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ORIGINAL ARTICLE

HLA GAMMA BLOCK MATCHING IN UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION AND THE DEVELOPMENT OF GRAFT VERSUS HOST DISEASE

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Abstract: The human leukocyte antigen (HLA) genes routinely typed for hematopoietic stem cell transplantation (HSCT) are HLA-A, -B, -C, -DRB1 and -DQB1. HLA mismatches (MM), which have been associated with many post-transplantation complications, including acute graft-versus-host disease (GvHD), sometimes occur when a fully matched donor is not available. Gamma block (GB) is located in the central HLA region between Beta and Delta blocks and contains many inflammatory and immune regulatory genes, including the C4 gene. C4 was proposed as a marker when predicting haplotype matching due to positive linkage disequilibrium (LD) with its surrounding loci. Our aim was to investigate the association between GB matching in patients and their 9/10 HLA matched unrelated donors (UD) and the occurrence of GvHD. Patients and their UDs were typed using the PCR-SSP kit that detects 25 SNPs within GB. Gamma block mismatch occurred in 25 (75.8%) of the 33 studied patient-UD pairs. There was a significant difference in GvHD occurrence between Gamma type matched (GT-M) and mismatched (GT-MM) patient-UD pairs (p=0.0302). The probability of GvHD occurrence had also shown an increase, although insignificant, along with the number of GT-MM between patient-UD pairs (p=0.0913). These results suggest that GT matching could be useful in reducing the risk of post-HSCT complications.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is most often the treatment of choice for patients with hematological diseases and malignancies. Human leukocyte antigen (HLA) mismatches have been shown to have the strongest impact on the outcome following unrelated HSCT[1]. HLA genes typed for patient-donor matching prior to transplantation in a routine procedure are HLA-A, -B, -C, -DRB1 and -DQB1, also called the classical or traditional HLA genes. If a donor within the patient's family is unavailable, unrelated donors (UD) for HSCT are found through national or international stem cell registries[2]. Patient-donor matching is performed comparing traditional HLA genes, but HLA mismatches have also been associated with the development of graft versus host disease (GvHD) and higher risk of disease relapse. However, the HLA region includes many other non-HLA genes that regulate immune and inflammatory responses[3].

Linkage disequilibrium (LD) is one of the main features of HLA genes. LD implies a non-random association of alleles at two or more neighbouring HLA loci. The discovery of conserved haplotypes (also called ancestral haplotypes) led to the explanation of HLA gene inheritance and description of a block-like pattern of LD[4, 5]. Recombination within the HLA region occurs infrequently and usually at specific "hot spots" of recombination. These hot spots represent the basis for defining the block structure of the HLA region, with similar patterns found throughout the human genome[6]. Among the human population, different ethnic groups are characterized by haplotypes that carry specific genomic sequences containing various sequence motifs, multibase deletions/insertions and substitutions. Those

sequences are highly conserved and can be divided into four major genomic blocks – the Alpha, Beta, Gamma and Delta block. The Alpha block contains the HLA-A gene, the Beta block contains HLA-B and -C genes, while the Delta block contains HLA-DR and -DQ genes[7].

The Gamma block (GB) is located between the Beta and Delta blocks, which contain the commonly typed classical HLA genes. The Gamma block contains more than 60 genes that encode proteins of diverse function, among which many have a role in immune and inflammatory responses and are not usually typed for matching in HSCT. The most studied among them are tumour necrosis factor (TNF) and three components of the complement system – C2, C4 and factor B (Bf). The complement system is the principal effector mechanism of humoral immunity, and its main role is the clearance of immune complexes, opsonisation and cell lysis. Components C2 and C4 participate in the classical pathway of activation of the complement system, while Bf is a part of the alternative pathway. Most human chromosomes carry two C4 genes – C4A and C4B, which are both highly polymorphic. Partial or complete deficiency of C4 is associated with the occurrence of various autoimmune and immune complex diseases[8]. In a study conducted by Petersdorf et al in 2007, HLA haplotype matching was shown to be associated with an increased risk of severe acute GvHD[3]. The Gamma block region, which includes the C4 gene, could be used as a marker when predicting haplotype matching due to its positive LD with its surrounding loci[2]. The C4 gene is not usually typed for genetic matching between the patient and donor in HSCT, but due to its evident role in disease development and haplotype matching, some scientists had suggested including non-HLA genes of the GB as an alternative or addition to traditional HLA typing[9]. GB typing is performed using the Olerup Gamma-Type™ kit that consists of a panel of sequence-specific primers (SSP) for 25 single nucleotide polymorphisms (SNPs) located within the C4 gene of the GB.

