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MDM2 SNP309 G allele decreases risk but does not affect onset age or survival of Chinese leukaemia patients

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ABSTRACT

Although mutations in *p53* are rare in leukaemia, MDM2, the negative regulator of p53, is often overexpressed. Recently, a single nucleotide polymorphism (SNP) in the *MDM2* promoter - within the oestrogen-receptor-binding region - resulting in either a G or T allele was shown to affect its transcription, with elevated MDM2 being produced when it is a G allele. Expectedly, SNP309G females were found to be at a higher risk of accelerated onset of cancers. We have therefore analysed, in a pilot study, whether the status of *MDM2* SNP309 and *p53* codon-72 polymorphism, which was also shown to affect cancer predisposition, would affect cancer risk, onset age, overall survival and response to therapy in Chinese leukaemia patients. *p53* SNP was not associated with any of the parameters. However, in contrast to expectations, the *MDM2* SNP309G allele was associated with reduced risk of leukaemia. No other association was found between SNP309 and other parameters in both males and females. Thus, the data highlights ethnic differences in the effects of this SNP on cancer risk.

1. Introduction

p53 is a critical tumour suppressor gene that is mutated in almost 50% of all human cancers.[1,2] However, leukaemia represents a class of cancers that does not contain significant levels of p53 mutations.[3] Nonetheless, in cases where there are no direct *p53* mutations, the p53 pathway has been suggested to be defective due to alterations in either upstream or downstream regulators.[4,5] Leukaemia indeed provides an example for such a phenomenon, whereby MDM2, the critical negative regulator of p53, is often overexpressed.[6–12] Hence, it is conceivable that MDM2 overexpression could weaken the p53 pathway, thereby contributing to leukaemiogenesis.

Single nucleotide polymorphisms (SNPs) in *p53* and genes in the p53 pathway have been identified, and have been proposed to affect tumourigenecity.[4,5,13] Of note is the codon 72 polymorphism of *p53* that has been intensely studied. This SNP, resulting in either a C or G nucleotide, leads to either a Proline (Pro) or Arginine (Arg) residue at

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codon 72, respectively. Both these variants have been shown to preferentially affect the different functional properties of p53. For example, the Arg variant has been shown to be more potent in apoptosis induction whereas the Pro variant was shown to be better in inducing cell-cycle arrest and in its ability to repair damaged-DNA.[14–16] Not unexpectedly, these variants have been associated with cancer predisposition, though there is no uniform agreement to which variant predisposes individuals to higher risk of cancer.[16–19] In leukaemias, whereas the Pro variant increased the risk of chronic myeloid leukaemia (CML), the Arg allele was associated with increased risk of chronic lymphocytic leukaemia (CLL).[20,21] By contrast, no association of p53 codon 72 polymorphism was noted with acute myeloid leukaemia (AML), the B-type CLL (B-CLL)[22–24] and even CLL.[25]

Similarly, a SNP in the promoter of *MDM2* - the SNP309 - results in either a G or T allele.[26] This SNP is found in the Sp-1 binding site of the promoter, which lies in the oestrogen-receptor-binding region.[27] The G allele results in a higher affinity site for Sp-1 binding, and hence leads to enhanced *MDM2* transcription.[27] It has therefore been postulated that females carrying the G allele in particular are more sensitised to cancer formation and initial reports have indicated that in familial cancers, SNP309G carriers succumbed to earlier onset of cancers.[27] Furthermore, various reports have suggested a correlation between the G allele and earlier onset of many cancers, though lack of correlation has also been reported.[28–31] In lymphoid malignancies, the role of this SNP has not been extensively studied, except for two reports that either showed lack of correlation with survival in mantle cell lymphoma and CLL, or an association of the G allele with early onset age in childhood acute lymphoblastic leukaemia (ALL), particularly in Caucasian and Black populations but not in Hispanic populations.[12,25,32]

There is an ethnicity-based bias for the prevalence of both the codon 72 *p53* and the *MDM2* SNP309 polymorphsims. The Arg variant of p53 has been shown to be prevalent in Caucasian populations living further away from the equator whereas the Asians and Africans tend to contain more of the Pro carriers.[33] Similarly, the *MDM2* SNP309T allele was found to be enriched in the Caucasians who contain extremely low frequencies of the G allele, in contrast to the Asians and Azhkenazi Jews who have a significantly higher percentage of the SNP309 G allele.[27,34]

