0.05 and out of Hardy-Weinberg Equilibrium ($P<10^{-5}$). A dataset of 617,049 informative autosomal SNPs was identified. All filtering procedures were performed with PLINK software [14].

Association tests were performed between patients with rejection (GR) and patients with functional graft (FG). At the SNP level, we used the function *--fisher* of *PLINK* software, and we corrected results for multiple tests with the Bonferroni method. We also used a second cut-off of $P<10^{-5}$ to detect potential associations. At the haplotype level, we used the *--hap-assoc* function to estimate haplotype frequencies with the E.M algorithm [15] and to compute $\chi 2$ test between cases and controls (Affymetrix, Inc.). With the aim to associate candidate SNPs with known and predicted regulatory elements, we used the Regulome database [16]. Finally, the regression analysis to investigate the influences of covariants on transplant outcome (conditioning regimens, Pesaro class risk, and age at transplantation) was performed using the function *-gml* as implemented in the R package [17].

## Results

### Identification of SNPs differentially expressed in β-Thalassemic patients who reject HLA-identical HSCT graft

A total of 110 β-Thalassemic patients transplanted from genotypically HLA-identical 12/12 allele-matched siblings were studied. To define genetic risk factors associated with rejection, β-Thalassemia patients who rejected transplants (Graft Rejection, GR, n=15) were compared to patients who developed either Complete Chimerism (CC, n=85) or Persistent Mixed Chimerism, in which donor and recipient cells stably coexist (PMC, n=10) (Table 1). Patients belonging to these two last groups are henceforth referred to as functional graft (FG, n=95). Unlike patients who reject the graft, patients who develop CC or PMC are indeed disease free and transfusion independent [18].

Initially, SNPs were genotyped and analysed in a first group of 45 transplanted patients (GR, n=9 *vs.* FG, n= 36) using the Affymetrix 6.0 array, allowing the analysis of a total of 617,049 informative SNPs located on autosomes. After Bonferroni correction for multiple tests, (correction for 617,049 test, cut-off P value = $8.1\times10^{-8}$), no SNPs showed a statistically significant difference between GR and FG patients. We then chose a nominal *P* value of $<1\times10^{-5}$ as cut-off to detect potential differences in allele frequency between GR and FG patients. Using this criterion, we found 6 SNPs located in chromosomes 1, 2, 6, and 11 (Table 2, and Figure 1S). In addition to these SNPs, we found also 37 SNPs with a nominal *P* value $<1\times10^{-4}$ (Table 1S).

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### SNPs in BAT2 (A/G) and BAT3 (T/C) genes are predominant in β-Thalassemic patients who rejected allo-HSCT graft

All but one SNPs with potential differences in allele frequency between GR and FG patients (Table 2) were located either in inter-genic regions, in genes with unknown function, or not directly associated to immunologic functions. We therefore further characterized this unique SNP - rs11538264 ($P$=4.26×10$^{-6}$) - located in the *BAT2* gene encoding for proline-rich coiled-coil 2A protein (PRRC2A) in proximity of the TNF-α and TNF-β loci of chromosome 6 in HLA class III region [19]. Interestingly, this SNP is in linkage disequilibrium (LD) ($R^2$ = 0.86) with the SNP (rs10484558) located in *BAT3* gene, at 12.3 Kb downstream *BAT2* gene, encoding for a large proline-rich protein, (BAG6) with a nominal $P$ value of 3.01×10$^{-5}$ (Table S1).

SNP rs11538264 (*BAT2*) leads to the A or G alleles, whereas SNP rs10484558 (*BAT3*) leads to the C or T alleles, being the allele G for *BAT2* and the allele T for *BAT3* the most represented in human populations, with frequencies greater than 80% in the populations analysed [20]. In contrast, the A allele of SNP rs11538264 *(BAT2*) and the C allele of SNP rs10484558 *(BAT3*) were significantly more frequent in the GR (n=9) than the FG (n=36) patient group (43.8% *vs.* 0.00%, and 43.8% *vs.* 1.8%, respectively). This result is even more significant considering that the frequency of the allele A (rs11538264, *BAT2*) and of the allele C (rs10484558, *BAT3*) was 4.2% in the parents of transplanted β-Thalassemic patients, and of 5% in a small (n=10) cohort of independent individuals from the same ethnic group, analysed in parallel. These data are in line with the frequencies of the allele A (rs11538264, *BAT2*) and of the allele C (rs10484558, *BAT3*) reported in databases obtained in wide populations (Table S2) [20].

