

## Interleukin-31 Polymorphisms and Serum IL-31 Level in Patients with Mastocytosis: Correlation with Clinical Presentation and Pruritus

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**Data on interleukin-31 (IL-31) involvement in the pathogenesis of mastocytosis, and its impact on pruritus development in the disease, are limited. The aim of this study was to analyse distinct IL-31 gene polymorphisms in 127 patients (age 0.5–76 years) with mastocytosis and their correlation with clinical presentation, pruritus and serum IL-31 levels. In patients with mastocytosis, the frequency of IL-31 IVS2+12AA genotype and IVS2+12A allele was higher than in control subjects and they were linked to an increased risk of development of mastocytosis. In adult patients, but not in children, -2057AA genotype was also associated with an increased risk of occurrence of mastocytosis. Pruritus affected 83.3% of 78 adult patients with mastocytosis, and a positive correlation between serum IL-31 levels and pruritus was found in these patients. In conclusion, distinct polymorphic variants of the IL-31 gene may be involved in the pathogenesis of mastocytosis, and IL-31 may be involved in the induction of pruritus in patients with mastocytosis.**

**Key words:** mastocytosis; interleukin 31; polymorphism; pruritus; tryptase.

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Mastocytosis is an uncommon and heterogeneous disease, defined as 1 of the 8 subcategories of myeloproliferative neoplasms (MPN) according to the 2008 WHO classification of tumours of haematopoietic and lymphoid tissues (1). Mastocytosis results from a clonal, neoplastic proliferation of morphologically and immunophenotypically abnormal mast cells (MCs) that accumulate in various organs (2, 3). The disease may be limited to the skin (cutaneous mastocytosis, CM), but it may also involve extracutaneous organs (systemic mastocytosis, SM) including the bone marrow (BM), skin, liver, spleen, lymph nodes and gastrointestinal tract (2–4). Most patients present typical skin lesions that may coexist with pathology of internal organs (SM<sub>skin</sub>+). Childhood-onset mastocytosis is usually a skin-limited disease that spontaneously regresses with age, whereas

adult-onset mastocytosis presents with multi-organ involvement and persistent course (5–7). CM is defined by the presence of typical skin lesions in the absence of involvement of extracutaneous tissues. CM clinically manifests as maculopapular type (MPCM), diffuse type (DCM) and mastocytoma of the skin (4). The diagnosis of SM is based on WHO criteria (1–3). The major criterion of SM is the histological demonstration of multifocal, dense infiltrates of MCs (>15 MCs in aggregates) detected in sections of BM and/or other extracutaneous organs. Minor criteria include: (i) in biopsy sections of BM or other extracutaneous organs, >25% of MCs in the infiltrate are spindle-shaped or have atypical morphology or, of all MCs in BM aspirate smears, >25% are immature or atypical; (ii) detection of an activating point mutation at codon 816 of *KIT* in BM, blood or other extracutaneous organs; (iii) MCs in BM, blood or other extracutaneous organs express CD2 or/and CD25 in addition to normal MCs markers; (iv) serum total tryptase persistently exceeding 20 ng/ml (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid) (3). The presence of the major criterion and at least one of the minor criteria, or more than 2 minor criteria is required for the diagnosis of SM (1–3).

The pathogenesis of mastocytosis is complex and not sufficiently understood (2–6). It has been proved that the key role is played by somatic activating D816V *KIT* mutation in exon 17, which results in dysregulation of the normal development and proliferation of MCs (3, 6–13). It has also been reported that interleukin-13 (IL-13) promoter gene polymorphism – 1112C/T and Q576R polymorphism in the IL-4 receptor  $\alpha$ -chain are associated with clinical presentation of mastocytosis (12, 13).

IL-31, related to the IL-6 cytokine family, is secreted by CD4<sup>+</sup>T cells, particularly activated T<sub>H</sub>2 cells, peripheral blood and skin-homing CD45RO<sup>+</sup> CLA<sup>+</sup> (cutaneous lymphocyte-associated antigen-positive) T cells and MCs (14–17). IL-31 receptor complex has been identified on activated monocytes, macrophages, eosinophils, basophils, dorsal root ganglia, and keratinocytes (15). IL-31 plays a significant role in the induction of chronic inflammation and regulates numerous processes of innate and adaptive immunity in tissues exposed to environmental factors (15). Recent studies demonstrate that IL-31 is involved in the pathogenesis of

chronic skin inflammation and pruritus in patients with atopic dermatitis, chronic spontaneous urticaria, allergic contact dermatitis, prurigo nodularis, primary cutaneous lymphomas and myeloproliferative disorders including mastocytosis (15–25). However, the significance of IL-31 and its gene polymorphism in the pathogenesis and induction of pruritus in mastocytosis is unknown.

