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Phenotypic and Genotypic Characteristics of Mastocytosis According to the Age of Onset

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Abstract

Adult's mastocytosis is usually associated with persistent systemic involvement and c-kit 816 mutation, while pediatrics disease is mostly limited to the skin and often resolves spontaneously. We prospectively included 142 adult patients with histologically proven mastocytosis. We compared phenotypic and genotypic features of adults patients whose disease started during childhood (Group 1, n = 28) with those of patients whose disease started at adult's age (Group 2, n = 114). Genotypic analysis was performed on skin biopsy by sequencing of c-kit exons 17 and 8 to 13. According to WHO classification, the percentage of systemic disease was similar (75% vs. 73%) in 2 groups. C-kit 816 mutation was found in 42% and 77% of patients in groups 1 and 2, respectively (p = 0.001). 816 c-kit mutation was associated with systemic mastocytosis in group 2 (87% of patients with systemic mastocytosis vs. 45% with cutaneous mastocytosis, p = 0.0001). Other c-kit activating mutations were found in 23% of patients with mastocytosis' onset before the age of 5, 0% between 6 and 15 years and 2% at adults' age (p < 0.001). In conclusion, pathogenesis of mastocytosis significantly differs according to the age of disease onset. Our data may have major therapeutic relevance when considering c-kit-targeted therapy.

Introduction

Mastocytosis is a heterogeneous disease characterized by mast cell accumulation in various organs. Some cases are complicated with organ insufficiency and/or symptoms due either to mast cell infiltration or mediators release. Tissues that are commonly involved are skin, bone marrow, gastrointestinal tract, liver and skeletal systems[1]. The majority of mastocytosis cases occur in children (65%)[2], mostly as isolated cutaneous forms while less than 20% are complicated by a systemic dissemination[3]. Most of the cases resolve by puberty[3]. In contrast, patients with mastocytosis starting at the adult’s age present more often persistent systemic involvement.

Stem cell factor (SCF) is the major growth factor for mast cell survival and differentiation and interacts with its cognate receptor KIT, a tyrosine kinase encoded by the c-kit gene. C-kit proto-oncogene activation results in mastocytes accumulation and abnormal migration and activation in various tissues[4,5]. In neoplastic mast cell lines, valine or tyrosine substitution for an aspartate in c-kit codon 816 results in constitutive phosphorylation and activation of KIT[6]. In early reports, Asp816Val was found in peripheral blood of 27% of 55 adult patients with mastocytosis mainly of systemic forms or associated with clonal haematological disorders[7–9]. Currently, it is well admitted that this mutation was also reported in skin and bone marrow in the vast majority of adult patients with systemic or cutaneous mastocytosis[7–11]. In contrast, mutations in the c-kit gene are considered as rare in children[7–10]. However, a recent study reports presence of 816 mutations in 11 out of 13 adults with the pediatric onset of cutaneous mastocytosis and in all children with systemic mastocytosis (SM)[12]. Taken together these findings may explain differences in clinical presentation and outcome according to age of onset, and particularly the resolution of the disease in children’s mastocytosis. The finding of these mutations may have important

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implications for pathogenesis understanding, prognosis and therapeutics particularly regarding the potential use of c-kit inhibitors.

To our knowledge, no large study has attempted to describe characteristics of adult's mastocytosis according to the age of disease's onset. In this report, we compared phenotype and characteristics of adult's mastocytosis according to the age of histologically confirmed mastocytosis according to their childhood genotype in a large prospective cohort of adults’ patients with mast cell lineage disease, TMPE: telangiectasia macularis eruptive perstans. AHNMD: systemic mastocytosis with an associated haematological non-aggressive systemic mastocytosis, SSM: smouldering systemic matocytosis, SM-CM: cutaneous mastocytosis, ISM: indolent systemic mastocytosis, ASM: aggressive systemic mastocytosis, ISM: indolent systemic mastocytosis, ASM: aggressive systemic mastocytosis, SM-AHNMD: systemic mastocytosis with an associated haematological clonal non-mast cell lineage disease, TMPE: telangiectasia macularis eruptive perstans.

