bjh short report

Comparative analysis of IL6 and IL6 receptor gene polymorphisms in mastocytosis

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Summary

Mastocytosis is a rare disease with reported high interleukin-6 (IL6) levels influencing disease severity. The present study investigated polymorphisms within the genes that encode IL6 and its receptor (IL6R) in relation to mastocytosis development in a case-control design. Analysis of the *IL6R* Asp358Ala polymorphism showed that carriers of the AA genotype had a 2·5-fold lower risk for mastocytosis than those with the AC or CC genotypes. No association with mastocytosis was found for the *IL6*−174G/C polymorphism, however, it may influence the effect of *IL6R* polymorphism. To the best of our knowledge this is the first study analysing *IL6/IL6R* polymorphisms in mastocytosis.

Keywords: mastocytosis, IL6, IL6R, genetic polymorphism.

Mastocytosis is a rare disease defined by autonomous proliferation and activation of mast cells resulting from activating mutations in *KIT*, which encodes the receptor for stem cell factor (Orfao *et al*, 2007). Mastocytosis diagnosis and classification has been well defined and the disease has been recently included in the World Health Organization (WHO) group of myeloproliferative neoplasms (MPNs) (Horny *et al*, 2008; Valent *et al*, 2007). Clinical manifestations of the systemic form (SM) are caused by tissue infiltration of proliferating mastocytes into different organs and the release of their mediators while the cutaneous form (CM) is a localized type of the disease. The presence of activating *KIT* mutations alone is not sufficient to account for the different clinical forms of mastocytosis, suggesting that other mutations or polymorphisms in genes important in regulation of mast cell turnover are likely to influence the clinical outcome of the disease. One such candidate gene might be *IL6*, which encodes the multifunctional cytokine, interleukin-6 (IL6). Recent data indicate that mastocytosis patients may

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exhibit elevated plasma IL6 levels, which are in close correlation to disease severity (Brockow *et al*, 2005).

Beside various clinical factors (age, gender, inflammation, etc.), IL6 level may be influenced by different genetic variations. A G to C substitution at position −174 of the *IL6* gene promoter was shown to decrease *IL6* transcription *in vitro* and, in accordance, differences in IL6 serum levels between carriers of the wildtype and variant alleles of the polymorphism were reported (Fishman *et al*, 1998; Rivera-Chavez *et al*, 2003), however others failed to reproduce these findings (Qi *et al*, 2006). A functional polymorphism (Asp358Ala) in exon 9 of the *IL6R* gene, resulting in the substitution of one of the two amino acids at the site of proteolytic cleavage, leads to altered shedding of membrane-bound IL6 receptor (IL6R) (Galicia *et al*, 2004). Given that soluble IL6R (sIL6R) binds to IL6, circulating IL6 levels may be influenced by circulating receptor levels via sequestration (Rose-John *et al*, 2006). Accordingly, many studies also found a minor allele (C) of this polymorphism was associated with higher levels of sIL6R and IL6 (Galicia *et al*, 2004; Reich *et al*, 2007).

The aim of the present case-control study was to compare the frequency of *IL6* −174 G/C and *IL6R* Asp358Ala polymorphisms between mastocytosis patients and healthy controls to evaluate their role in heritable mastocytosis risk.

Materials and methods

DNA samples and data were collected from 66 patients; 19 from Vienna, 24 from Gdansk and 23 from Budapest [male/female ratio: 26/40; mean age: 42·9 years (range 18–80)]. Patients were diagnosed according to the WHO criteria: 27 had CM, 27 had indolent systemic mastocytosis (ISM), three had smoldering systemic mastocytosis (SSM), six had SM in association with a haematological disease other than mastocytosis (SM-AHNMD), two had the aggressive systemic (ASM) form of the disease and one presented with mast cell leukaemia (MCL).

The control group consisted of 99 subjects. Exclusion criteria for controls were malignancies and immunopathological disorders. Mean age was 68 years (range 37–91) with a 45/54 male/female sex ratio. All patients and controls gave informed consent and the study was approved by the national scientific ethical committee (ETT TUKEB 12236-45/2004-1018EKU).