The aim of the study was to assess the frequency of IL-31 -1066G/A, -2057G/A, and IVS2+12A/G gene polymorphisms in patients with mastocytosis and determine whether there are any differences between patients with mastocytosis and the control group and between patients with various forms of the disease, including CM, indolent SM (ISM), adult- and childhood-onset mastocytosis. Further aims were to determine the relationship of distinct *IL-31* genetic variant polymorphisms with serum IL-31 levels and pruritus, and to assess the correlation between serum IL-31 levels and pruritus intensity in adult patients with mastocytosis.

## MATERIALS AND METHODS

### Participants

Two groups of patients with mastocytosis were enrolled in the study: a group of 127 patients participating in analysis of polymorphic variants of the IL-31 gene (-1066G/A, -2057G/A, IVS2+12A/G) and a group of 78 patients in whom the evaluation of serum IL-31 levels was performed. In all cases, CM was confirmed by histological examination of the skin sample. SM was diagnosed strictly according to current WHO criteria and current recommendations (1, 4–6). Therefore, bone marrow biopsy with histological examination, basal serum tryptase levels, assessment of mast cells morphology and immunophenotype and *KIT* D816 mutation analyses were performed in all adult patients. Mutational analysis was performed using an allele-specific PCR. Only patients with cutaneous involvement and without associated allergic diseases and/or other diseases provoking pruritus were enrolled in the study. Clinical evaluation of patients and collection of blood samples used to realize the purposes of the study were performed during the diagnostic period before starting mastocytosis therapy. All the patients enrolled in the study were diagnosed at the Department of Dermatology, Venereology and Allergology and at the Department of Allergology and Pneumology of the Medical University of Gdansk, which has become the Polish Center of Excellence of the European Competence Network of Mastocytosis since 2005. The control group comprised 97 healthy subjects (age range 18–52 years; mean age  $27.7 \pm 7.54$  years) with no history of allergic diseases, mast cell mediator-related symptoms, anaphylaxis, other diseases provoking pruritus and systemic therapy. All of them were free of any skin diseases. All patients and healthy control subjects gave written informed consent for clinical examination and blood analysis. The study was approved by the medical ethics committee of the Medical University of Gdansk and was conducted according to the principles of the Declaration of Helsinki.

Determination of distinct IL-31 polymorphism was performed in a group of 127 patients with mastocytosis, including 57 children (30 males, 27 females) and 70 adults (15 males, 55 females), and a group of 97 healthy control subjects (23 males and 74 females). The paediatric subgroup (age range 0.5–16 years; mean age  $4.45 \pm 3.97$  years) included 45 MPCM, 8 mastocytoma and 4 patients with DCM. The adult subgroup comprised 38 patients with CM (age range 18–66 years; mean age  $35.87 \pm 13.23$  years)

and 32 patients with indolent SM associated with skin involvement (ISMskin+; age range 18–76 years; mean age  $40.77 \pm 13.71$  years). In all adults with mastocytosis, morphology of skin lesions corresponded to MPCM.

Serum IL-31 levels were analysed in a group of 78 adult patients with mastocytosis (24 males and 54 females) and 60 healthy control subjects (14 males and 46 females). The group of patients consisted of 24 CM (age range 21–63 years; mean age  $35.33 \pm 12.56$  years) and 54 ISMskin+ patients (age range 18–77 years; mean age  $44.37 \pm 13.44$  years). All of them presented MPCM.

### Determination of IL-31 -1066G/A, -2057G/A, and IVS2+12A/G gene polymorphisms

Genomic DNA was prepared from whole-blood samples using Blood DNA Prep Plus according to the manufacturer's instructions (A&A Biotechnology, Gdańsk, Poland). Analysis of the polymorphic variants of the IL-31 gene was performed by amplification refractory mutation system PCR (ARMS-PCR). ARMS-PCR method using self-designed specific sequences of oligonucleotides and internal amplification control of growth hormone 1 (GH1) gene fragment: GH1-F GCCTTCCCAACCATTCCTTA and GH1-R TCACGGATTCTGTTGTGTTTC, was applied. PCR conditions were as follows: initial denaturation for 5 min at 94°C; 34 cycles of 40 s at 94°C, annealing step for 60 s at 64°C for IL-31 -2057, 62°C for IL-31 -1066, 53°C for IL-31 IVS2+12 and 90 s at 72°C; final elongation at 72°C for 5 min. PCR products were separated in 2% agarose gels and stained with ethidium bromide.