We validated the presence of SNPs rs11538264 and rs10484558 by means of Sanger sequencing in 25 patients previously genotyped by microarray, and we extended the analysis to additional 65 β-Thalassemic patients who underwent allo-HSCT (n=6, GR and n=59, FG patients). The frequency of the allele A (rs11538264, *BAT2*) and of the allele C (rs10484558, *BAT3*) in this latter population resulted higher in the GR compared to the FG patient group, but it did not reach the statistical significance, possibly because numerically limited (data not shown). Since these additional transplanted β-Thalassemic patients were identical to those analysed in microarrays in terms of disease, ethnicity, and treatment, we established the allele and genotype frequencies of the total samples (n=15, GR and n=95, FG patients). Overall, GR patients (n=15) carried significantly higher frequencies of the allele A of rs11538264 (*BAT2*) (9/30 (30%) *vs*. 5/190 (2.6%); $P$=6.34×10$^{-6}$; OR=15.86), and of the allele C of rs10484558 (*BAT3*) (9/30 (30%) *vs*. 6/190 (6.3%); $P$=1.44×10$^{-5}$; OR=13.1) compared to FG patients (n=95) (Table 3). Furthermore, the respective genotype frequencies A/A, A/G, and G/G of the *BAT2* - SNP rs11538264, and C/C, C/T, and T/T of *BAT3* - SNP rs10484558, were differentially represented in the GR compared to the FG patient group, being the genotype A/A (*BAT2* - SNP rs11538264) and C/C (*BAT3* - SNP rs10484558) never detected in the FG patients (Table 2).

### The BAT2/BAT3 A/C haplotype is associated with graft rejection after allo-HSCT for β-Thalassemia

SNP rs11538264 (*BAT2*) and SNP rs10484558 (*BAT3*), separated by 12.3 Kb, are in strong Linkage Disequilibrium (LD) ($R^2$=0.92 considering the total sample). Haplotypes estimates by EM algorithm revealed only three (AC, GC, and GT) of the four possible haplotypes, with haplotype AT not found in the present patient cohort and with haplotype GC showing a frequency less than 1%. In line with the allele frequency observations, haplotype AC had a significantly higher frequency in GR (n=15) compared to FG (n= 95) patients (30.0% (9/30) *vs.* 2.6% (5/190), nominal $P=1.15\times10^{-8}$, adjusted $P$=0.0071, Table 3). The haplotype containing the predominant alleles (GT) had a prevalence of 70.0% (21/30) in GR patients and 96.8% (184/190) in FG patients (nominal $P=5.94\times10^{-8}$, adjusted $P$=0.037, Table 3). To associate the identified SNP rs11538264 (*BAT2*) and SNP rs10484558 (*BAT3*) with known and predicted regulatory DNA elements, we used the RegulomeDB database [16]. Results indicate SNP rs11538264 as an eQTL (Expression Quantitative Trait Loci) influencing the expression level of non-classical HLA-C gene, as reported by [21] (GHSdatabase:http://genecanvas.ecgene.net/uploads/ForReview/ghs_probe_express030510.zip).

With the aim to test the influence of additional risk factors of rejection, we performed regression analysis considering as independent variables: conditioning regimens, Pesaro risk class, and age comparing patients with different SNPs. Results were not significant for conditioning protocols, age at transplantation and the Pesaro risk class 3, in which the majority of the analyzed patients belonged to (n=68, Table 1). Notably, risk class often shows a high association with post-transplantation complication and mortality, but in regression analyses previously performed rejection was never associated with this specific Pesaro risk class[10]. Based on these analyses, we concluded that patients carrying different BAT2 and BAT3 SNPs are similar with respect to other risk factors of rejection after allo-HSCT.

## Discussion

By genome wide analysis we have identified two single nucleotide polymorphisms (rs11538264 and rs10484558) in the *BAT2* and *BAT3* genes, within the HLA class III region, in strong linkage disequilibrium, that are statistically associated, at the haplotype level (adjusted P=0.0071), with graft rejection after HLA-identical HSCT. For the first time, an association between the haplotype AC, of *BAT2* and *BAT3* genes, and rejection was indeed detected after HLA-identical sibling HSCT in β-Thalassemia patients. In addition, evaluation of the BAT2/BAT3 SNPs also defines an haplotype (GT) which is significantly, although not exclusively, associated with functional grafts.

Both BAT2 and BAT3 belong to the B-associated transcripts; *BAT2* encodes a proline-rich coiled-coil 2A protein whose function has not yet been determined, and *BAT3* encodes a large proline-rich protein that acts as regulator of histone H3K4 demethylation, subsequent gene transcription [22], and regulation of apoptosis [23-26]. More recently, it has been observed that the *BAT2* transcript, together with interferon (IFN)-γ and TNF-α transcripts, was up-regulated in peripheral blood mononuclear cells (PBMC) of two patients with an on going GvHD after allo-HSCT [27], suggesting a possible role of BAT2 in inflammatory responses

after allo-HSCT. Notably, the SNP rs11538264 (A/G) of *BAT2* described here is a non-synonimous variation, causing a VAL1774MET substitution that may impact on the biological function of the protein. Our results are strengthened by the evidence that the SNPs rs11538264 could be involved in regulation of expression levels of HLA-C in monocytes [21].