Table 1. Characteristics of 142 adults with mastocytosis according to their age of onset.

| Age of onset | Group 1: ≤15 years (n = 28) | Group 2: >15 years (n = 114) | P |
|--------------|----------------------------|-----------------------------|---|
| N (%)        | N (%)                      |                             |   |
| Females      | 19 (68)                    | 74 (65)                     | 0.700 |
| Age          | 32 ±10                     | 49 ±13                      | <0.0001 |
| Age of onset | 7 ±6                       | 36 ±13                      | <0.0001 |
| Duration of mastocytosis evolution (years) | 25 ±13 | 13 ±11 | <0.0001 |
| WHO classification | CM 7 (25) | 31 (27) |   |
| ISM          | 17 (60)                    | 72 (63)                     |   |
| ASM          | 3 (11)                     | 6 (5)                       |   |
| SSM          | 1 (3)                      | 3 (3)                       |   |
| SM-AHNMD     | 0 (0)                      | 2 (2)                       | 0.820 |
| SM           | 21 (75)                    | 83 (73)                     | 0.800 |
| Hepatomegaly | 5 (15)                     | 14 (11)                     | 0.524 |
| Splenomegaly | 4 (12)                     | 10 (8)                      | 0.459 |
| Adenomegaly  | 1 (8)                      | 7 (7)                       | 0.890 |
| Clinical cutaneous form | Urticaria pigmentosa 24 (86) | 104 (91) | 0.381 |
| Diffuse cutaneous mastocytosis | 6 (21) | 32 (33) | 0.316 |
| TEMPE        | 4 (14)                     | 13 (14)                     | 0.841 |
| Organ involvement | Gastrointestinal 5 (18) | 12 (11) | 0.284 |
| Bone marrow  | 16 (57)                    | 75 (68)                     | 0.414 |
| Liver        | 3 (11)                     | 3 (3)                       | 0.058 |
| Skeletal involvement | 11 (39) | 47 (41) | 0.839 |
| Systemic symptoms | Flush 19 (68) | 60 (54) | 0.188 |
| Weakness     | 15 (54)                    | 71 (67)                     | 0.188 |
| Anaphylactoid shock | 7 (26) | 17 (13) | 0.200 |
| Abdominal pain | 15 (54) | 47 (46) | 0.456 |
| Diarrhoea    | 12 (43)                    | 56 (52)                     | 0.396 |
| Nausea       | 6 (26)                     | 12 (26)                     | 1.000 |
| Skeletal pain | 15 (54) | 42 (40) | 0.184 |
| Elevated tryptase serum levels (>20 ng/ml) | 12 (48) | 70 (69) | 0.045 |

CM: cutaneous mastocytosis, ISM: indolent systemic mastocytosis, ASM: aggressive systemic mastocytosis, SSM: smouldering systemic mastocytosis, SM-AHNMD: systemic mastocytosis with an associated haematological clonal non-mast cell lineage disease, TEMPE: telangiectasia macularis eruptive perstans.

Table 2. Exons 8 through 13 and 17 mutations according to mastocytosis age of onset.

| Age of onset | WT | Other mutations | Mut 816 |
|--------------|----|----------------|---------|
| Group 1A (n = 12) | 3 (25) | 2 (25) | 6 (50) |
| Group 1B (n = 14) | 9 (64) | 0 (0) | 5 (36) |
| Group 2 (n = 112) | 24 (21) | 2 (2) | 86 (77) |

Data are expressed in number (percentage), 816: substitution of valine in 816 codon of c-kit, Other mutations: mutation in exons 8 through 13, WT: absence of mutation in exons 8 through 13 and 17. Complete genotype was not available for 4 816 WT patients; 1 patient in group 1A, 1 in group 1B and 2 in group 2.

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Results

A hundred and forty-two patients with mastocytosis were recruited, 28 patients in group 1 (13 in group 1A and 15 in group 1B) and 114 in group 2. For the whole population, thirty-five percent were male, with a mean age of 46±14 years at the time of inclusion. Seventy-three percent had historically confirmed (bone marrow or other histologically proven organ involvement) systemic mastocytosis (SM) with 63% ISM, 6% ASM, 3% SSM and 1% SM-AHNMD. The most frequently involved organ was bone marrow, concerning 64% of the patients, then skeletal system (40%), gastrointestinal system (12%) and liver (4%). Considering skin lesions, 90% of the patients presented with urticaria pigmentosa, 27% with diffuse cutaneous mastocytosis, and 14% with TEMP. Clinical examination showed hepatomegaly for 12% patients, splenomegaly for 9% and adenomegaly for 7%. The most frequent symptoms were asthenia (65% of the patients), flush (56%), diarrhoea (50%), abdominal pain (47%), skeletal pain (43%), and anaphylactic shock or blackout (17%). Tryptase level was above 20 ng/ml for 65% of the patients.

