Oncogenic mutations in melanomas and benign melanocytic nevi of the female genital tract

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Background: The genetic heterogeneity of melanomas and melanocytic nevi of the female genital tract is poorly understood.

Objective: We aim to characterize the frequency of mutations of the following genes: *BRAF*, *NRAS*, *KIT*, *GNA11*, and *GNAQ* in female genital tract melanomas. We also characterize the frequency of *BRAF* mutations in female genital tract melanomas compared with melanocytic nevi.

Methods: Mutational screening was performed on the following female genital tract melanocytic neoplasms: 25 melanomas, 7 benign melanocytic nevi, and 4 atypical melanocytic nevi.

Results: Of the 25 female genital tract melanoma specimens queried, *KIT* mutations were detected in 4 (16.0%), *NRAS* mutations in 4 (16.0%), and *BRAF* mutations in 2 (8.0%) samples. Two of the tumors with *KIT* mutations harbored double mutations in the same exon. No *GNAQ* or *GNA11* mutations were identified among 11 melanomas screened. *BRAF* V600E mutations were detected in 7 of 7 benign melanocytic genital nevi (100%) and 3 of 4 atypical genital nevi (75%).

Limitations: Our study is limited by the small sample size of this rare subset of melanomas.

Conclusion: *KIT*, *NRAS*, and *BRAF* mutations are found in a subset of female genital tract melanomas. Screening for oncogenic mutations is important for developing and applying clinical therapies for melanomas of the female genital tract. (J Am Acad Dermatol 2014;71:229-36.)

Key words: BRAF; female genital melanomas; female genital nevi; KIT; NRAS; oncogenic mutations.

Over the past decade, the development of targeted therapies has significantly changed the clinical approach to patients with advanced melanoma. Improvements in targeted therapies rely on a fundamental understanding of the genetic heterogeneity of cancer. Recent studies have revealed different patterns of genetic mutations in known melanoma oncogenes depending on anatomic site[1] (ie, chronic sun-damaged skin vs nonchronic sun-damaged skin vs acral vs mucosal). We hypothesize that additional genetic heterogeneity can be appreciated even within this

Abbreviations used:

BRAF:	v-raf murine sarcoma viral oncogene homolog B
KIT:	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, CD117
NRAS:	neuroblastoma RAS viral (v-ras) oncogene homolog

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classification. For example, of melanomas arising in mucosal sites, previous studies have suggested that the frequency of *KIT* gene mutations may be higher in melanomas of the reproductive mucosal sites compared to melanomas of the sinonasal mucosa.[2,3] These studies have generally been performed as single-institution studies and on a small number of clinical samples. Our current understanding of the genetic characteristics of subtypes of mucosal melanomas is limited and warrants additional investigation.

In particular, melanomas of the female genital tract present many unique clinical challenges. The lack of effective screening methodologies results in tumors that are frequently diagnosed at advanced stages and are associated with poor outcomes.[4,5] The desire to temper aggressive, potentially noncurative surgical interventions with more conservative approaches may narrow therapeutic margins. The complex pelvic lymphatic drainage patterns, particularly for women with multifocal mucosal disease, may blunt the diagnostic accuracy of sentinel lymph node biopsy. The rare nature of these cancers (0.23% of all melanomas and 18% of mucosal melanomas[6]) has challenged rigorous query into the associated oncogene patterns.

The current standard of care for managing melanoma of the female genital tract involves local excision using margins based upon the measured depth of invasion or Breslow thickness. Sentinel lymph node biopsy, nodal dissection, radiotherapy, and chemotherapy are also considered in the patient-specific context. However, in recent years, kinase inhibitors have proven effective for some patients with advanced disease. For example, vemurafenib and dabrafenib are drugs that have been shown to be effective for *BRAF*-mutant melanomas (V600E/K substitutions).[7-9] *KIT* kinase inhibitors, such as imatinib, sunitinib, and sorafenib, have yielded responses in *KIT*-mutant melanomas, and *NRAS*-mutant melanomas are being targeted with mitogen-activated protein kinase kinase inhibitors in ongoing clinical trials.[10-15] In a recent report, a patient with vulvar melanoma that experienced progression to lymph node metastasis after interferon-alpha therapy achieved disease stabilization for 8 months on imatinib.[16] As shown in these clinical experiences, genetic alterations in melanoma subtypes may have both prognostic and therapeutic significance.[17] Mucosal melanomas have been previously described to exhibit heterogeneity in their oncogenic aberrations,[2,3,18-29] but the extent to which anatomic location correlates with this genetic diversity remains largely unexplored. To our knowledge, there are no studies focused specifically on oncogenic mutations in melanomas of the female genital tract. Therefore, we screened for such oncogenic mutations in *BRAF*, *NRAS*, *KIT*, *GNA11*, and *GNAQ*.