We have therefore evaluated if both the polymorphisms in *p53* and *MDM2* would affect the risk of leukaemia formation, the age of onset, response to therapy and overall survival, in Chinese Asians living in Singapore, who are descendants from Southern China and who have similar *MDM2* SNP frequencies as the Chinese from China.[34] The data presented indicate that contrary to expectations, it is the G allele of the *MDM2* SNP309 that was associated with a decreased risk of leukaemia. *p53* status did not affect any of the parameters. Importantly, there was no gender bias, suggesting that the effects of *MDM2* SNP309 may be manifested in an ethnicity-based manner and does not support the hypothesis that the G allele always predisposes or accelerates the onset of cancers in females.

2. Materials and methods

2.1. Samples

Genomic DNA were prepared from peripheral blood as described by standard procedures using the Qiagen kit, from a total of 160 healthy[35] and 44 Singaporean Chinese leukaemia patients, with the approval of the National Cancer Centre and Department of Heamatology, Singapore General Hospital ethics committees, and used for genotyping. Data on stage and age of onset of disease, etc. were obtained from the hospital records with ethics approval.

2.2. Genotyping and sequencing

Genomic DNA from peripheral blood samples was analysed for the genetic variation in codon 72 in exon 4 of the *p53* gene, by PCR analysis followed by *BstU1* digestion, using primers spanning exon 2 to 4, as follows: Forward: 5′-tcagacactggcatggtgtt-3′ and Reverse: 5′-aagcctaagggtgaagagga-3′, which give rises to a 819bp product covering exons 2–4. The *p53 arg* allele has a unique *BstUI* site that is absent in the *pro* allele, resulting in bands of different sizes, as follows: 267bp, 552bp (Arg/Arg), 819bp (Pro/Pro) and 267bp, 552bp, 819bp (Arg/Pro).

Mutations in *p53* were determined by sequencing using PCR of the whole p53 coding region, from exon 2-11 including all introns, using the following primers: Ex5-Ex6 - Forward: 5′-ttctttgctgccgtgttcca-3′ and Reverse: 5′-aggtcaaataagcagcagga-3′ (562bp); Ex7-Ex9 - Forward: 5′-acagagcgagattccatctc-3′ and Reverse: 5′-ctgatggcaaatgccccaat-3′ (967bp); Ex10 - Forward: 5′-ctcaggtactgtgtatatac-3′ and Reverse: 5′-tggaatcctatggctttcca-3′ (224bp); Ex11 - Forward: 5′-aggcccttcaaagcattggt-3′ and Reverse: 5′-actcattgcagactcaggtg-3′ (1471bp). Sequencing primers for exons 2–4 are the same as used for PCR analysis, indicated earlier. *MDM2* promoter SNP309 was determined by sequencing reactions, essentially as described.[27]

2.3. Statistical analysis

Fisher's exact test was used to check Hardy–Weinberg equilibrium for controls, compare the genotype distribution between healthy subjects and leukaemia cases, and the effects of SNPs on response to treatment. Kruskal–Wallis method was employed to investigate the effects of SNPs on age of onset of disease. Log-rank test was performed to compare the equivalence of Kaplan–Meier curves among different genotypes.

Odds ratios (ORs) together with 95% confidence intervals (CIs) were estimated to investigate the role of SNPs in leukaemia. The role of individual SNP in leukaemia was assessed appropriately based on the relationship of OR_1 (p53: Arg versus Pro, MDM2: C/C versus A/A), OR_2 (p53: Het versus Pro, MDM2: C/A versus A/A) and OR_3 (p53: Arg versus Het, MDM2: C/C versus C/A) to reflect a biological model of gene effect.[36]

1. Recessive model: $OR_1 = OR_3 \neq 1$ and $OR_2 = 1$;
2. Dominant model: $OR_1 = OR_2 \neq 1$ and $OR_3 = 1$;
3. Overdominant model: $OR_2 = \frac{1}{OR_3} \neq 1$ and $OR_1 = 1$;
4. Codominant model: $OR_1 > OR_2 > 1$ and $OR_1 > OR_3 > 1$ (or $OR_1 < OR_2 < 1$ and $OR_1 < OR_3 < 1$).