### Evaluation of serum IL-31 levels, serum tryptase levels and pruritus severity

Serum IL-31 levels were measured using an enzyme-linked immunoabsorbent assay (ELISA) standard kit (Bender MedSystems GmbH, Vienna, Austria). All procedures were performed according to the manufacturer's protocol. Total serum tryptase levels were measured using a fluoroimmunoassay (ImmunoCAP Tryptase; Thermo Fisher Scientific, Uppsala, Sweden) in all children and adults with mastocytosis enrolled in the study. The assessment of pruritus severity was performed using a visual analogue scale (VAS). Spontaneous itch severity in the last 24 h was measured at the beginning of the visit at the Department of Dermatology before physical examination and provoking Darier's sign.

### Statistical analysis

Statistical analysis was performed with the use of the STATISTICA 10 software package (StatSoft, Tulsa, OK, USA). Pearson's  $\chi^2$  test was employed to examine the significance of the differences in the observed alleles and genotypes between groups. A logistic regression model was used to calculate the odds ratios (ORs) and the 95% confidence intervals (95% CIs). Mann-Whitney *U* test was used to compare the median values. Correlation coefficients were determined using Spearman's rank correlation test. *p*-values below 0.05 were considered to be statistically significant.

## RESULTS

### Genetic variant polymorphisms in relation to clinical presentation and pruritus

The results of this study indicate that the frequency of AA genotype for the IL-31 IVS2+12 was higher in all patients with mastocytosis (children and adults) than in control subjects (50.4% vs. 29.9%,  $p=0.002$ ) and

it was linked with an increased risk of mastocytosis development (OR 2.38;  $p=0.002$ ). The same was true for allele A of IVS2+12 (OR 1.71;  $p=0.009$ ) (Table I), whereas the IVS2+12AG genotype was linked with a decreased risk of mastocytosis occurrence compared with control subjects (OR 0.46;  $p=0.006$ ).

Comparison of the frequencies of distinct IL-31 gene polymorphisms between all patients with adult-onset mastocytosis and controls revealed higher frequencies of: IVS2+12AA genotype and IVS2+12A allele, and -2057AA genotype (Table II). Moreover, IVS2+12AA genotype (OR 2.345,  $p=0.009$ ), IVS2+12A allele (OR 1.694,  $p=0.032$ ) and -2057AA genotype (OR 4.043,  $p=0.045$ ) were linked with an increased risk of mastocytosis occurrence in adults (Table II). Furthermore, the IVS2+12AG genotype was linked with a decreased risk of mastocytosis occurrence compared with control subjects (OR 0.47;  $p=0.019$ ).

There were no statistically significant differences between CM and ISMskin+ in respect of IL-31 -1066G/A, -2057G/A, IVS2+12A/G gene polymorphism frequencies ( $p=0.816$ ,  $p=0.676$ ,  $p=0.158$ , respectively) in adult mastocytosis patients. However, in adult patients with CM, IVS2+12AA genotype was more frequent than in ISMskin+ and the difference was close to statistical significance (60.5% in CM vs. 37.5% in ISMskin+;  $p=0.055$ ).

Comparison of the frequencies of distinct IL-31 gene polymorphisms between adult patients with CM and controls revealed higher frequencies of IVS2+12AA genotype (60.53% in CM vs. 29.90% in controls;  $p=0.001$ ), IVS2+12A allele (80.26% vs. 63.92%;  $p=0.009$ ) and -2057AA genotype (13.16% in CM vs. 3.09% in controls;  $p=0.026$ ). IVS2+12AA genotype, IVS2+12A allele and -2057AA genotype were linked with an increased risk of CM occurrence in adults (OR 3.59,  $p=0.001$ ; OR 2.29,  $p=0.010$ ; OR 4.75,  $p=0.034$ , respectively). Moreover, IVS2+12AG genotype was less frequent in patients with CM than in control subjects (39.47% vs. 68.04%,  $p=0.002$ ) and it was linked with decreased risk of CM occurrence compared with control subjects (OR 0.31,  $p=0.003$ ).