BAT3 has recently been shown to be critically involved in regulating HLA class II expression [28]: IFN-γ induces BAT3 expression that facilitates the nuclear import of the class II transactivator (CIITA), with subsequent activation of HLA class II gene expression in antigen-presenting cells (APCs). Moreover, BAT3 is primarily expressed by T helper (Th)1 cells and protects them from cell death [29]. Interestingly, *BAT2* and *BAT3* polymorphisms have been associated with increased incidence of type 1 diabetes and have been hypothesized to be involved in the inflammatory process of pancreatic beta-cell destruction [30, 31]. In addition, it has been shown that several isoforms of BAT3 exist and that their expression is cell-specific, which may contribute to a specific activity in a given cells [32]. These findings, together with the described role of BAT3 as chaperone in regulating the pattern of HLA class II gene expression in APCs, suggest that BAT3 may be critically involved in modulating effector T cell responses.

Although we could not test in our β-Thalassemia patients whether the allelic variant of *BAT3* resulted in an altered protein and function, sequence analysis of the genetic variants and bioinformatic analyses with RegRNA [33] and ESEFinder [34] predicted the disruption of an exon splicing enhancer, binding site for the splicing factor SRp20, caused by the C allele of the rs10484558 SNP *(BAT3*) [35], and the introduction of two novel binding sites for the SRSF1 and SRSF5 splicing factors, respectively. It can thus be hypothesized that alteration of the putative enhancer results in an inefficient inclusion of the seventh exon of the BAT3 transcript encoding the first of the two proline rich regions of the protein, and may limit the protein-protein interactions. Future studies are warranted in order to elucidate whether, for example, the *BAT3* C allele encodes a protein with modulated chaperone activity, which may lead to increased HLA class II expression in APCs and/or to enhanced Th1 cell activation in the context of allo-HSCT rejection.

In conclusion, by genome wide analysis we have identified two polymorphisms (rs11538264 and rs10484558) in the *BAT2* and *BAT3* genes within the HLA class III region, in strong LD. The haplotype containing the A allele of SNP rs11538264 *(BAT2*) and the C allele of SNP rs10484558 *(BAT3*) is associated with graft rejection after HLA-identical sibling HSCT in β-Thalassemic patients. In addition to providing a potentially useful indication for individualized prophylaxis against rejection in β-Thalassemic patients carrying the relevant polymorphisms, the identified haplotype for the *BAT* genes may represent new molecular markers for risk assessment in allo-HSCT also for other non-malignant hematologic diseases and, possibly, organ transplantation. An important question to be addressed in future studies is whether these polymorphisms are specifically associated with graft rejection after allo-HSCT in β-Thalassemia or can be also relevant in other clinical settings of allo-HSCT. Moreover, it could also be important to test whether the identified BAT2 and BAT3 SNPs would be associated to graft rejections after allo-HSCT for other clinical indications or after allo-HSCT with different HLA disparities.

Therefore, the general relevance of these polymorphisms as predictive markers of rejection beyond the present context of allo-HSCT in β-Thalassemia, has to be addressed in future studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

**Characteristics of HLA-related HSC transplanted β-Thalassemic patients.**

| **Patients** | **n (%)** |
|---|---|
| Male | 59 (53.6%) |
| Female | 51 (46.4%) |
| Age transplantation; years; median (range) | 10.5 (2-27) |
| **Conditioning Protocols** * | **n (%)** |
| PC 26 | 46 (41.8%) |
| PC 26MOD | 24 (21.8%) |
| PC 6 | 30 (27.3%) |
| PC 6.1 | 10 (9.1%) |
| **Pesaro Class risk** | **n (%)** |
| 1 | 6 (5.4%) |
| 2 | 36 (32.7%) |
| 3 | 68 (61.8%) |
| **Transplantation Outcome** | **n (%)** |
| Graft Rejection (GR) | 15 (13.6%) |
| Persistent Mixed Chimerism (FG) | 10 (9,1%) |
| Complete Chimerism (FG) | 85 (77.3%) |