3. Results

3.1. *MDM2 SNP309, but not p53 codon 72 polymorphism, affects risk of leukaemia*

The leukaemia group analysed consisted of 44 Chinese patients, categorised as follows: 26 AML patients, 13 ALL patients and 5 BAL (biphenotypic acute leukaemia) patients (Table 1). Median age of onset was 49.5 years for females and 41.0 years for males, and 43.5 years overall. Of the 32 who were treated with curative intent by the respective standard induction chemotherapy for AML and ALL, 19 responded to treatment and achieved remission, while the remaining 13 were refractory to chemotherapy (Table 1).

We first determined if there were significant differences in the distribution of the various genotypes. Comparison of *p53* codon 72 polymorphic genotypes revealed no significant differences between the proportion of Arg/Arg, Pro/Pro and Arg/Pro frequencies between healthy and leukaemia patients (% of Arg/Arg versus Pro/Pro versus Arg/Pro: healthy/leukaemia → 35.0/29.5 versus 20.0/13.6 versus 45.0/56.8) ($p = 0.419$) (Fig. 1A). No significant effect of *p53* on leukaemia risk was detected, with ORs of Arg/Arg versus Pro/Pro, Arg/Pro versus Pro/Pro and Arg/Arg versus Arg/Pro being 1.24 (0.43, 3.57), 1.85 (0.69, 4.95), and 0.67 (0.31, 1.42), respectively (Table 2a). Analysis of mutations was performed throughout the coding region of *p53* (from exon 2–11) by sequencing, which did not reveal the presence of any mutations in all the samples (data not shown). These data suggest that mutations or alterations in p53 codon 72 status do not contribute directly to risk of leukaemia.

By contrast, there were significant differences noted in the distribution of the various *MDM2* genotypes, with a significant increase of the T/T proportion in the leukaemia group and a corresponding decrease of the G/T proportion (% of G/G versus T/T versus G/T: healthy/leukaemia → 31.3/32.5 versus 18.7/37.5 versus 50.0/30.0) ($p = 0.025$) (Fig. 1B). The ORs of G/G versus T/T, G/T versus T/T and G/G versus G/T were 0.52 (0.21, 1.24), 0.30 (0.12, 0.72) and 1.73 (0.73, 4.11), respectively, which suggested that the variant G allele might decrease the risk of leukaemia in a dominant mode (G/T+G/G versus T/T: [0.38 (0.18,0.82)]) (Table 2b). Together, these results indicate a negative correlation between the G allele of *MDM2* SNP309 with leukaemia risk.

Table 1 – Summary of leukaemia patient status

Ethnic Group	Chinese
Median Age (yrs)	43.5 (females: 49.5; males: 41.0)
n	44 (22 females, 22 males)
Cancer type:	
AML	26 (9 females, 17 males)
BAL	5 (4 females, 1 males)
ALL	13 (9 females, 4 males)
No. receiving Curative intent treatment:	32
Response to treatment:	
Remission	19
Refractory	13