There were no statistically significant differences between adult ISMskin+ patients and control subjects in respect of IL-31 -1066G/A,

**Table I. Genotype and allele frequencies of distinct interleukin-31 (IL-31) gene polymorphisms in all mastocytosis patients and healthy control group**

IL-31 gene polymorphism	Genotype and allele	Mastocytosis <i>n</i> = 127 <i>n</i> (%)	Control <i>n</i> = 97 <i>n</i> (%)	<i>p</i> -value <sup>a</sup>	Odds ratio (95% CI)	<i>p</i> -value
-1066G/A	GG	55 (43.31)	37 (38.14)	0.44	1.24 (0.72–2.12)	0.44
	GA	59 (46.46)	48 (49.48)	0.65	0.88 (0.52–1.50)	0.65
	AA	13 (10.23)	12 (12.37)	0.60	0.81 (0.35–1.86)	0.62
	G	169 (66.53)	122 (62.89)	0.42	1.17 (0.79–1.73)	0.42
	A	85 (33.47)	72 (37.11)			
-2057G/A	GG	58 (45.67)	49 (50.52)	0.47	0.82 (0.48–1.40)	0.47
	GA	58 (45.67)	45 (46.39)	0.91	0.97 (0.57–1.65)	0.91
	AA	11 (8.66)	3 (3.09)	0.09	2.97 (0.81–10.96)	0.10
	G	174 (68.50)	143 (73.71)	0.23	0.77 (0.51–1.17)	0.23
	A	80 (31.50)	51 (26.29)			
IVS2+12A/G	AG	63 (49.61)	66 (68.04)	<b>0.006</b>	<b>0.46 (0.27–0.80)</b>	<b>0.006</b>
	AA	64 (50.39)	29 (29.90)	<b>0.002</b>	<b>2.38 (1.36–4.16)</b>	<b>0.002</b>
	GG	0 (0.00)	2 (2.06)	0.10	0.15 (0.007–3.16)	0.22
	G	63 (24.80)	70 (36.08)			
	A	191 (76.20)	124 (63.92)	<b>0.009</b>	<b>1.71 (1.14–2.58)</b>	<b>0.009</b>

<sup>a</sup>Pearson's  $\chi^2$  test. Significant values are shown in bold. CI: confidence interval.

-2057G/A and IVS2+12A/G gene polymorphism frequencies ( $p=0.599$ ,  $p=0.342$ ,  $p=0.547$ , respectively).

In paediatric patients with mastocytosis, frequency of AA genotype for the IL-31 IVS2+12 was higher than in control subjects and it was linked to an increased risk of mastocytosis development (OR 2.43,  $p=0.01$ ) (Table III). The same was revealed with allele A for IVS2+12 (OR 1.73,  $p=0.037$ ). IVS2+12AG genotype was linked with a decreased risk of mastocytosis occurrence compared with control subjects (OR 0.45,  $p=0.021$ ). There was no statistically significant difference between childhood-onset mastocytosis and control subjects in respect of IL-31 -1066G/A and -2057G/A gene polymorphism frequencies (Table III).

Further statistical analysis revealed that there were no statistically significant differences between pruritic and non-pruritic mastocytosis patients in respect of IL-31 -1066G/A, -2057G/A, IVS2+12A/G gene poly-

**Table II. Genotype and allele frequencies of distinct interleukin-31 (IL-31) gene polymorphisms in all adult-onset mastocytosis patients and controls**

IL-31 gene polymorphism	Genotype and allele	Adult-onset mastocytosis <i>n</i> = 70 <i>n</i> (%)	Controls <i>n</i> = 97 <i>n</i> (%)	<i>p</i> -value <sup>a</sup>	Odds ratio (95% OR)	<i>p</i> -value
-1066G/A	GG	30 (42.86)	37 (38.14)	0.54	1.22 (0.65–2.27)	0.54
	GA	34 (48.57)	48 (49.48)	0.91	0.96 (0.52–1.78)	0.91
	AA	6 (8.57)	12 (12.37)	0.43	0.66 (0.24–1.86)	0.44
	G	94 (67.14)	122 (62.89)	0.42	1.2 (0.76–1.91)	0.42
	A	46 (32.86)	72 (37.11)			
-2057G/A	GG	29 (41.43)	49 (50.52)	0.24	0.69 (0.37–1.29)	0.25
	AG	33 (47.14)	45 (46.39)	0.92	1.03 (0.56–1.91)	0.92
	AA	8 (11.43)	3 (3.09)	<b>0.032</b>	<b>4.043 (1.03–15.83)</b>	<b>0.045</b>
	G	91 (65.00)	143 (73.71)	0.09	0.66 (0.41–1.06)	0.09
	A	49 (35.00)	51 (26.29)			
IVS2+12A/G	AG	35 (50.00)	66 (68.04)	<b>0.02</b>	<b>0.47 (0.25–0.89)</b>	<b>0.019</b>
	AA	35 (50.00)	29 (29.90)	<b>0.008</b>	2.345 (1.24–4.44)	<b>0.009</b>
	GG	0 (0.00)	2 (2.06)	0.23	0.27 (0.01–5.73)	0.40
	G	35 (25.00)	70 (36.08)			
	A	105 (75.00)	124 (63.92)	<b>0.031</b>	1.694 (1.05–2.74)	<b>0.032</b>

