Cytokine gene polymorphisms in systemic mastocytosis

Thesis

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Budapest

2014

Introduction

The genome of any member of the human species differ from another. Genes are not genetically identical among human populations. If a single nucleotid difference occurs in more than 1% of the population, we refer to it as a polymorphism. These geentic variations can lead to the wide variety of human feaures.

The investigation of the geentic background of rare human diseases is an excellent way to improve our knowledge about the pathophysiology , susceptibility, and to find th ebest therapeutic approach, once the patient already suffers from the illnes.

Mastocytosis is characterized by the inappropriate growth or differentiation of mast cells. It is a good example for a rare disease, where genetics can help our understanding.

Lots of factors can contribute to the disorder of mast cells, including the interleukin 6 cyokin, and its receptor, so we invetigated SNP in the coding genes of these two proteins. In addition to that, I also measured the blood level of other humoral factors at the first visit of the patient, and sequentially during the treatment.

Objectives

The aim of this study was to investigate the actors that can contribute to the development of a rare disease, called mastocytos. In order to gain an insight into the genetic background of this illness we investigated genetic polymorphisms in genes encoding interleukins, and different members of the complement system. These are important components of the humoral immunity, which are known to effect the development and differentiation of mast cells.

Previous studies have already implicated, that SNP int he gene of IL6 and Il6 receptor can have an effect ont he serum concentration of this cytokin. In this study, I focused on the allele distribution of such polymorphisms.

If we consider the differential diagnostic of mastocytosis, it is crucial to exclude the possibility of the lack of the C1 inhibitor protein (hereditary or acquired).

There is a lack of systematic measurment of the different parameteres of the complent system, however a growing number of international publications focuse on this illness. That is

the reason why we found it important to measure the following parameters: CH50, C3, C4 (as the most commonly investigated complement members in association with diseases), and the C1-INH level and activity, C1q, and the level of the antibodies against these factors.

The results of the presents study shows report of an international patients group. We were able to collect 66 samples of patient service, examine and analyze in a collaboration of hematologist colleagues from Austrian, Poland and Hungarian. The increasing number of cases in the future we would like to reiterate our investigations. An increase in the number of cases could only be realized by cooperation of national and international centers.

The disease is rare. Therefore, it was important in two cases explained in greater detail. Presentation of these medical records the following happens. I am going to show the change of the level of these parameters, in two medical cases.

During my doctoral work at the Semmelweis University Mastocytosis Network, I also payed attention on the age, sex and serum tryptase level of the Hunagrian patients, registrated before the beginning of study.

I wanted to answer the following questions:

- is there any difference in the allele frequency of -174 G/C polymorphism in the IL6 gene between healthy and ill people?

- is there any difference in the allele frequency of the ASP358Ala (A/C) polymorphism in the IL6 receptor gene between healthy and ill people?

- Can we show the difference of CH50, C3, C4 , C1-INH, C1q level among controll and ill population?

- How is the CH50, C3, C4 , C1-INH level and activity C1q level, and anti C1q antibody level changing during the development of the disease?

- How does the age, sex and Se tryptase distribution look like in people with mastocytosis (or in different subgroups of them)

Methods

For the investigation of the genetic variations, all in all 66 patients were included, from three different countries: Hungary (Budapest, Mastocytosis Network), Austria (Wien) and Poland(Gdansk). All of them meet the requirements of the WHO criteria for the different subgroups of mastocytosis. (26 men, 40 women, average age: 42.9, subgroups: 27 cutaneous mastocytosis, 27 indolent systemis mastoctosis, 3 smoldering , 6 systemic mastocytosis combined with hematologic disease, 2 aggressive systemic mastocytosis, and 1 mast cell leukemia.)

The investigation of the complement factors, was only carried out ont he Hungarian samples. This meant 46 patients (19 men, 27 women, average age : 49.18 years, 25 cutaneous form, 21 systemic form). Bceause of the small number of samples, we did not divide them int o further subgroups.