Comparison of patients’ characteristics between groups 1 and 2 is reported in table 1. As expected patients whose disease started in adulthood had a shorter duration of mastocytosis evolution. Patients with childhood onset mastocytosis had less frequent serum tryptase elevation and tended to present more frequent liver involvement. Gender, presence of systemic involvement, type of skin lesion, bone marrow infiltration, gastrointestinal involvement, hepatomegaly, splenomegaly, adenomegaly, weakness, anaphylactoid shock or blackout, nausea, abdominal pain, diarrhoea, osteoporosis and prevalence of X-ray bone lesions did not statistically differ between two groups. Hematological and liver biological parameters did not differ according to the age of onset (data not shown). Familial mastocytosis was found in 12 patients (8%).

Genotypic characteristics of patients are reported in table 2. Sixty eight percent of the patients presented a D816X mutation of c-kit gene in the exon number 17. Patients with an adult onset mastocytosis had more frequently D816X mutation (77%) in group 2 vs. 42% of patients in group 1, p<0.001). Elevated tryptase serum levels (>20 ng/ml) were more frequently observed in the group of patients with the presence of 816 c-kit mutation (88%) vs those without (44%) (p<0.0001). Presence of D816X c-kit mutation was not associated with duration of mastocytosis evolution (16.25±12.25 years in patients without mutation vs. 14±11.65 in patients with the mutation, p = 0.328).

Mutation screening from exons 8 through 13 was performed for 816 wild type patients except 1 patient in group 1A, 1 patient in group 1B and 2 in group 2 because the amount of RNA was not sufficient and a new biopsy was denied. Non-816 mutations were
mostly found in group 1A (25% in group 1A vs. 0% in group 1B vs
2% in group 2, p = 0.002). In group 1A, 2 patients presented with
Del419D deletion in exon 8 and 1 with K509I mutation in exon 9. In
group 2, 1 patient presented Del419D deletion in exon 8 and 1 patient with V560G mutation in exon 11. We report the main
characteristics of these patients with unusual c-kit mutations in table 3.

We studied the association between genotype and phenotype in
groups 1 and 2, respectively (table 4). No association between
genotype and mastocytosis form according to the WHO
classification were evidenced in group 1, 71% vs 57% were
mutated for 816 c-kit

Table 3. Description of 5 adult patients with mastocytosis not related to 816 c-kit mutation.

| Mutation | Exon | Gender | Age of onset | WHO classification | Familial form |
|----------|------|--------|--------------|--------------------|--------------|
| K509I    | 9    | M      | <1 year      | CM                 | No           |
| Del419D  | 8    | M      | <1 year      | ISM (bone marrow)  | No           |
| Del419D  | 8    | F      | 5 years      | ISM (bone marrow)  | No           |
| Del419D  | 8    | F      | 41 years     | ISM (bone marrow, gastrointestinal) | No |
| V560G    | 11   | F      | 53 years     | CM                 | No           |


Discussion

It is currently admitted that pediatrics and adult is mastocytosis
exhibit different clinical and genotypic features[2]. To our
knowledge, however, only one retrospective study has compared
phenotypic characteristics of mastocytosis according to the age of
onset [16]. The authors reported more often significant clinical
improvement and more frequent gastrointestinal symptoms in
childhood onset patients. However, patient’s age was unknown,
the search for systemic involvement was not investigated in all
cases and a long-term follow up was available only for a subgroup
of patients.

In the present report, we have demonstrated that although
exhibiting genotypic differences, clinical features of adult patients
with mastocytosis were similar regardless their adult or pediatric
onset.