<sup>a</sup>Pearson's  $\chi^2$  test. Significant values are shown in bold. CI: confidence interval.

morphisms ( $p=0.52$ ,  $p=0.24$ ,  $p=0.98$ , respectively). The same was true when groups of adults and children were evaluated separately (data not shown). There were no statistically significant differences in pruritus scores between genotypes for the -1066G/A, -2057G/A, IVS2+12A/G gene polymorphisms ( $p=0.39$ ,  $p=0.49$ ,  $p=0.59$ , respectively). The analogous analyses performed separately for children and adult patients revealed no statistically significant differences (data not shown).

#### Serum IL-31 level in relation to clinical presentation and genetic variant polymorphisms

The median and mean  $\pm$  standard deviation (SD) serum IL-31 levels were 4.875 and  $5.114 \pm 1.46$  pg/ml (range 3.1–12.94 pg/ml), respectively, in 78 adult patients with mastocytosis (CM and ISMskin+) and they were significantly higher ( $p < 0.001$ ) compared with serum IL-31 in healthy control subjects (median 0.0 pg/ml; mean  $\pm$  SD  $0.235 \pm 0.734$  pg/ml; range 0–4.1 pg/ml) (Fig. 1).

In patients with CM, the median and mean  $\pm$  SD serum IL-31 levels were 4.52 pg/ml and  $4.68 \pm 1.05$  pg/ml (range 3.4–7.5 pg/ml), respectively. Corresponding values in ISMskin+ patients were 5.01 pg/ml and  $5.31 \pm 1.58$  pg/ml (range 3.1–12.9 pg/ml), respectively. There were no statistically significant differences in serum IL-31 levels between CM and ISMskin+ patients ( $p=0.573$ ).

In 62 *KIT D816V*-positive adult patients median and mean  $\pm$  SD serum IL-31 levels were 4.98 pg/ml and  $5.25 \pm 1.51$  pg/ml (range 3.1–12.9 pg/ml), respectively. In 16 *KIT D816V*-negative adult patients the median and mean  $\pm$  SD serum IL-31 levels were 4.17 pg/ml and  $4.58 \pm 1.18$  pg/ml (range 3.4–7.5 pg/ml), respectively. Serum IL-31 levels were significantly higher in *KIT D816V*-positive patients than in *KIT D816V*-negative ones ( $p=0.024$ ). The comparison between *KIT D816V* positive and *KIT D816V* negative ones in respect of frequencies of IL-31 -1066G/A, -2057G/A,

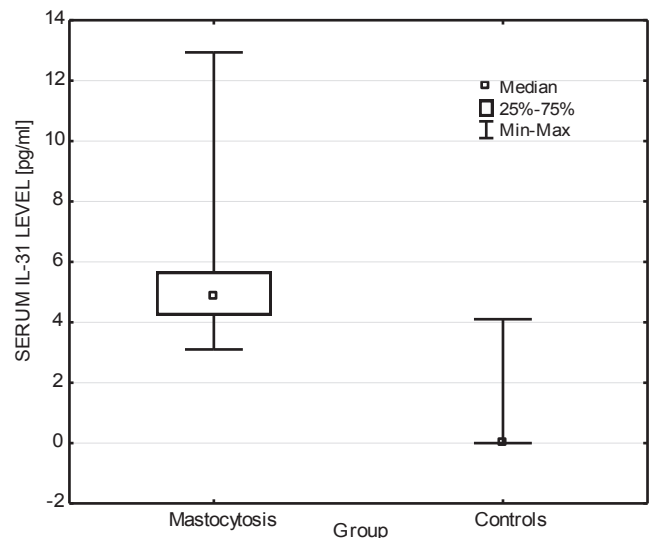


Fig. 1. Serum interleukin-31 (IL-31) level in adult patients with mastocytosis compared with healthy controls ( $p < 0.001$ ).

and IVS2+12A/G gene polymorphisms revealed no statistically significant differences in adult patients with mastocytosis ( $p=0.66$ ,  $p=0.77$ ,  $p=0.80$ , respectively).