Even though data on the subject of GB matching in HSCT are limited, some studies have shown statistically significant results in association to GvHD occurrence, which could play a role in donor selection in HSCT. Some recent studies provided data showing that haplotype matching between patients and their donors has reduced the risk of severe GvHD, but they did not identify a significant association between GB matching and HSCT outcomes[2, 10]. On the other hand, a study by Park et al suggested that C4 mismatch was a high-risk factor for the development of GvHD in unrelated HSCT[11]. A recent study by Maskalan et al also found GB matching to be strongly associated with GvHD occurrence following HSCT[12]. The study consisted of 51 patient-UD pairs who were 10/10 HLA matched. This study is a follow-up, with the aim of investigating GB matching between patients and their respective 9/10 HLA matched UDs to evaluate the impact of GB matching on the occurrence of GvHD.

MATERIAL AND METHODS

Study population

The study population consisted of 33 adult patients and their UDs, collected for HLA typing at the Tissue Typing Centre Zagreb while preparing for HSCT. The patients and their UDs underwent HSCT in the period of 2011-2020 at the University Hospital Centre Zagreb, at the Department of Internal Medicine, Division of Hematology. Informed and signed consent was obtained for both patients and donors in accordance with the Declaration of Helsinki. The research was approved by the ethical committee of the University Hospital Centre Zagreb.

Patients underwent allogeneic transplantation due to a diagnosis of acute myelogenous leukaemia and myelodysplasia syndrome (AML, MDS; N=20) or other diagnosis requiring such treatment (N=13). All patients (11 female and 22 male) were transplanted from a 9/10 HLA allele-matched UDs (18 female and 15 male), with a mismatch at HLA-A locus only. The majority of the patients (N=25) were treated with a reduced intensity conditioning (RIC) regimen. The rest of the patients (N=8) were treated with a myeloablative conditioning (MAC) regimen. Patients received bone marrow grafts (N=6) or peripheral blood stem cell grafts (N=27), mobilized from donors with granulocyte-colony-stimulating factor (GCSF) (10 μg/kg per day). No manipulation of the graft was performed in any of the cases. Patient and donor characteristics, along with HSCT variables, are shown in Table 1.

Table 1. Characteristics and HSCT variables of patient and unrelated donor pairs (N=33)

Patient and donor characteristic	n	%
Patient age (yrs.): median (range)	54 (20-66)	
Donor age (yrs.): median (range)	27 (19-50)	
Gender – patient/donor:		
female-female	5	15.1
female-male	6	18.2
male-female	13	39.4
male-male	9	27.3
Diagnosis		
AML+MDS	20	60.6
other	13	39.4
Conditioning regimen		
MAC	8	24.2
RIC	25	75.8
Stem cell source		
BM	6	18.2
PBSC	27	81.8

Legend: yrs. - years; n - number; AML - acute myelogenous leukaemia; MDS - myelodysplastic syndrome; MAC - myeloablative conditioning; RIC - reduced intensity conditioning; BM - bone marrow; PBSC - peripheral blood stem cell

DNA isolation

Genomic DNA was isolated from whole blood containing EDTA using the NucleoSpin Blood commercial kit (Macherey-Nagel, Duren, Germany).

HLA typing

Patients and UDs were typed at high resolution level using the standard Polymerase Chain Reaction – Sequence-Specific Primer (PCR-SSP) protocol for Olerup SSP typing kits and the Polymerase Chain Reaction - Sequence Based Typing (PCR-SBT) protocol for Olerup SBT typing kits (Olerup SSP AB, Sweden; Conexio Genomics, Australia), at the Tissue Typing Centre Zagreb.