Overall the percentage of SM was 73% and rates of SM did not
differ according to the age of onset. In previous reports, SM was
reported to be more frequent in adult mastocytosis patients than in
children with mastocytosis, regardless of their outcome. Worobec
et al. reported 65 patients with mastocytosis; 90% of the 55 adults
studied presented SM, whereas only 30% of the 10 children
studied presented SM [7]. In agreement with those findings,
previous reports have also shown that bone marrow involvement was
significantly more frequent in adult (45%–90%)[17] [18] than in
pediatric (18%) [19] patients. These discrepancies with the
results of our study might be explained by the fact that pediatric
patients with systemic involvement may not resolve at puberty and
therefore may not differ from mastocytosis beginning at adult’s
age. Alternatively, adult patients with mastocytosis related to
pediatric onset may have evolved from a cutaneous to a systemic
disease because of the longer course of the disease. However, this
hypothesis is unlikely since tryptase levels, a marker of mast cell
burden, was significantly lower in the group 1.

Our work elucidated that c-kit genotype differed with the age of
mastocytosis onset. D816X mutation in exon 17 of c-kit was more
frequent in patients with adult onset mastocytosis than in those
with childhood onset. No large previous study had compared c-kit
genotype in mastocytosis according to age of onset. However,
several studies compared c-kit genotype in adults and children
patients with mastocytosis. Most of the studies reported a higher
prevalence of D816X c-kit mutations in adults than in children
with mastocytosis. Longley et al. reported that all of 10 adult
patients (with adult onset) with cutaneous or systemic mastocytosis
presented D816 mutation on skin biopsy, whereas only 13% of the
15 children with mastocytosis age of onset between 0 and 12 years
had the mutation [9]. In another study, 816 mutation was present
in skin for 6 out of 6 adults patients with adult onset (5 patients
with SM and 1 with CM), and none of the 11 children patients
with CM[10]. A recent study reported 93% of D816V c-kit mutation
in bone marrow of 113 adult patients with systemic mastocytosis,
however, no data on skin biopsy and age of disease’s onset was
available in this study [20]. Overall these studies suggest that 816 c-kit
mutation prevalence is high in adult patients and low in pediatric
patients. However, Yanagihori et al reported D816X c-kit mutation
for 11 out of 13 adults with CM in whom disease started during
infancy [12]. In line with this report, in our study we found that 816
mutations was higher (40%) than expected in the population of
adults patients with pediatric onset. These findings strongly suggest
that 816 mutations in pediatric patients may be associated to a
higher probability of persistence at adult age.

Here, non-816 c-kit mutations were rarely found in adults’ onset
patients (2%), whereas it represented 25% of patients with age of
onset below 5 years old and 0% of patients with an age of onset
between 5 and 15 years. We studied exons 17 and 8 through 13
but not the whole c-kit gene because a previous study of our group
targeted the entire gene in 50 children and did not find any
mutation in other exons of c-kit gene (unpublished personal data).
Three patients presented Del419D in exon 8 of c-kit, 2 patients had
a disease onset before the age of 5 and 1 after 15, all presented SM
including one with gastrointestinal involvement. This mutation

| WHO classification | Group 1 | Group 2 |
|--------------------|--------|--------|
|                     | wt816  | mut 816| wt816  | mut 816|
| CM (n = 38)         | 5 (71) | 2 (29) | 17 (55) | 14 (45) |
| p = 0.788           |        |        |        |        |
| SM (n = 104)        | 12 (57)| 9 (43) | 11 (13) | 72 (87) |
| p = 0.668           | <0.001 |        |        |        |

Data are expressed in number (percentage).
was recently reported in a patient that presented familial gastrointestinal stromal tumor and mastocytosis [21]. In the case reported here, the mutation was not found in peripheral blood mononuclear cells, ruling out a germline mutation. One of our patients with nonfamilial CM from group 1A presented a K509I mutation in exon 9. Another recent study reported K509I mutation in a case of familial mastocytosis [22]. An adult onset patient with CM/ISM presented V560G mutation in exon 11, which has previously been reported in 2 adults, 1 CM and 1 SM [10]. Taken together, these results suggest that in pediatric patients below the age of 15 other mutations than the classical 816 of the c-kit gene may occur. Therefore, pathogenesis of mastocytosis clearly differs according to age’s onset.