Further statistical analysis revealed that there were no statistically significant differences in serum IL-31 levels in respect of IL-31 -1066G/A, -2057G/A, IVS2+12A/G gene polymorphisms in all adult patients with mastocytosis ( $p=0.75$ ,  $p=0.36$ ,  $p=0.31$ , respectively) and healthy control subjects ( $p=0.35$ ,  $p=0.21$ ,  $p=0.92$ , respectively). The analyses were also performed separately for CM and ISMskin+ groups of patients and revealed no statistically significant differences (data not shown).

#### Serum IL-31 levels in relation to pruritus

The prevalence of pruritus in adult patients with mastocytosis was 83.3% (it was found in 65 of 78 patients). In ISMskin+ patients, pruritus was found to be significantly more frequent (50/54,

92.6%) than in CM ones (15/24, 62.5%;  $p=0.001$ ). The median and mean  $\pm$  SD pruritus scores (VAS) for all adult patients with mastocytosis were 5.00 and  $4.22 \pm 2.69$ . In ISMskin+ patients (median and mean  $\pm$  SD VAS: 5.0 and  $5.18 \pm 2.44$ ), pruritus was significantly more severe than in the CM ones (median and mean  $\pm$  SD VAS: 2.0 and  $2.04 \pm 1.85$ ;  $p=0.000002$ ).

The median and mean  $\pm$  SD serum IL-31 levels in adult mastocytosis patients with pruritus were 5.05 and  $5.27 \pm 1.53$  pg/ml (range 3.10–12.94 pg/ml), whereas the corresponding values in patients

Table III. Genotype and allele frequencies of distinct IL-31 gene polymorphisms in all childhood-onset mastocytosis patients and controls

IL-31 gene polymorphism	Genotype and allele	Childhood-onset mastocytosis <i>n</i> = 57 <i>n</i> (%)	Controls <i>n</i> = 97 <i>n</i> (%)	<i>p</i> -value <sup>a</sup>	Odds ratio (OR)	<i>p</i> -value
-1066G/A	GG	25 (43.86)	37 (38.14)	0.49	1.27 (0.65–2.46)	0.49
	GA	25 (43.86)	48 (49.48)	0.50	0.80 (0.41–1.54)	0.50
	AA	7 (12.28)	12 (12.37)	0.99	0.99 (0.37–2.68)	0.99
	G	75 (65.79)	122 (62.89)	0.61	1.13 (0.70–1.84)	0.61
	A	39 (34.21)	72 (37.11)			
-2057G/A	GG	29 (50.88)	49 (50.52)	0.97	1.01 (0.53–1.95)	0.97
	GA	25 (43.86)	45 (46.39)	0.76	0.90 (0.47–1.74)	0.76
	AA	3 (5.26)	3 (3.09)	0.50	1.74 (0.34–8.93)	0.51
	G	83 (72.81)	143 (73.71)	0.86	0.95 (0.57–1.61)	0.86
	A	31 (27.19)	51 (26.29)			
IVS2+12A/G	AG	28 (49.12)	66 (68.04)	<b>0.02</b>	<b>0.45 (0.23–0.88)</b>	<b>0.021</b>
	AA	29 (50.88)	29 (29.90)	<b>0.009</b>	<b>2.43 (1.23–4.78)</b>	<b>0.01</b>
	GG	0 (0.00)	2 (2.06)	0.28	0.33 (0.02–7.04)	0.48
	G	28 (24.56)	70 (36.08)			
	A	86 (75.44)	124 (63.92)	<b>0.036</b>	<b>1.73 (1.03–2.91)</b>	<b>0.037</b>

<sup>a</sup>Pearson's  $\chi^2$  test. Significant values are shown in bold.

without pruritus was 4.38 pg/ml and  $4.34 \pm 0.70$  pg/ml (range 3.40–5.86 pg/ml). Serum IL-31 levels in pruritic cases of mastocytosis were significantly higher than in non-pruritic cases ( $p=0.009$ ). Positive correlation between serum IL-31 levels and pruritus scores (VAS) were found in 78 adult patients with mastocytosis (Spearman  $r=0.39$ ,  $p=0.0004$ ; Fig. 2).