The controll group contained 99, non-related people from the Caucasian population, without any malignant or immunological disorders. The controll population was in Hardy Weinberg equlibrium. During the case-sontroll study, we always used to the whole controll population's age, and sex fitted subgroups.

The handling of blood, DNA, cytological, hystological samples always met the requirements of the relevant technical proposals. The sampling was conducted at the SE 3rd Department of Internal Medicine. The blood samples were evaluated in the general laboratory of SE 3rd Department of Internal Medicine, DNA genotyping and PCR was done inthe research laboratory of the same institue, while the histological investigation took place in the SE1st Pathology Department.

The Asp358Ala SNP was investigated by fluorogenic TaqMan assay, with 5nuclease activity. The -174 polymorphism (G/C, rs 1800795) was genotyped by PCR-RFLP, where the following primer pair was used for the amplification of a 299 basepair long segment: 5'-TTGTCAAGACATGCCAAAGTG-3' and 5'-TCAGACATCTCCAGTCCTATA-3'.

The parameters, that are most often under observation in patients with complement disorders, are CH50, C3, and C4, and in patients with heredotary or acquired C1 inhibitor deficiency are C1-INH level and activity, antibodies against C1-INH, C1q, and C1q antibodies. We decided to examine these factors in our blood samples by ELISA method.

Our data were analyzed by MedCalc 9.3.3.0. computer program. To test the existence of the Hardy-Weinberg equilibrium, and the association of the SNPs, chi square probe was conducted. „Graphard Prims v. 4.0" és „Statistical Package for the Social Sciences (SPSS) v. 13.0" softwers were used for this purpose.

Results

IL-6 gene -174 G/C SNP allele frequencies analys

After measuring the allele frequencies of the IL-6 gene -174 G/C SNP, we could not detect significant association with mastocytosis. Among the patients, GG" genotype was observed in 18 people (27,3%), „GC" in 32 (48,5%),and „CC" in 16 people (24,2%). The „G" allele's frequency in healthy people was 51,5% (68), and the „C" allele: 48,5% (64). Among the ill people, 36 samples had „GG" genotype (36.4%), 49 had „GC" (49.5%), 14 had „CC" (14.1%), with the following allele distribution: „G" 121 (61,1%), „C" 77 (38,9%). From these results, the calculated p value is 0-1988 and 0.0843.

IL-6R gene Asp358Ala (A/C) SNP allele frequencies analys

In case of the IL-6R gene Asp358Ala (A/C) SNP , the „AA" genotype and the „A" allele had significantly lower frequency in patients. (P=0,0317 and P=0,0229 respectively.) The ODDS ration for the development of mastocytosis in people with the „AA" genotype was 0.4019 (95% confidence inerval (CI)= 0,2013-0,8021; P=0,0088.) People with „C" allele („AC"and „CC" genotypes) suffered more often from mastocytosis, (OR=2,488; 95% CI=1,247-4,967; P= 0,00888).

We took a step further, and analysed the two above mentioned SNPs haplotaypes. The highest freqencyu of mastocytosis could be observed, when the „GC" allele in the IL-6 gene (-174) and the IL-6R gene „AC" alleles occured together.

During the investigation of the factors of the complement system, we could not detect any significant differences between the median and average values of the patients, and the normal interval.

After the investigation of the putative effect of IL 6 polymorphisms, we wanted to measure the level of different components of the complement system. In addition to the

investigation of CH50-, C3-, C4 level, we measured the level and activity of C1-INH, as well as C1-INH antibodies, and the C1q, C1q antibody level. We could not detect any significant alterations among the people affected by the disease. We showed the result of the monitoring of these parameters through two cases. I would like to present two medical case reports:

1st patient was a. male patient. In the patients history was renal carcinoma. The patient came for the 40 years of clinic symptoms, maculopapulary lesions, pruritus, diarrhea and recurring, pleural fluid. The patients bone marrow aspirate smear, infiltration with mastocytes, and theis clusters could be verified. A quarter of these cells had altered morphology. The CD 117, CD 2, CD 25 triple co-expression was determined by flow cytometry. The trypase level of the patient was 661 ng/ml , which is a fold higher than normal (20 ng/ml). The DNA test showed a silent mutation int he 17th exon of the c-KIT gene, (798 codon 81350 locus). Taking these into consideration, one major criterium, and three of the four minor criteria was true for the patient. During the follow-up of the man, the complement parameters did not change with the acute states of the patient.