Prospective studies in pediatric population is currently performed to determine whether frequency of these unusual mutations might be a prognostic indicator.

In conclusion, despite similar clinical presentation, the high frequency of non mutated c-kit gene in case of childhood’s onset contrasted with a high frequency of 816 mutation in mastocytosis cases starting at the adult’s age. These findings may have important therapeutic implications when targeted therapy against kinase activity of c-kit is considered.

**Methods**

**Patients**

Adult patients (>18 years old) with mastocytosis were recruited prospectively through the French mastocytosis network (AFIRM « French Association for the Initiatives of Research on Mastocyte and Mastocytosis», protocol “Physiopathological and clinical study of mastocytosis in adult patients”) to compare phenotypic and genotypic features of patients according to their age of onset. Data collection was conducted in 15 medical centers between January 2000 and December 2004.

Patients were included after written informed consent was obtained. The study was approved by the ethical committee of Pitie-Salpetriere university hospital (Paris, France) and data were computerized in accordance with the French Commission Informa- tique et Libertés. All patients had a histologically proven mastocytosis. In all cases skin biopsies and bone marrow biopsies/or aspiration were performed. The diagnosis of cutaneous involvement by mastocytosis was based on the association of both clinical symptoms compatible with cutaneous mastocytosis and the presence of a mast cells multifocal accumulation in skin biopsy according to usual histopathological criteria[13,14]. In most of the cases TEMEP doesn’t show a significant increase in mast cells and the diagnosis was made in association with clinical datas. However, some subtle features can be useful in the histological diagnosis such as a slight increased number of fusiform and loosely arranged mast cells situated around dilated superficial capillaries. Immunohistochemistry with anti-CD25 was performed on thirty mastocytosis skin samples out of this series, including all various forms of cutaneous mastocytosis, such as urticaria pigmentosa, TEMEP and systemic mastocytosis with skin involvement. All cases were negative.

Bone marrow involvement was defined by bone marrow aspiration and/or biopsy with a dense focal or diffusse mast cell infiltrate with spindle-shaped cells [15]. However, in several cases, foci of spindle mast cells located mainly around blood vessels or bone trabeculae were observed which allowed the bone marrow to be classified as involved. Mast cells expressed CD117 and tryptase but in our hands, were rarely stained with CD25 antibody, but were usually closely associated with foci of small B and T-cells.

Liver and/or gastrointestinal involvements were histologically proven. The presence of mast cells within the sinusoids defined liver involvement and the presence of a dense mast cells infiltrate in lamina propria defined gastrointestinal involvement [15]. Clinical, biological and histological data were recorded through a Clinical Records Form (CRF). The following criteria were collected: age at data collection, age of onset of cutaneous involvement based on available written medical reports from a pediatrician or patient’s parents, age at diagnosis, gender, cutaneous clinical form (mastocytoma, urticaria pigmentosa, telangectasia macularis eruptiva perstans (TMEP)), flush, fatigue, anaphylactoid shock, abdominal pain, diarrhea, presence of SM and date of onset, hepatomegaly, splenomegaly, adenomegaly, bone pain, osteoporosis (defined as T score &lt;−2), radiographic skeletal lesion, the presence of bone marrow involvement on bone marrow aspiration and/or biopsy, gastrointestinal, liver involve- ment, blood cell count, liver enzymes and tryptase serum levels. Bone involvement included osteoporosis and/or compatible radiological bone lesions. Patients were classified according to WHO classification [1], in CM, indolent systemic mastocytosis (ISM); smouldering systemic mastocytosis (SSM), aggressive systemic mastocytosis (ASM) or systemic mastocytosis with an associated haematological clonal non-mast cell lineage disease (SM-AHNMD). In cases in which bone marrow aspirate and/or biopsy did not disclose mast cell involvement and that no other histologically organ involvement was proven (liver, gastrointestinal tract), patients were considered to have CM. Group 1 included patients with mastocytosis age of onset ≤15 years old (because this corresponds to the cut off between pediatrics and adult’s population in the French health system). This group was divided in group 1A that included patients with age of onset ≤5 years old and group 1B >5 and ≤15 years old. Group 2 included patients with mastocytosis age of onset >15 years old.