The PCR-SSP method is based on using primers with a complementary sequence to the specific HLA allele sequence. Each HLA locus is determined by a specific number of reactions in the PCR-SSP typing set. Each reaction tests for the presence of a single allele or gene group of HLA using two sets of primers – for the control gene and for the specific gene/allele of HLA. The results are read using a 1.5% agarose gel electrophoresis and analysed using the Helmberg Score™ computer program[13].

The PCR-SBT method, also called the Sanger sequencing method, is based on using primers that are specific for HLA allele region amplification. Each PCR reaction amplifies a single HLA locus and uses a mixture of deoxynucleotides and labelled dideoxynucleotides that randomly get added to the DNA chain by DNA polymerase. The results are read using capillary electrophoresis and analysed using the Assign™ program[14].

Gamma block typing

Gamma block typing was done using a commercially available Olerup Gamma-Type PCR SSP typing kit (Olerup SSP AB, Sweden). The kit contains 25 sequence specific primers for SNPs within the C4A/C4B gene in the Gamma block of the central HLA region. The detection of amplicons was performed on a 2% agarose gel by gel electrophoresis. A positive Gamma-Type™ reaction is observed and recorded when both the target amplicon and internal control amplicon are amplified, while a negative reaction should amplify only the internal control amplicon (Figure 1). Gamma-Type™ profiles were compared between patient and donor samples to determine Gamma block matching. The results were entered into the Gamma-Type™ Matching Pairs Worksheet which allowed automatic comparison of patient and donor results for matching status.

Statistical analysis

The number of observed matched or mismatched patient-UD pairs was determined by direct counting and presented in numbers and percentages. The studied group of 33 recipient-donor pairs was divided into subgroups which were compared using the Fisher's exact test. The association of Gamma type region match or mismatch (and number of mismatches) with GvHD occurrence was calculated using the Kaplan-Meier estimator (MedCalc, version 19.2.6). The difference between subgroups and statistical significance (P) was calculated using the log-rank test included in the Kaplan-Meier algorithm. The significance level was set to $P < 0.05$.

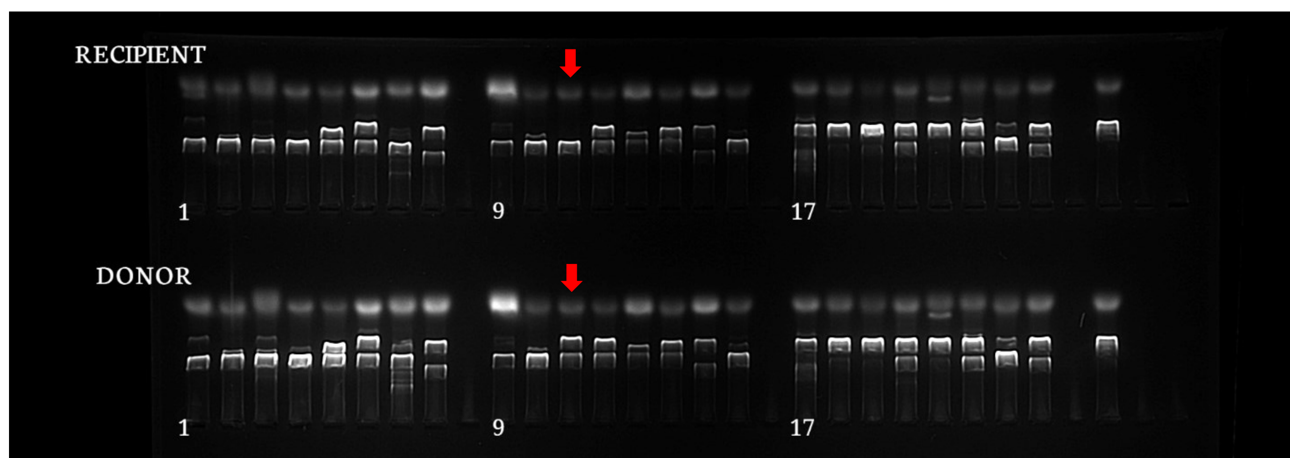

Figure 1. Gel electrophoresis results of Gamma type (GT) testing for mismatched (MM) recipient and donor pair. Positive GT matched reactions are observed where 2 bands are amplified by gel electrophoresis, both the internal control and target amplicon – positions 5, 6, 8, 12, 13, 14, 15, 20, 22 and 24, as shown above. The red arrows show a GT mismatch reaction on position 11 where the donor's sample gives a positive GT reaction, while the recipient's sample shows no amplification of the target amplicon on the same position.