2nd patient was a female patient, with type 2 diabetes mellitus, hypothyroidism, IgG kappa monoclonal gammopathy and essential thrombocytemy whos bone biopsy was clearly infiltrated with mastocytes. The trypase level of the patient was 200 ng/ml, which is highly over the normal value (less than 20ng/ml.)Taking these values into consideration, the patient met the requirements of a major and a minor criterium. In case of O.B., thrombocythemia joined to mastocytosis. Molecular genetic investigations found a c-KIT mutation, at the 17th locus: D816V. During the complement analysis, C1-inhibitor (INH)-, and C1q antibodies were not detected, the C1q serum level was a bit higher than the normal range. The low amount of CH50, C3, C4 levels bought us to the idea, to test, wether complemetn consumption could play a role in this patient. We conducted the complement consumption test, but consumption could not be detected.

I also summarized the the distribution of the age, sex and serum tryptase level, detected in patients of the Semmelweis University Mastocytosis Network, before the beginning of the study.

If we considered the WHO classification, among the Hungarian patients (66 people) **,** 27 were sufefring from cutaneous mastocytosis (40,91%), while systemic mastocytosis occured in 39 cases (59,09%) .

The sex distribution was the following: 26 men (39.39%), and 40 women (60.61%).

As we were taking a look at the age of the patients, we could see, that the xoungest patients usually suffered from cutaneous form the disease and the eldest patients had systemic mastocytosis combined with hematologic disease (65.7 years was the average age of this subgroup), or aggressive systemic mastocytosis (77.5 years average age). The serum trypatse level could be observed in 46 samples (27 women and 19 men) . All in all, the average level was 210,48 ng/ml, while the minimum and maximum measured values were 2,14 and 2357 ng/ml respectively.

The highest tryptase level (2357 ng/ml) was measured in a patient with aggressive systemic mastocytosis, the lowest (2,41 ng/ml) in case of cutaneous mastocytosis.

Mean values could only be determined in three subgroups (only these contained more than one case): cutaneous, indolent, and smoldering mastocytosis. Among these subgroups, an order of the tryptase level could be detected this way: the cutaneous form had the lowest value: 21,5 ng/ml, the indolent form the medium (112,0 ng/ml), and the smoldering the highest 937 ng/ml. These results are in great agremment with pervious studeis in literature, which show connection between the tryptase blood level, and the severity of the disease.

Conclusions

In case of the G/C SNP, located -174 basepairs of the IL6 gene, we could not detect significant difference between the allele frequencies in the controll group, or the people suffering from mastocytosis.

Contrarily, we showe significant difference in case of the missense Asp358Ala (A/C) variation in the IL6R gene .

Evalueating the results about the two SNPs together showed, that . the most abundant haplotype among the people with mastocytosis was the „GC” (IL6 gene), and „AC” (IL6R gene).

Significant alterations could be detected neither of the CH50-, C3-, C4 level, nor the activity of C1-INH, C1-INH antibodies , and the C1q, C1q antibody levels.

During the study of the disease course, we could not detect the change of the above mentioned lood parameters.

We also verified in one clinical case, that the low CH50-, C3-, C4 concenrations were not a result of complement consumption.

The analysis of the Hungarian population with mastocytosis, we found that the aggressive form of the disease usually occur above 70. We found that vast majority of the patients are female, which is characteristic for immunological disorders.

Publications

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