*PC26, protocol 26, was a conditioning regiment for β-Thalassemic patients in class 3 (according to the Pesaro classification) consisting of a pre-transplant treatment starting at day −45 with 3 mg/Kg Azatioprine (Az), at day −17 with 30 mg/Kg of Hydroxyurea (HU) and from day −16 to day −12 with 30 mg/$m^2$ Fludarabine (Flu), followed by 14 mg/kg Bu and a reduced dose of Cy (160 mg/kg). PC26 MOD, protocol 26 modified, was a conditioning regiment for β-Thalassemic patients in class 3 (according to the Pesaro classification) and was equal to PC26, with the addition of 10 mg/kg TT. PC6, protocol 6, was a conditioning regimen for β-Thalassemic patients in class 1 or 2 (according to the Pesaro classification) based on 14 mg/kg Busulfan (Bu) and 200 mg/kg Cyclophosphamide (Cy). PC6.1, protocol 6.1, was a conditioning regimen for β-Thalassemic patients in class 1 or 2 (according to the Pesaro classification) based on 14 mg/kg Bu, 200 mg/kg Cy and 10 mg/kg Thiothepa (TT).

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**Table 2**

**Allele frequency differences between GR and FG patients of SNPs showing with nominal P value < $1\times10^{-5}$.**

| Chr | SNP | Location (Bp) | Allelic Variants | P value | Adjusted P value | *Gene* | Left Gene | Right Gene |
|---|---|---|---|---|---|---|---|---|
| 1 | rs1831870 | 57627203 | G/A | $9{,}3\times10^{-7}$ | 0,574 | *DAB1* | **C8B** | *LOC729423* |
| 1 | rs10889030 | 57628701 | T/A | $2.88\times10^{-6}$ | 1,000 | *DAB1* | *C8B* | *LOC729423* |
| 6 | rs11538264 | 31603189 | G/A | $4.26\times10^{-6}$ | 1,000 | *BAT2* | *SNORA38* | *BAT3* |
| 11 | rs10891245 | 111167792 | T/G | $6.29\times10^{-6}$ | 1,000 | *FLJ45803* | *C11orf53* | *LOC120376* |
| 11 | rs12792445 | 111176351 | C/T | $6.29\times10^{-6}$ | 1,000 | *LOC120376* | *FLJ45803* | *POU2AF1* |
| 2 | rs983970 | 126140847 | A/G | $6.59\times10^{-6}$ | 1,000 | - | *CNTNAP5* | *LOC150554* |

SNPs with putative association ($P < 10^{-5}$) in GR patients as compared to FG patients. Nominal *P* value from association study and adjusted *P* values obtained with Bonferroni correction (correction for 617,049 test, cut-off *P* value = $8.1\times10^{-8}$) are shown.

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**Table 3**

**Allele and genotype frequencies of rs11538264 (*BAT2)* andrs10484558 (*BAT3)* genes in HLA-related HSC transplanted β-Thalassemic patients with different outcomes.**

| Gene | | | Number (Frequency %) of patients | | Nominal P value | Adjusted P value |
|---|---|---|---|---|---|---|
| | | | Graft Rejection | Functional Graft | | |
| ***BAT2*** | **Allele** | A | 9/30 (30.0) | 5/190 (2.6) | $6.34\times10^{-6}$ | 1.000 |
| | | G | 21/30 (70.0) | 185/190 (97.4) | | |
| | **Genotype** | A/A | 1/15 (6.7) | 0/95 (0.0) | | |
| | | A/G | 7/15 (46.7) | 5/95 (5.3) | | |
| | | G/G | 6/15 (40.0) | 90/95 (94.7) | | |
| ***BAT3*** | **Allele** | C | 9/30 (30.0) | 6/190 (3.2) | $1.44\times10^{-5}$ | 1.000 |
| | | T | 21/30 (70.0) | 184/190 (96.8) | | |
| | **Genotype** | C/C | 1/15 (6.7) | 0/95 (0.0) | | |
| | | C/T | 7/15 (46.7) | 6/95 (6.3) | | |
| | | T/T | 6/15 (40.0) | 89/95 (93.7) | | |
| ***BAT2/BAT3*** | **Haplotypes** | AC | 9/30 (30.0) | 5/190 (2.6) | $1.15\times10^{-8}$ | 0.007 |
| | | GC | 0/30 (0.0) | 1/190 (0.50) | 0.690 | 1.000 |
| | | GT | 21/30 (70.0) | 184/190 (96.8) | $5.94\times10^{-8}$ | 0.037 |

The allele, genotype and haplotype frequencies for BAT2 and BAT3 polymorphisms in 110 β-Thalassemic transplanted patients (n=15, GR and n=95, FG patients). Nominal *P* value from association study and adjusted *P* values obtained with Bonferroni correction (correction for 617,049 test, cut-off *P* value = $8.1\times10^{-8}$) are shown.