RESULTS

The patients and their UDs were 9/10 HLA allele-matched, with a mismatch at HLA-A locus only.
Gamma block mismatch occurred in 25 (75.8%) of the 33 studied patient-UD pairs. The number of mismatches varied from 1 to 5 SNPs with SNP on the position 11 mismatched in the majority of the cases (N=10), followed by mismatch at SNP on the position 12 (N=8).

A total of 7 Gamma type mismatched (GT-MM) pairs had one MM, 5 pairs each had two and three MMs, 7 patient-UD pairs had four MMs, while only 1 pair had five MMs. The number of Gamma block mismatches in patient-UD pairs is shown in Figure 2. Overall, 9 SNP reactions showed no mismatches throughout the study. The specific SNP reactions in which mismatches occurred, along with the percentage of the mismatch present in the studied group are given in Figure 3.

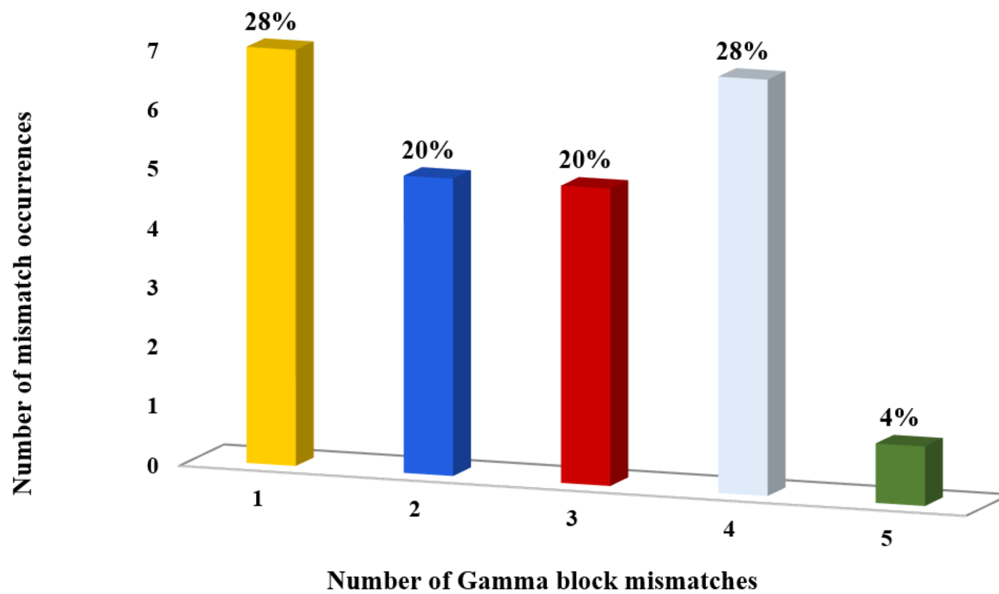

Figure 2. The number of Gamma block mismatches between patient and unrelated donor pairs (N=25)