## DISCUSSION

MCs that are abnormally accumulated in various tissues, particularly in the skin and bone marrow of patients with mastocytosis, are a significant source of various cytokines including IL-31 (2, 11, 24). This cytokine signals through a heterodimeric receptor complex on activated cells (15, 16). After binding of the receptor complex, IL-31 activates Janus kinase (JAK) and then activates pathways of signal transducers and activators of transcription (STATs), mitogen-activated protein-kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K). To our knowledge, genetic variations of IL-31 polymorphisms in patients with mastocytosis in relation to pathogenesis, serum IL-31 levels and clinical manifestation of the disease have never been investigated.

We found that the frequency of AA genotype for the IL-31 IVS2+12 as well as allele A for IVS2+12 were higher both in children and adults with mastocytosis than in control subjects and were linked with an increased risk of mastocytosis development. It is worth pointing out that our study group included children with CM as well as adults with CM limited to the skin and ISM<sub>skin+</sub>. These forms of mastocytosis are assessed as benign variants of the disease, characterized by a normal life expectancy (3).

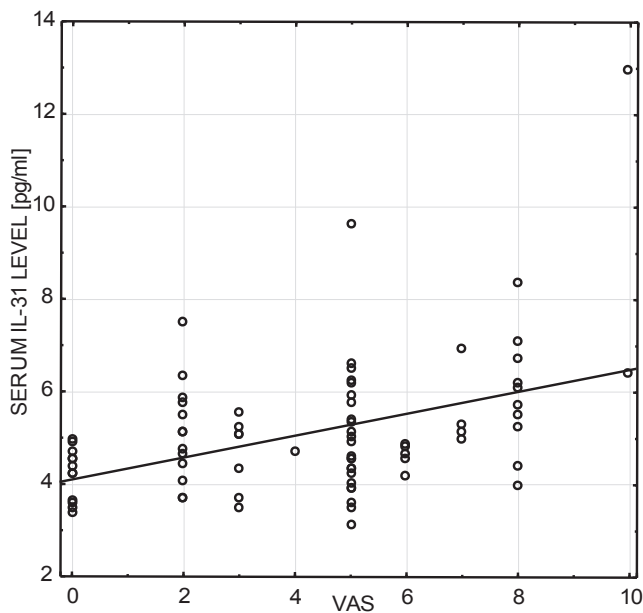


Fig. 2. Correlation between visual analogue score (VAS) and serum interleukin 31 (IL-31) level in adult patients with mastocytosis (Spearman's  $r=0.39$ ,  $p=0.0004$ ).

Therefore, AA genotype for the IL-31 IVS2+12 and allele A for IVS2+12 could be considered as genetic features associated with benign mastocytosis presenting with skin involvement. Nevertheless, further investigations based on a larger group of patients, including those with advanced forms of SM, such as smouldering SM (SSM), aggressive SM (ASM), mast cell leukaemia (MCL) and SM-AHNMD, would be required to prove this initial hypothesis. We also found that IVS2+12AG genotype was linked with a decreased risk of mastocytosis occurrence in children and adults. Based on the obtained data, it is difficult to assess the clinical relevance of this finding. It is not surprising that there were no significant differences between adult patients with CM and ISM<sub>skin+</sub> in respect of IL-31 genetic polymorphisms and IL-31 serum levels. It has been demonstrated that the majority of adult patients with mastocytosis have ISM and that the skin involvement can be the first clinical manifestation reflecting an early stage of systemic disease (26, 27). Our study revealed that in adult patients with mastocytosis IL-31 -2057AA genotype is associated with an increased risk of mastocytosis occurrence. There was no significant relationship between -2057AA genotype and risk of mastocytosis occurrence in children. This finding supports the concept that childhood-onset and adult-onset mastocytosis may differ with regard to some pathogenetic aspects. Although it has been proved that paediatric mastocytosis is also a clonal disease associated with activating *KIT* mutations, all factors influencing clinical presentation of childhood-onset mastocytosis remain currently unsettled (9, 28). Clinical observations show that paediatric mastocytosis is a skin-limited, regressive disease in the majority of cases (7, 29–31). Conversely, in adults, SM characterized by persistent multi-organ involvement is the prevalent form of the disease (3–5). In light of these clinical data, the results of our study suggest that the IL-31 -2057AA genotype could be considered as a genetic feature of adult-onset mastocytosis associated with skin involvement. Apart from mastocytosis, IL-31 polymorphisms have been studied in numerous skin diseases and asthma (18, 20, 21, 23, 32, 33). Lan et al. (32) reported a relationship between specific alleles of IL-31 and AD, but not with a non-atopic hand dermatitis. Interestingly, Sokołowska-Wojdyło et al. (18) showed that the GA genotype for the IL-31 -2057 and AA genotype and A allele for the -1066 are linked with an increased risk of AD development. In contrast to our findings in mastocytosis, no differences in allele and genotype frequencies of IL-31 have been reported between CTCL and controls (21) and between asthma and controls (33). Our results show that there were no statistically significant differences in serum IL-31 levels in respect of IL-31 -1066G/A, -2057G/A, IVS2+12A/G gene polymorphisms in adult patients with mastocytosis. Interestingly, serum IL-31 levels were significantly higher in *KIT D816V*-positive adult