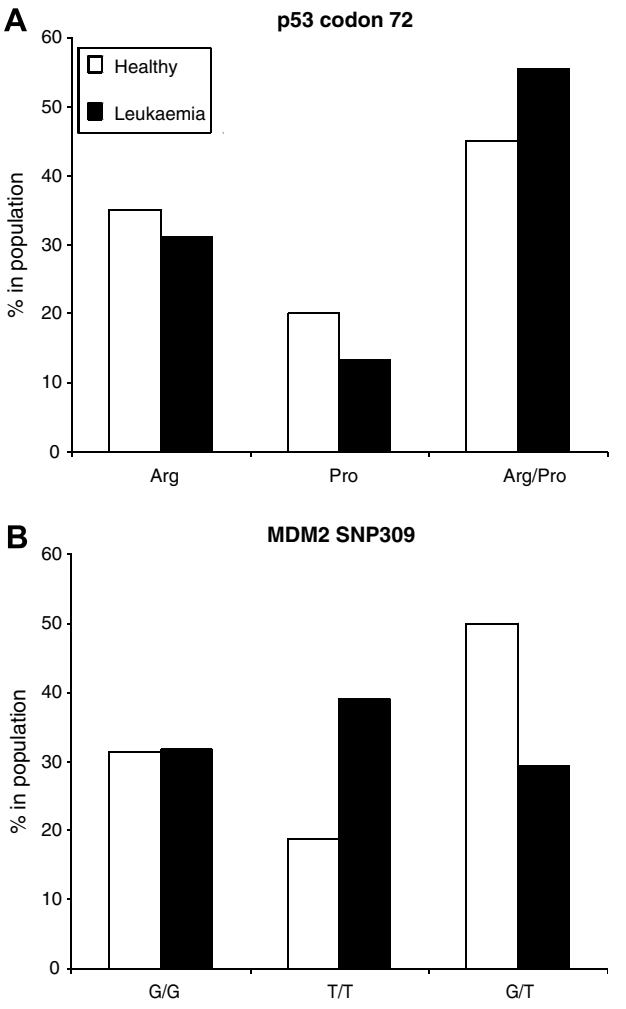

Fig. 1 – MDM2 SNP309, but not codon 72 polymorphism of *p53*, affects leukaemia risk. Genotypic analysis of *p53* codon 72 polymorphism (A) and SNP309 of MDM2 (B), comparing the frequency of the various genotypes in healthy controls and leukaemia cases. $n = 160$ for healthy and $n = 44$ for leukaemia cases. No significant differences were noted for *p53* ($p = 0.419$) but the differences were significant for *MDM2* ($p = 0.025$).

3.2. *MDM2 SNP309 does not affect the age of onset or overall survival*

Since p53 polymorphism did not affect leukaemia risk, we focused on evaluating the role of *MDM2* SNP309 on the onset age of leukaemia and overall survival. Kruskal–Wallis analysis indicated no association of any of the *MDM2* genotypes with age of onset in the aggregate cohort ($p = 0.659$)(Fig. 2A, top panel). When stratified by gender, we did not also observe any significant differences in the age of onset ($p = 0.417$ for males; $p = 0.985$ for females) (Fig. 2A, bottom panel), indicating that this SNP may not affect leukaemia susceptibility in a gender specific manner.

When overall survival was analysed in aggregate, no significant differences were seen with respect to the various *MDM2* genotypes ($p = 0.872$) (Fig. 2B, top panel). Median survival was 6 months for all patients (Fig. 2B, top panel). When

Table 2a – Effects of p53 SNP on the risk of leukaemia

	Het versus Pro	Arg versus Pro	Arg versus Het
OR (95% CI)	1.85 (0.69, 4.95)	1.24 (0.43, 3.57)	0.67 (0.31, 1.42)

Table 2b – Effects of MDM2 SNP on the risk of leukaemia

	G/T versus T/T	G/G versus T/T	G/G versus G/T	G/T + G/G versus T/T
OR (95% CI)	0.30 (0.12, 0.72)	0.52 (0.21, 1.24)	1.73 (0.73, 4.11)	**0.38 (0.18, 0.82)**