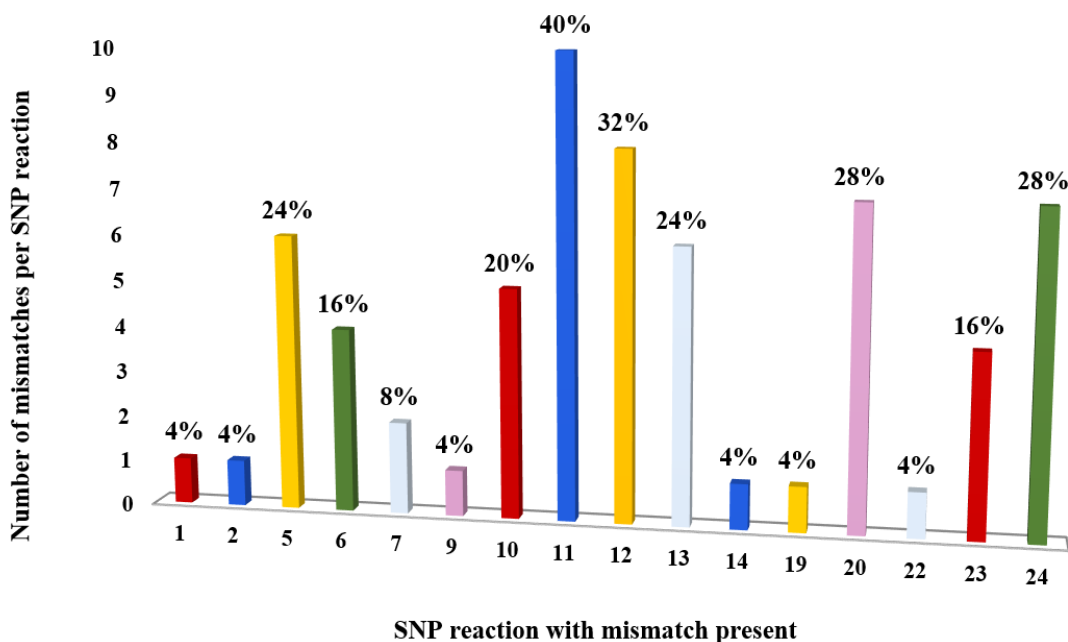

Figure 3. Number of mismatches per SNP reaction, excluding 9 SNP reactions with no mismatches among patient-unrelated donor pairs (N=16). The percentage shows the presence of a specific SNP mismatch among 25 patient-unrelated donor Gamma Type mismatched pairs. SNP - Single nucleotide polymorphisms

There was a significant difference in GvHD occurrence between Gamma type matched and mismatched patient-UD pairs, given in Figure 4. A significantly higher incidence of GvHD was observed among GT-MM patient-UD pairs compared to GT-M patient-UD pairs (p=0.0302; HR: 2.74; CI: 1.1011-6.8145). Although not statistically significant, the number of mismatches present between patients and their respective UDs had shown a tendency of a higher probability of GvHD occurrence as the number of GT-MM increases between patient-UD pairs, as shown in Figure 5.

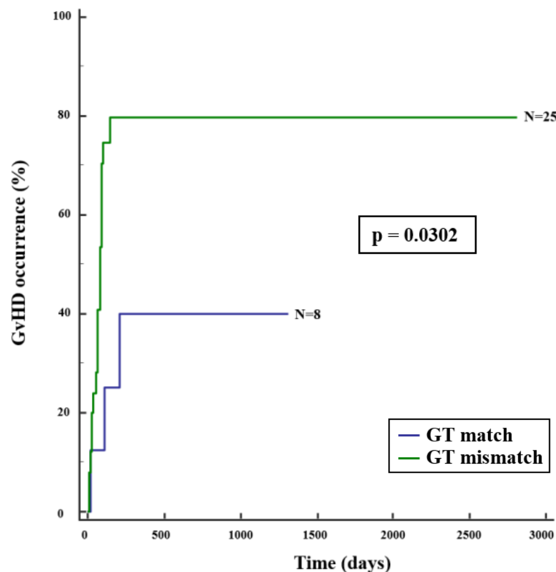

Figure 4. The occurrence of GvHD in relation to Gamma type match/mismatch in patient and unrelated donor pairs after hematopoietic stem cell transplantation. GvHD - graft versus host disease; GT - Gamma type

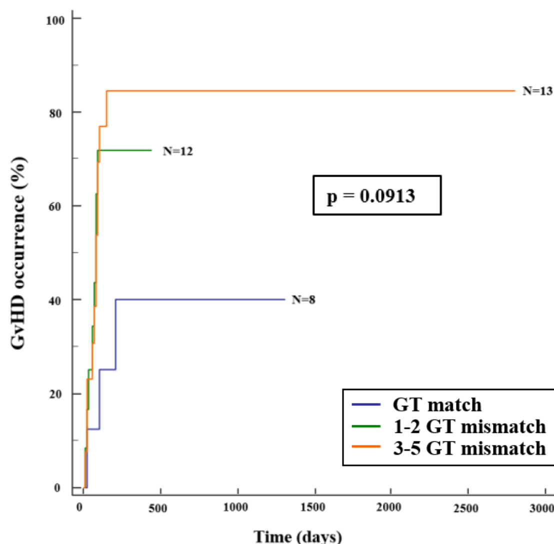

Figure 5. The occurrence of GvHD in relation to Gamma type match/number of mismatches in patient-unrelated donor pairs. The graph shows a higher probability of GvHD occurrence as the number of GT mismatches increases between patient/donor pairs, with a tendency to be significant. GvHD - graft versus host disease; GT - Gamma type

The highest incidence of GvHD was observed in patients with more than 3 GT-MM with their UD (p=0.0913; HR: 3.57; CI: 1.3197-9.6501) in comparison to patients with 1 or 2 GT-MM or no GT-MM.