CAPSULE SUMMARY

- Different patterns of genetic mutations may occur in melanomas arising in different anatomic sites.
- Of 25 female genital melanomas screened, *KIT* mutations were detected in 4 of 25 (16.0%), *NRAS* mutations in 4 of 25 (16.0%), and *BRAF* mutations in 2 of 25 (8.0%) samples.
- A subset of female genital melanomas may benefit clinically from targeted therapies.

In addition to the therapeutic challenge of treating female genital tract melanomas, there is also the challenge of clinical screening for their precursor lesions. While genital nevi are not uncommon, cytologic and architectural atypia challenges accurate histopathologic characterization and leads to confusion of their identity as possible precursors of melanoma. As such, we sought to clarify whether benign melanocytic nevi (with and without atypia) might be precursor lesions for invasive melanoma by comparing their *BRAF* mutation pattern to female genital tract melanomas. This investigative focus seeks to guide the clinical management of atypical genital nevi, which are an overall poorly understood group of melanocytic lesions in the spectrum of nevi of special sites.

METHODS

After obtaining institutional review board approval for this retrospective study, 11 cases of melanoma arising from the female genital tract with retrievable tumor material and 11 control cases of benign gynecologic melanocytic lesions were identified from the Duke Melanoma Database and the Duke University Tumor Registry. Fourteen additional cases of melanoma arising in the female genital tract that have not been previously published were obtained from the pathology archives of Oregon Health and Science University. Deidentified clinical information was obtained for each subject, including patient demographic information (eg, age and race) and clinical features (eg, ulceration, anatomic location, immunosuppressed status, and nodal positivity).

All cases were reviewed microscopically by a pathologist to confirm the diagnosis and to identify areas rich in lesional cells (ie, nevoid cells or

melanoma, depending on the case). Tumor-rich areas were then isolated by macrodissection from parallel unstained sections (minimum 60% tumor cellularity), and DNA was prepared as previously described.[30] Eleven of the melanomas were screened for mutations in *BRAF*, *NRAS*, *KIT*, *GNA11*, and *GNAQ* using a combination of multiplex polymerase chain reaction studies and mass spectroscopy (Sequenom; San Diego, CA).[30] The complete list of mutations screened by this approach was previously published as a supplemental table in reference 30. This approach covers all hotspot regions of these genes, but does not cover some known KIT exon 11 insertions and deletions. Therefore, additional screening of this exon was carried out with high-resolution melting curve analysis using an LC480 LightCycler (Roche, Mannheim, Germany). The remaining 14 melanoma cases were screened for mutations in *BRAF* (exon 15), *NRAS* (exons 1 and 2), and *KIT* (exons 11, 13, and 17) using standard, bidirectional Sanger sequencing. All mutations identified on the Sequenom system were also confirmed by Sanger sequencing. All melanocytic nevi were screened for *BRAF* using Sanger sequencing.

Two melanomas were found to harbor dual KIT gene mutations. To determine whether these mutations were on the same allele, we used a combination of an amplicon-based library preparation and semiconductor-based next generation DNA sequencing (Ion Torrent Personal Genome Machine; ThermoFisher Scientific, Waltham, MA), as previously described.[31] It should be noted that this type of sequencing was not available when the study was initiated, and was only used to examine the 2 tumors with dual mutations.