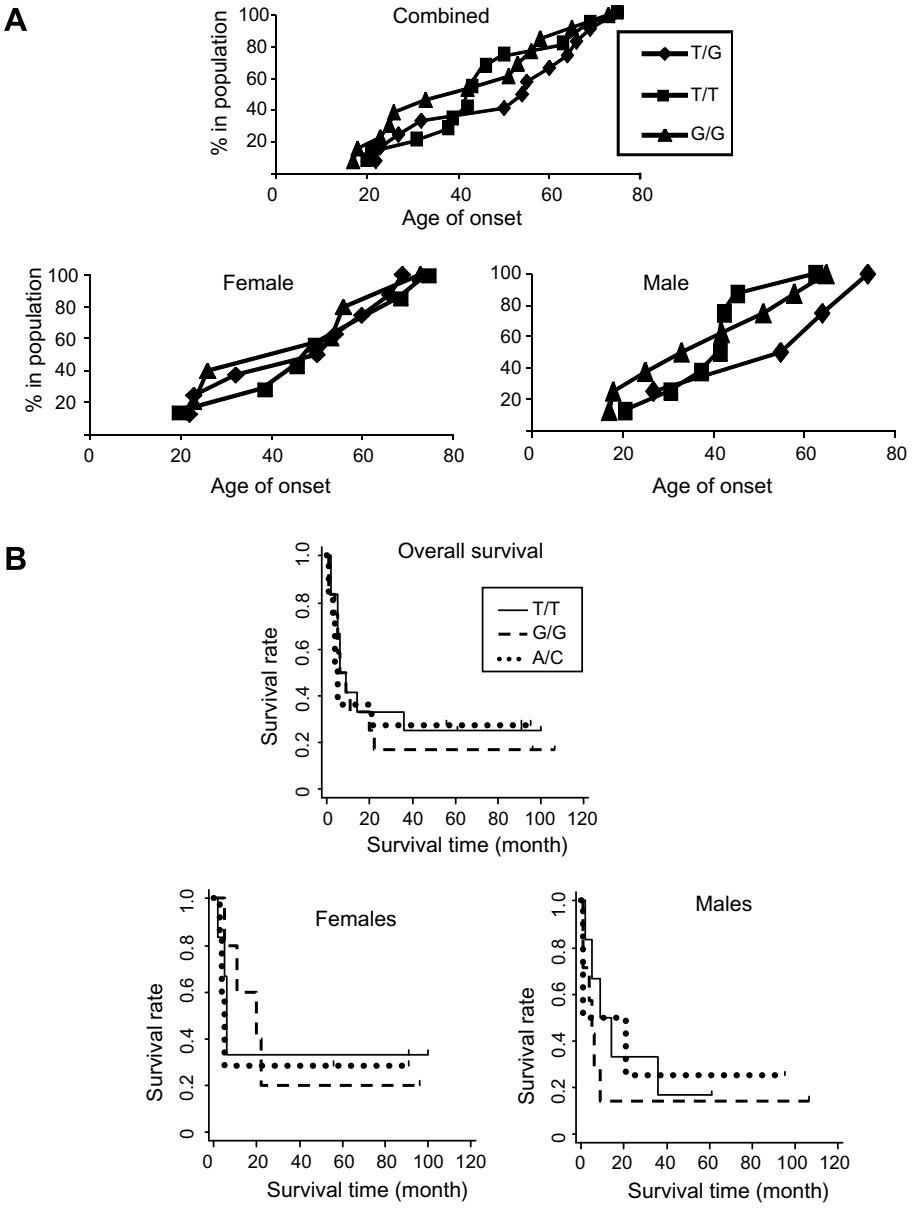

Fig. 2 – MDM2 SNP309 polymorphism does not affect onset age or survival of leukaemia patients. (A) The cumulative incidence of cancer cases in the population was plotted as a function of age of onset of leukaemia in the total population (combined)(top panel), or individually as males and females (lower panel). No significant differences were noted with respective to the different *MDM2* genotypes. *n* = 15 for T/T, *n* = 13 for G/G and *n* = 12 for G/T. (B) Kaplan–Meier survival curves for all patients (top) with different *MDM2* genotypes. Curves for females and males are shown separately below.

Table 3 – Genotypic status of treatable patients

Response	Genotype							
	p53				MDM2			
	n	pro/pro (%)	pro/arg (%)	arg/arg (%)	*n*	G/G (%)	G/T (%)	T/T (%)
Remission	19	5.3	57.9	36.8	18	27.8	33.3	38.9
Refractory	13	7.7	53.8	38.5	11	27.3	27.3	45.5

segregated based on gender, log-rank tests did not detect any significant differences in survival among different *MDM2* genotypes either for male ($p = 0.798$) or female patients ($p = 0.702$) (Fig. 2B, lower panel). Taken together, these results indicate that the *MDM2* SNP309 does not affect age of onset or overall survival, either in the male or the female population.

3.3. *Effect of p53 or MDM2 polymorphisms on response to treatment*

We finally evaluated if the response to curative chemotherapy for acute leukaemia is influenced by the polymorphisms in *p53* and *MDM2*. Comparison of p53 codon 72 polymorphic genotypes revealed no significant differences between the proportion of Arg/Arg, Pro/Pro and Arg/Pro frequencies between those who achieved remission and those who were refractory to treatment (% of Arg/Arg versus Pro/Pro versus Arg/Pro: remission/refractory → 36.8/38.5 versus 5.3/7.7 versus 57.9/53.8) ($p = 1.000$) (Table 3). Similarly, *MDM2* genotypes did not influence response to treatment as no significant differences were observed between the various genotypes of the remission and refractory groups (% of G/G versus T/T versus G/T: remission/refractory → 27.8/27.3 versus 38.9/45.5 versus 33.3/27.3) ($p = 1.000$) (Table 3). These data therefore indicate that the polymorphisms in *p53* or *MDM2* do not affect response to treatment of leukaemia patients.