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples were collected from each patient and control, and genomic DNA was extracted from white blood cells by the salting-out procedure. The −174G/C polymorphism of the *IL6* gene (rs1800795) was analysed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the Asp358Ala single nucleotide polymorphism (SNP) of the *IL6R* gene *(A>C,* rs2228145, previously: rs8192284*)* was genotyped using a TaqMan SNP Genotyping Assay (Life Technologies, Carlsbad, CA, USA) as described previously (Aladzsity *et al*, 2009).

Deviation from Hardy–Weinberg equilibrium was evaluated and association analysis was performed by chi-square test using the GRAPHPAD PRISM v.4.0 and Statistical Package for the Social Sciences (SPSS) v.13.0 software. All tests were two-tailed and $P < 0{\cdot}05$ was considered to be statistically significant.

Results

There was no significant difference between mastocytosis patients and controls in the frequency of −174G/C polymorphism of the *IL6* gene (Table I).

For the Asp358Ala SNP of the *IL6R* gene, the AA genotype and A allele was found to be less frequent in mastocytosis patients when compared to controls ($P = 0{\cdot}0317$ and $P = 0{\cdot}0229$, respectively) (Table I). The odds ratio (OR) for developing mastocytosis of AA genotype carriers was 0·4019 [95% confidence interval (CI) = 0·2013–0·8021; $P = 0{\cdot}0088$], whereas C allele carriers (AC + CC) had a higher risk (OR = 2·488; 95%CI = 1·247–4·967; $P = 0{\cdot}0088$) for the disease.

Analysis of the combined effect of the two studied polymorphisms showed that the AA genotype of *IL6R* polymorphism, together with the G allele of *IL6* polymorphism, were less frequent in mastocytosis patients than in controls ($P = 0{\cdot}00026$) with an OR of 0·2348 (95%CI = 0·1043–0·5289; $P = 0{\cdot}0003$) (Fig 1).

Discussion

The aim of the present study was to evaluate the role of two polymorphisms influencing circulating IL6 level in heritable mastocytosis risk by comparing mastocytosis patients and controls. Analysis of the *IL6R* Asp358Ala polymorphism showed that frequencies of the A allele and the AA genotype were significantly lower in mastocytosis patients in comparison to healthy controls. Carriers of the AA genotype had an approximately 2·5-fold lower risk for mastocytosis than those

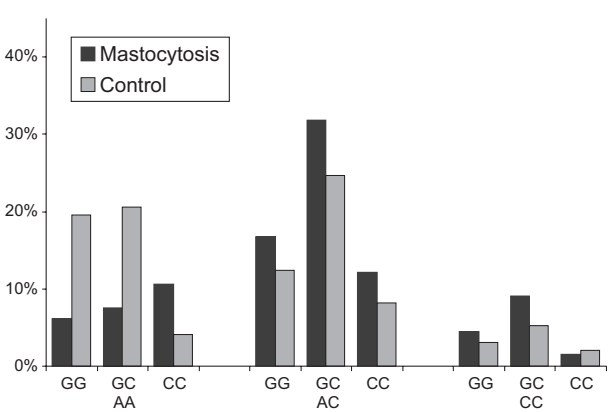

Fig 1. Combined distribution of *IL6* and *IL6R* polymorphisms in mastocytosis patients and controls. *Upper line* on the *x*-axis shows genotypes of *IL6* −174G /C polymorphism. The *lower line* shows genotypes of *IL6R* Asp358Ala (A/C) single nucleotide polymorphism. Differences between carriers of both *IL6R* AA and *IL6* GG/GC versus all other genotypes were evaluated by chi-square test.

Table I. Allele and genotype frequencies of *IL6* and *IL6R* polymorphisms in mastocytosis patients and controls.