DISCUSSION

Previous studies have shown that HLA matching plays an important role in the outcomes following HSCT between patients and UD pairs[1]. For that reason, it has become the gold standard when searching for available and matching donors. However, even among HLA matched patient-UD pairs, there may be mismatches in other MHC genes which could also have an effect in the development of GvHD. Negative outcomes are less common when there is a donor available within the patient's family, as MHC haplotypes are shared by descent[3]. The C4 gene of the Gamma block region has shown potential for indicating MHC haplotype matching due to strong LD with the surrounding loci [2].
In this retrospective study of 33 patient-UD pairs who underwent HSCT, we investigated the role of Gamma type matching on the development of GvHD following the procedure. We hypothesized that GT-MM could increase the risk of developing GvHD. If so, it could be considered as a useful marker, lowering the risk of complications post HSCT.
Our results showed a significant association between GT-MM and a higher risk of GvHD occurrence. Out of 25 GT-MM patients, 20 developed GvHD. The results given in a previous study conducted by Maskalan et al also suggested a positive correlation between GvHD occurrence and GT-MM. In their study of 51 unrelated 10/10 HLA matched patient-donor pairs, out of the 36 that were GT-MM, 19 (52.78%) developed GvH[12]. Hogan et al reported that among 225 patients who were HLA matched and GT matched with their UD had a lower risk of developing GvHD than those who were HLA matched, but GT mismatched[15]. On the other hand, Askar et al conducted a study of 714 patients that were HLA matched and did not report any significant association between GB SNP nor C4 SNP mismatches with HSCT outcomes[10]. In 2020, another group found no significant correlation between the occurrence of GvHD and C4 mismatches, although those results were not in accordance with their research of the same subject conducted in 2014[16].
Considering that all patient-donor pairs in our study group were unrelated, mismatches within the MHC Gamma region were expected. The number of GB mismatch occurrences varied from 1 to a total of 5. Interestingly, the occurrence of 4 mismatches appeared to be as common as only 1 mismatch (28%). It should be noted that a single mismatch is sufficient to identify a pair as a GT-MM, and we did not repeat some reactions that yielded indeterminate results due to a shortage of reagents. We noticed a significant number of reactions had a positive result (mismatch) for SNP on positions 11 and 12, occurring as positive for 10 and 8

reactions, respectively. We have tried to investigate whether there was a connection between a higher occurrence of GvHD and the presence of a mismatch at the SNP positions 11 or 12, and we did find some statistical significance for SNP 11, but not 12. On the other hand, there were no mismatches present in 9 out of the 25 SNP reactions. Moyer et al suggested that some SNP positions might be more relevant for the outcome of unrelated HSCT[17], but because we used a commercial kit whose manufacturer did not disclose which SNPs correlate to which exact genomic positions, we cannot comment on the suggestion.

Some limitations of our study should be noted. While our study group was very homogenous, the sample size included only 33 patient-UD pairs. They were not typed for HLA-DPB1, even though there are reports of a higher risk of negative outcomes following unrelated HSCT when a mismatch in HLA-DPB1 is present[18]. Our studied group was a 9/10 HLA match, and all had a mismatch at the HLA-A locus. There are some studies that indicate that an HLA-A locus mismatch itself has an impact on GvHD development, which should also be considered[19]. Furthermore, in our research we focused solely on the occurrence of GvHD post-HSCT, with no regard to the 5-year survival or disease relapse in patients.

In conclusion, existing data on this subject is still quite limited and the results are inconsistent. Making assumptions should be done with caution, but there are indications that GT matching could be useful to reduce the risk of complications for patients that underwent HSCT, with a strong requirement for further investigations in larger cohorts.

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