A systematic review of the literature surrounding benign nevi and melanoma of the female genital tract was performed. The search was performed on April 7, 2013 using the PubMed database. Search terms included the following MeSH terms: "nevi and melanomas," "genitalia, female," "mucous membrane," "tumor markers, biological," "genetics," and "genetic phenomena." The search algorithm was also inclusive of any literature containing the following key terms in either the title or abstract: "pigmented lesion(s)," "mole(s)," "nevus," "nevi," "melanoma(s)," "vulva(r)," vagina(l)," "labia(l)," "clitoral," "clitoris," "gynecologic," "mucosal," "KIT," "oncogene," and "genetic(s)." This comprehensive search yielded 498 articles, with 19 articles (3.8%) containing subject matter relevant to this study. To be included, articles were required to be written in English and to illustrate oncogene or mutational analysis of benign nevi, atypical nevi, or melanomas localized to the female genital tract. Articles with questionable relevance were reviewed. In addition, the reference sections of selected studies were reviewed for potentially inclusive articles missed during the initial search. Included articles were reviewed independently by the first (D.T.), second (J.K.), and senior authors (K.N.; Table I).

The frequency of *BRAF*, *NRAS*, and *KIT* mutations in our series of gynecologic melanomas was compared with the cumulative published frequencies of these oncogenes in primary and metastatic cutaneous melanomas.

RESULTS

Mutations in *KIT* or *NRAS* are found in a subset of melanomas arising from the female genital tract

Mutational screening of the candidate oncogenes *BRAF*, *NRAS*, *KIT*, *GNA11*, and *GNAQ* was performed on 25 melanomas arising from the female genital tract. Data were compiled from patient tumors at Duke University (n = 11) and Oregon Health and Science University (n = 14; Table II). Patient ages ranged from 29 to 82 years (median, 68 years of age). Primary melanomas of the female genital tract included those arising from the vagina (n = 8), vulva (n = 9), labia (n = 2), introitus (n = 1), and cervix (n = 1); metastatic locations included inguinal nodes (n = 3) and 1 cutaneous metastatic deposit. *KIT* mutations were detected in 4 of 25 samples (16.0%; see Table II). Interestingly, 2 of the tumors harbored double mutations in the same exon (KIT exon 13, K642E and Y646H; KIT exon 11, W557R and L576P). In both cases, next generation sequencing revealed that the mutations were on the same allele.

NRAS mutations were detected in 4 of 25 samples (16.0%). These include 1 melanoma of the labia (*NRAS* A59T), 2 melanomas of the vulva (*NRAS* G13D and *NRAS* Q61K), and 1 melanoma of the vagina (*NRAS* G12V). Mutations in *NRAS* and *KIT* were mutually exclusive.

BRAF mutations were detected in 2 of 25 samples (8.0%), representing a vaginal melanoma (*BRAF* K601E) and a cutaneous metastasis of a primary female genital tract melanoma (*BRAF* V600E). Interestingly, the cutaneous metastasis also had mutations in *KRAS* (G13D) and *PIK3CA* (E545K), which were detected as part of a broader screening panel performed on this particular sample (a combination of multiplex polymerase chain reaction studies and mass spectroscopy). Fifteen tumors lacked detectable mutations in the *KIT*, *NRAS*, or *BRAF* oncogenes. No *GNAQ* or *GNA11* mutations were identified among 11 melanomas that were screened.

Table I. Literature review summarizing oncogenic mutation frequency in female genital melanomas