4. Discussion

This pilot study to assess if the common polymorphisms in *MDMD2* and *p53* would affect leukaemia risk in the Chinese population living in Singapore revealed that besides the protective role of the *MDM2* SNP309G allele against leukaemia risk, other parameters such as age of onset, survival and response to treatment were not affected. An important finding from this work is that in contrast to expectations, the *MDM2* SNP309 G allele did not predispose to leukaemia nor accelerate the onset of the disease, but rather reduced the risk of the disease independent of gender, highlighting that ethnicity may play a critical factor in the manifestation of the effects of the polymorphic alleles.

The different polymorphic variants of *p53* have been shown to predispose to various cancers, and in leukaemia, association between the *pro* allele and CML and the *arg* allele with CLL have been reported (20,21). However, risk of AML was not associated with this polymorphism, and similarly, our results confirm the lack of any correlation between AML/ALL risk and p53 polymorphic status. Moreover, other parameters such as survival and response to treatment were also not found to be associated with *p53* polymorphic status. Furthermore, we did not detect any mutations in the coding region of the entire *p53* gene in all the samples. Finally, we did not observe any combined effect of the SNPs in *p53* and *MDM2* examined in this study, suggesting that p53 may not be a critical regulator of AML and ALL leukaemiogenesis. Nonetheless, we cannot formally exclude the possibility that other alterations that may affect the p53 pathway, which have not been examined here, may affect leukaemiogenesis.

By contrast, the G allele of *MDM2* SNP309 was seen to reduce the risk of AML and ALL. These results are perplexing as the G allele is associated with increased production of MDM2, and hence, is thought to lead to reduced p53 function, and therefore, cancer predisposition. However, since p53 was not seen to affect leukaemia risk in this context, the data suggests that other MDM2-dependent functions may reduce leukaemia risk. How this may be is unclear. Overexpression of MDM2 has also been shown to promote oncogenesis independent of p53,[37] though this may not be applicable in this case. One could therefore envisage that the G allele of *MDM2* may lead to elevated MDM2 that may be able to actively degrade other essential substrates that are necessary for leukaemiogenesis. Alternatively, it is also possible that MDM2-mediated apoptosis, which has been suggested to occur in some instances via the transcriptional repression of p65RelA eventually leading to suppressed NFkappaB activity,[38] may be relevant in this context, especially in association with the G allele. Such possibilities require further investigation. Nonetheless, though radical, this idea of the *MDM2* G allele conversely affecting cancer status was similarly noted in our recent study of Chinese breast cancer patients. Women with the *MDM2* G allele were found to have delayed onset of cancer compared to those of the T/T genotype.[34] At present, the molecular mechanisms are unclear as to why the G allele, with the expected elevated production of MDM2, would either reduce the risk of cancer or delay the onset age, as opposed to the T allele. One underlying theme that emerges is that such a pattern is noted in the Chinese population, where the basal frequency of the G allele is much higher than in the healthy Caucasian counterparts.[34] It is in the latter populations that the G allele was found to accelerate the onset of cancers, especially in familial cases. Hence, it appears that ethnicity may influence the effect of the *MDM2* G allele on cancer risk. A recent report analysing childhood ALL noted that though the G allele was associated with early onset age in Caucasian and Black populations, this was not the case in Hispanic populations.[12] Therefore, emerging evidence points to other ethnicity-based factors that may confound the effects of individual polymorphic variants of genes in the p53 pathway.

In conclusion, results from the pilot study presented here indicate that both the common *p53* and *MDM2* SNPs do not

affect most parameters associated with AML and ALL. However, the *MDM2* SNP309 G allele appears to reduce the risk of leukaemiogenesis in the Chinese population. The molecular mechanisms of how the G allele leads to reduced cancer risk need to be ascertained in future work.

Conflict of interest statement

None declared.

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