	Mastocytosis (n = 66)	Controls (n = 99)	P value*
IL6 −174 G/C			
Genotype			
GG	18 (27·3%)	36 (36·4%)	0·1988
GC	32 (48·5%)	49 (49·5%)	
CC	16 (24·2%)	14 (14·1%)	
Allele			
G	68 (51·5%)	121 (61·1%)	0·0843
C	64 (48·5%)	77 (38·9%)	
IL6R Asp358Ala			
Genotype			
AA	16 (24·2%)	43 (44·3%)	0·0317
AC	40 (60·6%)	44 (45·4%)	
CC	10 (15·2%)	10 (10·3%)	
Allele			
A	72 (54·5%)	130 (67%)	0·0229
C	60 (45·5%)	64 (33%)	

The genotype frequencies are in Hardy–Weinberg equilibrium.
*chi-square test.

with the AC or CC genotypes (OR = 0·4019). The *IL6*−174G/C polymorphism showed no association with mastocytosis but a protective effect of *IL6R* AA genotype was found to be elevated in the presence of the G allele of the *IL6*−174G/C polymorphism. The risk for developing mastocytosis was more than 4-fold lower (OR = 0·2348) in carriers of both *IL6R* AA and *IL6* GG/GC genotypes than the rest of the patients.

Interleukin-6 is a proinflammatory cytokine involved in host defence mechanisms. Binding of IL6 to IL6 receptor activates mast cell signalling through the Janus kinase (JAK) and Btk pathways, which play an important role in the pathogenesis of mastocytosis. It has been reported that patients with myeloproliferative neoplasms have elevated levels of IL6, regarding JAK1 hyperactivation, therefore might benefit from JAK1/2 inhibitor therapy (Panteli *et al*, 2005; Tefferi, 2000; Cardama *et al*, 2010;.). Wickenhauser *et al*, (1999) noted aberrant IL6/IL6R serum levels in polycythaemia vera – a representative MPN – of yet unknown significance while Brockow *et al*, (2005) have reported elevated levels of IL6 in mastocytosis. Studies on *IL6R* and *IL6* polymorphisms in MPNs – to our present knowledge – have not yet been reported.

Interleukin-6 level may be influenced by different genetic variations, two of which were selected for analysis in this study. The AA genotype of the *IL6R* Asp358Ala polymorphism, found to be a protective factor against mastocytosis development in this study, was previously reported to be associated with lower circulating sIL6R and IL6 levels. Hence, it is rational to hypothesize, that genotypes of this polymorphism influence mastocytosis development through decreasing IL6 levels, resulting in lower activation of mast cells. In addition, changes in sIL6R level may also have an important role as sIL6R is capable of sensitizing target cells toward IL6 and eliciting a biological response in cells that do not express membrane-bound IL6R but only the signal transducing gp130 subunit (Brockow *et al*, 2005; Rose-John *et al*, 2006). A direct role of the *IL6* −174G/C SNP in mastocytosis development was not found; however, it may have an influence on the effect of the *IL6R* Asp358Ala polymorphism.

This preliminary study has shown that the AA genotype of the *IL6R* Asp358Ala polymorphism may confer protection against mastocytosis disease development; however, large cohort studies are needed to confirm this finding. Therefore we are planning to analyse IL6 serum levels in parallel with *IL6* and *IL6R* gene polymorphisms in different mastocytosis subtypes in a larger patient cohort in a more extended international collaboration. Given that there are several *KIT* mutations in mastocytosis (Orfao *et al*, 2007), most of which have unknown consequences, future investigations should study their possible relationship with *IL6* and *IL6R* genetic polymorphisms as well.

Author contributions

Eszter Rausz, Szilágyi A and Judit Varkonyi designed the study and wrote the paper; Marschalko M, Hidvégi B, Szakonyi J are dermatologists in the network; Csomor J is the pathologist in the network; Lautner-Csorba Orsolya performed genotyping of *IL6R* polymorphism; Aladzsity I, Kokai M, Szilágyi A genotyped the *IL6* polymorphism and carried out statistical evaluation; Nedoszytko B, Lange M, Niedoszytko M, Valent P contributed patient samples and data; Falus A provided technical support to the study.

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