Authors	No. of cases/ anatomic site	*BRAF*		*NRAS*		*KIT*		Methods
		N (%) [site]	Mutations	No. (%) [site]	Mutations	No. (%) [site]	Mutations	Mutations
Carvajal et al[10]	13/Vulva and vagina	0/13 (0%)	NA	3/13 (23.1%) [vulvovaginal]	Exon 2 Q61L (1/13), exon 1 G12D (1/13), and exon 1 G13V (1/13)	7/13 (53.8%) [vulvovaginal]	Exon 11 L576 P (5/13), exon 11 Y553C (1/13), exon 18 V852I (1/13), and exon 13 K642E (1/13)	PCR, followed by Sanger sequencing, *KIT* exons 9, 11, 13, 17, 18; *NRAS* exons 1 and 2; *BRAF* exon 15
Cohen et al[22]	8/Vulva	0/8 (0%)	NA	Not tested	Not tested	Not tested	Not tested	PCR, followed by sequencing of *BRAF* exon 15; Mutector assay
Edwards et al[23]	8/Vulva	0/8 (0%)	NA	Not tested	Not tested	Not tested	Not tested	PCR, followed by sequencing using *BRAF* exon 15F; restriction length polymorphism analysis by TspRI
Handolias et al[24]	7/1 Vagina, 1 cervix, 4 vulva, and 1 labia	Not tested	Not tested	Not tested	Not tested	2/7 (29%) [1 vulva and 1 labia]	Exon 11 L576P (1/7); exon 13 K642 (1/7)	High-resolution melting-screen analysis; sequencing *KIT* exons 11, 13, 17
Omholt et al[2]	30/23 Vulva and 7 vagina	2/30 (6.7%) [both vulvar]	Exon 15 V600E (2/30)	3/30 (10%) [all vaginal]	Exon 1 G12D (1/30), exon 2 Q61L (1/30), and exon 2 Q61H (1/30)	8/30 (26.7%) [all vulvar]	Exon 11 W557R (1/30), exon 11 V559D (1/30), exon 11 V560D (1/30), exon 11 P573L (1/30), exon 11 L576P (2/30), exon 17 D820Y (1/30), and exon 17 N822K (1/30)	PCR; sequencing *KIT* exons 9, 11, 13, 17, 18; *NRAS* exon 15; *BRAF* exon 15
Satzger et al[25]	10/Female genital tract	0/5 (0%)	NA	Not tested	Not tested	3/9 (25%) [not specified]	Exon 11 579del (1/9), exon 18 184IV (1/9), and exon 11 L576P (1/9)	PCR; sequencing *KIT* exons 9, 11, 13, 17, 18; *BRAF* exon 15; also fluorescent melting curve analysis for *BRAF*

Torres-Cabala et al[21]	15/11 Vulva and 4 vagina-cervix	Not tested	Not tested	Not tested	Not tested	4/15 (26.7%) [3 vulvar and 1 vaginal]	Exon 11 L576P (1/15), exon 17 D816V (1/15), exon 13 K642E and exon 17 N822I (1/15), and exon 13 K642E (1/15)	PCR; sequencing *KIT* exons 11, 13, and 17
Wong et al[27]	8/1 Cervix, 4 vagina, and 3 vulva	1/8 (12.5%) [vulvar]	N581I	1/8 (12.5%) [vaginal]	Exon 2 Q61K	Not tested	Not tested	PCR; sequencing *BRAF* exons 11 and 15; *NRAS* exons 1 and 2
Schoenewolf et al[3]	16/Vulva and vagina	Not tested	Not tested	Not tested	Not tested	5/11 (45.5%) [vulvovaginal]	Exon 13 K642E (1/11), exon 11 L576P (2/11), exon 11 V560D (1/11), and exon 13 L641H (1/11)	PCR; sequencing *KIT* exons 9, 11, 13, 17, 18
Abu-Abed et al[28]	19/2 Vagina and 17 vulva	Not tested	Not tested	Not tested	Not tested	1/19 (5.3%) [vulvar]	Exon 11 L576P	PCR; sequencing *KIT* exons 11 and 13
Current study	25/8 Vagina, 9 vulva, 2 labia, 1 introitus, 1 cervix, 3 LN, and 1 metastasis	2/25 (8.0%) [metastasis, vagina]	Exon 15 V600E (metastasis) (1/25), exon 15 K601E (vagina) (1/25)	4/25 (16.0%) [1 vaginal, 3 vulva]	Exon 1 G12V (1/25), exon 1 G13D (1/25), exon 2 Q61K (1/25), and exon 2 A59T (1/25)	4/25 (16.0%) [1 vagina, 2 vulva, and 1 LN]	Exon 13 K642E and Y646H (1/25), exon 11 L576P (1/25), exon 11 W557R and L576P (1/25), and exon 13 K642E (1/25)	PCR and mass spectroscopy; sequencing *KIT* exons 11, 13, 17; *NRAS* exons 1 and 2; *BRAF* exon 15; high resolution melting curve analysis for exon 11 of *KIT*
Cumulative frequency (%)		5/97 (5%)		11/76 (15%)		34/129 (26%)		

BRAF, V-raf murine sarcoma viral oncogene homolog B; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, CD117; *LN*, lymph node; *NA*, not available; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *PCR*, polymerase chain reaction.