patients than in *KIT D816V*-negative ones, suggesting that IL-31 may be pathophysiologically associated with *KIT* mutation-dependent manifestations of mastocytosis.

Hartmann et al. (24) showed, for the first time, that patients with mastocytosis express increased serum IL-31 levels and that serum IL-31 levels correlate with the disease severity, tryptase level, and percentage of bone marrow infiltration in adult patients with mastocytosis. Our study focused on the relationship between serum IL-31 and pruritus in adult-onset mastocytosis associated with skin involvement. Pruritus is one of the most common mast cell mediator-related symptoms in patients with mastocytosis, reported in 84.2% of adult patients with mastocytosis with various categories of the disease (24). In our study group, pruritus was present in 83.3% of all adults with mastocytosis and it was more frequent in SM than in CM cases. IL-31 is also considered one of the itch mediators in atopic dermatitis (19, 34, 35), and IL-31 mRNA is significantly upregulated in the skin of atopic patients with pruritic skin inflammation in contrast to non-pruritic ones (35). On the other hand, skin challenge with IL-31 does not induce immediate itch in patients with atopic dermatitis and healthy controls (36). Recently published studies indicate that IL-31 may be involved in the pathogenesis of chronic spontaneous urticaria (37, 38). Interestingly, in cutaneous T-cell lymphomas, which are also associated with chronic itch, IL-31 does not correlate with pruritus intensity (21). Regarding the pruritogenic role of IL-31 in myeloproliferative disorders (MPD), Ischi et al. (16) proved that MCs release significantly elevated levels of itch mediators, such as histamine and IL-31, compared with normal MCs (16). Moreover, statistically significant correlation between the presence of pruritus and increased numbers of MCs, decreased apoptosis and elevated plasma levels of IL-31 has been revealed in patients with MPD (16). In our study we found significant positive correlation between serum IL-31 levels and intensity of pruritus measured by VAS. In part, our findings are in line with the results of Ischi et al. (16) and support the hypothesis that the serum IL-31 level plays a role in pruritus induction in mastocytosis associated with a skin involvement. In contrast to our results, Hartmann et al. (24) found that MCs activation symptoms, including pruritus, failed to affect levels of IL-31. The most probable explanation for these discrepancies is the differences between the studied groups. We included adult patients with CM and ISM<sup>skin+</sup>, whereas the group analysed by Hartmann et al. was highly heterogeneous because it consisted of children and adults with CM and various forms of SM (CM/ISM, SSM, SM-AHNMD) (24). Similarly to the aforementioned study, we found a significantly higher serum IL-31 level in patients with mastocytosis in comparison with controls. Presented results implicate that MCs may constitute a major cellular source for IL-31 in mastocytosis. Previously published studies on

the pathogenesis of itching, analysed together with our observations, suggest that IL-31 may be involved in the induction of pruritus in mastocytosis (35, 37–42). However, the results should be interpreted with care, since the pathophysiology of pruritus has been shown to be complex, extending far beyond the influence of mast cell-related mediators (36–39).

In conclusion, distinct polymorphic variants of the IL-31 gene may be involved in the pathogenesis of mastocytosis, and IL-31 may be involved in the induction of pruritus in patients with mastocytosis associated with skin involvement. Further investigation into these associations is required.

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