**Mutation screening**

Skin samples. A skin biopsy of 3 to 4 mm on mastocytosis cutaneous lesions was performed for each patient and directly incubated in RNA later (Qiagen) by the dermatologist in charge of the patient before sending it for centralization to the reference laboratory. Skin biopsy was systematically performed at inclusion in this study, therefore at various times from initial mastocytosis diagnosis. We previously assessed that nucleic acids in biopsies were stable for one week at room temperature (data not shown). Samples were stored at −80°C in such buffer upon arrival.

RNA preparation and c-kit D816V mutation detec- tion. Total RNA was extracted from skin biopsies using the Rneasy mini kit (Qiagen). Complementary DNA was synthesized by using random hexamers and oligo dT as oligonucleotide primer from 200 ng total RNA using the stratascript first-strand synthesis system (Stratagene) in a total volume of 50 μl as recommended by the manufacturer. Then, 2.5 μl of cDNA was introduced in each polymerase chain reaction (PCR).

c-kit gene was amplified by PCR using HotStartTaq™ DNA polymerase (Qiagen S.A. France). A total of 40 cycles was performed using either the 9700 or 2700 Gene Amp PCR Systems (Applied biosystems) at 94°C for 30 sec, 57°C for 30 sec and 72°C for 45 sec.

c-Kit coding sequences were amplified from complementary DNA with the PCR by using primer pairs indicated in table 1. For the specific detection of the mutation at the 816 position, we used the primer pairs (2295s & 2661r) indicated in table 5. Direct amplimer sequencing was carried out after PCR products were purified with the multiscreen HTS MNSV030050 purification system, (Millipore, Guyancourt France). They were directly sequenced with Big dye terminator V 1.1 (Applied biosystems) on an ABI Prism 3130 sequencer (Applied biosystems)
and analysed with the Seqscape software (Applied biosystems) using 2295s & 2647r sequencing primers described in table 5.

**Confirmation of D816 mutation detection.** D816V mutation was also confirmed by restriction digest analysis with BsmA1 and Ple1 restriction enzymes, which detect wild type and mutated form, respectively. Purified fluorescent primers (2295sF & 2661rF; see table 1) were used for PCR reactions. Size of restriction digest fragments (201 for Bsma1 fragment and 179 and 187 for Ple1 fragment) were directly determined on a 16 capillary sequencer (ABI Prism 3130 sequencer) with the GeneMapper software (Applied biosystems).

**Confirmation of the lack of 816 mutation.** In the case of detection of a WT sequence, an independent PCR was performed on cDNA. Same conditions as described above were used for the PCR reaction, except that 30 cycles were used. Then, PCR products were digested by BsmA1 enzyme in order to increase a putative mutated signal. The Bsma1 digested products were then amplified in a 25 cycles nested-PCR reaction using 2341s and 2600r primer pairs (see table 5). Same conditions of temperature and time as above were applied for this reaction. Purified nested PCR products were then sequenced with 2341s and 2600r primer pairs as described above and analysed with the Seqscape software.

**Exons 8 through 13 c-kit coding regions were sequenced for WT816 patients.** A PCR, using the same conditions, was performed on cDNA except that the c-Kit coding region was separated in five different fragments (PCRK1 to K5) for PCR reaction described in table 5. Each PCR fragment was then sequenced as described above using sequencing primer described in table 5.

**Statistical analysis**

Statistical analyses were performed using SAS version 9.1 and Excel.

Quantitative variables were summarized using the following descriptive statistics: number of observed and missing data, mean, standard deviation, median, minimum and maximum. Absolute and relative frequency distributions were provided for qualitative variables. We used Student’s T test for continuous variables with limited effective, and the Wilcoxon test for the nonparametric values. We confronted the values. For the discontinuous values, we used the chi2 test or Fisher’s exact test. Statistical significance was defined as p value $<0.05$.

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**Author Contributions**

Conceived and designed the experiments: OH OL PD AM FL. Performed the experiments: JL PD FP FF YY YZ SF DC. Analyzed the data: OH FL AC. Contributed reagents/materials/analysis tools: CB. Wrote the paper: OH OL PD FL FP. Other: Molecular Biology: HS MA. Data collection: AC CB. Collected the data: BS DG KH FS RD FS PC SB.

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