Table II. Oncogenic mutations detected in female genital melanomas in this study

Case no.	Melanoma site	Age, y	Mutations
1	Vagina	62	None detected
2	Vagina	70	None detected
3	Vagina	77	None detected
4	Vagina	75	None detected
5	Vagina	61	None detected
6	Vagina	62	*KIT* exon 13 K642E and Y646H (in *cis*)
7	Vagina	77	*NRAS* exon 1 G12V
8	Vagina	46	*BRAF* exon 15 K601E
9	Vulva	70	None detected
10	Vulva	52	None detected
11	Vulva	70	None detected
12	Vulva	75	None detected
13	Vulva	71	None detected
14	Vulva	58	*NRAS* exon 1 G13D
15	Vulva	29	*NRAS* exon 2 Q61K
16	Vulva	57	*KIT* exon 11 L576P
17	Vulva	67	*KIT* exon 11 W557R and L576P (in *cis*)
18	Labia	81	*NRAS* exon 2 A59T
19	Labia	82	None detected
20	Introitus	50	None detected
21	Cervix	39	None detected
22	Inguinal node	70	None detected
23	Inguinal node	70	None detected
24	Inguinal node	68	*KIT* exon 13 K642E
25	Cutaneous metastasis	57	*BRAF* exon 15 V600E, *KRAS* exon G13D, and *PIK3CA* exon 9 E545K

BRAF, V-raf murine sarcoma viral oncogene homolog B; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, CD117; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog.

Table III. Oncogenic mutations detected in female genital nevi in this study

Path	No. of samples	*BRAF* n/N (%)	*BRAF* Mutations
Intradermal nevus	4	4/4 (100)	V600E
Compound nevus	3	3/3 (100)	V600E
Atypical nevus, genital type	4	3/4 (75)	V600E
Total	11	10/11 (91)	

BRAF, V-raf murine sarcoma viral oncogene homolog B; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, CD117; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog.

The *BRAF* V600E mutation is commonly found in benign and atypical nevi of the female genital tract

We researched the frequency of *BRAF* mutations in 7 benign melanocytic genital nevi (3 compound nevi and 4 intradermal nevi) and 4 atypical genital nevi in parallel with the analysis of the 25 female genital melanomas. *BRAF* V600E mutations were detected in 7 of 7 benign melanocytic genital nevi (100%) and 3 of 4 atypical genital nevi (75%; Table III). These results indicate that *BRAF* V600E is common in benign and atypical genital nevi but not in invasive melanomas of the female genital tract.

DISCUSSION

Mutational analysis of mucosal melanomas to date has been quite limited.[18] Most mutational studies have focused on melanomas originating from various cutaneous sites, with relatively limited numbers of mucosal melanomas and even fewer of the female genital mucosa.[32]

Of the 25 melanoma cases in our study, 4 (16%) harbored *KIT* mutations, 4 (16%) had *NRAS* mutations, and 2 (8%) had *BRAF* mutations. The mutational frequencies we observed in *KIT*, *NRAS*, and *BRAF* in female genital melanoma are comparable to the frequencies previously reported for mucosal melanomas in general (of different anatomic sites).[16,17] However, the small number of samples analyzed in our study may not provide enough resolution to distinguish true differences in mutational frequency between mucosal melanomas in general compared with female genital melanomas. In fact, mucosal and glabrous epithelia are distinct at the histologic level. No *GNAQ* or *GNA11* mutations were identified among 11 melanomas that were screened. These genes are commonly mutated in uveal/choroidal melanomas, and rarely in cutaneous melanomas.[33,34] Of note, the majority of melanoma cases lacked a detectable driver mutation in the oncogenes most commonly associated with melanoma. This suggests that other genes yet to be determined, including those downstream of the *RAS–BRAF* pathway, may be important in the development of genital melanomas. In addition to genetic mutations, chromosomal changes leading to copy number variation has been reported to be higher in mucosal melanomas than melanomas arising from other sites in the body. The frequency of genome-wide copy number changes in female genital melanomas is not known, but beyond the scope of the current study. Whether anatomic subsites, such as glabrous sites (ie, nonhair-bearing, modified mucosa, such as the labia minora and vaginal/cervical mucosa) compared with nonglabrous sites (ie, hair-bearing, such as the labia majora) might differ in oncogene mutations remains an open question.

Based on our findings and those of previous publications (Table I), *BRAF* mutations are

uncommon in melanomas of the female genital tract. Moreover, when they do occur, they may be substitutions other than V600E (eg, K601E in our study; N581I in Wong et al[27]). The single V600E-mutant melanoma in our series also harbored *KRAS* and *PIK3CA* mutations. This is quite unusual, but it has previously been reported that paired primary and metastatic melanomas can have different oncogenic mutations, presumably because of metastatic outgrowths from a genetically heterogeneous primary tumor.[35] Interestingly, Turajlic et al[36] recently reported a *BRAF* V600E-mutant melanoma that showed primary resistance to a *BRAF* inhibitor because of coexisting *GNAQ* and *PTEN* mutations.[36] Our results, together with these studies, offer mechanistic insights into why melanomas of the female genital tract may not clinically respond to *BRAF* V600E-targeting therapies.

For benign genital nevi, a high percentage (91%) harbored *BRAF* V600E. This mutation has previously been documented in genital nevi by Nguyen et al[37]: 6 of 20 nevi (30%), with 3 of 13 (23%) of atypical genital nevi and 3 of 7 (43%) of genital nevi without atypia, had a *BRAF* V600E mutation. In comparison, our results showed a considerably higher incidence of *BRAF* V600E, present in 75% of genital nevi with atypia and 100% of genital nevi with atypia. The difference in results may be attributable to differences in genotyping methodologies or they may be related to the relatively small numbers of cases in both studies. While it is possible that *BRAF*-mutant nevi may evolve into *BRAF*-mutant melanomas, the finding that BRAF mutations are rare in gynecologic melanomas but common in melanocytic lesions of the vulva suggests that melanocytic nevi are unlikely to be precursors for most melanomas arising in this anatomic location.

Ten of 19 (53%) articles selected for review included mutational analyses of mucosal melanomas of various sites. From these studies, data pertaining strictly to melanoma of the female genital tract were extracted to calculate the cumulative published frequencies of the oncogenes of interest (Table I). All relevant cases of primary melanomas, lymph node metastases, local recurrences, and cutaneous metastases localized to the female genital tract were included. The results of the literature review combined with results of our study show that gynecologic melanomas most frequently feature *KIT* mutations (26%), less frequently harbor *NRAS* mutations (15%), and uncommonly have *BRAF* mutations (5%). It is therefore possible that the frequency of *KIT* mutations in female genital melanomas may be higher than that estimated from our study alone (16%). In calculating the cumulative frequency among the published literature, we note that the Abu-Abed et al study[28] reported a lower frequency of *KIT* mutations (5%) compared to other studies (range, 25-54%), which is only partially explained by their restricted sequencing of *KIT* exons 11 and 13. Additional limitations of combining mutational frequencies from a review of the literature include variability in sample preparation and mutation screening techniques (ie, melting curve analysis, polymerase chain reaction–based DNA sequencing, or high-throughput genotyping platforms) and limited access to quality controlled data.

As our understanding of the genetic heterogeneity of melanoma grows, investigation of the genetic subsets of melanoma becomes essential for informing targeted therapeutic options. Importantly, all of the *KIT* mutations identified in our study would be predicted to be sensitive to KIT kinase inhibitors, including imatinib, sunitinib, dasatinib, sorafenib, and regorafenib. The exceptions might be those tumors harboring 2 *KIT* mutations, because there are no preclinical data available on the drug sensitivity of these forms of doubly-mutated *KIT*. In addition, early clinical trial data suggest that *NRAS*-mutant melanomas may respond to mitogen-activated protein kinase kinase inhibitors.[38] In conclusion, the high frequency of clinically targetable mutations in primary melanomas of the female genital tract indicates an important role of genotyping in the clinical management of these tumors.

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