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Common chitotriosidase duplication gene polymorphism and clinical outcome status in sarcoidosis

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Abstract. *Background:* Chitotriosidase has been found to be useful as a sarcoidosis biomarker. In patients with better outcome lower values were observed. Some subjects have 24-base pair duplication in the chitotriosidase gene (CHIT1) that results in the production of inactive enzyme. This might influence the outcome of sarcoidosis and account for described observations. *Objectives:* The aim of this study was to correlate common CHIT1 duplication polymorphism and clinical outcome status in sarcoidosis (COS). *Methods:* This retrospective study comprised 180 patients with sarcoidosis. COS at 3, 5 and 10 years was determined and correlated with CHIT1 24-base pair duplication polymorphism. CHIT1 genotyping was done by the PCR method. *Results:* There was no significant correlation between CHIT1 24-base pair duplication polymorphism and COS at 3, 5 or 10 years but a subgroup analysis showed higher frequency of patients with Loefgren's syndrome (50% vs. 17.1%) and better COS in CHIT1 24-base pair duplication homozygotes vs. all other subjects in major COS groups (no, minimal and persistent disease) at 3 years (p=0.025) and borderline significant at 5 years (p = 0.090). *Conclusions:* In this study no correlation between CHIT1 24-base pair duplication polymorphism and COS was shown, but possible protective role of homozygous condition for CHIT1 24-base pair duplication polymorphism is suggested. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 194-199)*

Key words: sarcoidosis, polymorphism, chitotriosidase, clinical outcome status

Introduction

Sarcoidosis is a granulomatous inflammatory disease that typically affects mediastinal lymph nodes and lung parenchyma but almost any organ can be involved. It is presumed that environmental exposure triggers disease in genetically susceptible individuals. Wide range of genes has been associated with susceptibility to sarcoidosis and with clinical phenotype of sarcoidosis, but the strongest association is with human leukocyte antigen (HLA) genes, especially with HLA-DRB1 and DQB1 alleles (1-4). Haplotype HLA-DRB1*03/DQB1*02 has been strongly associated with Loefgren's syndrome (5, 6). HLA also has prognostic role in sarcoidosis, HLA DRB1*03 being associated with favorable course of sarcoidosis both in Loefgren's syndrome and non-Loefgren's patients (7). Some of genes associated with susceptibility and/or prognosis of sarcoidosis are tumor necrosis factor alpha (TNF-α) (8, 9), interleukin-10 (IL-10) (10, 11), transforming growth

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factor-beta1 (TGF-β1) (10, 12), angiotensin-converting enzyme (13, 14) and more but their role appears to be less strong or has not been consistently proven (15).

Chitotriosidase (CTO) is a human chitinolytic enzyme (chitinase) excreted by activated macrophages and polymorphonuclear neutrophils. CTO appears to be a part of the innate immune system against chitin containing pathogens, such as fungi and some parasites (16-18). The CTO expression in human macrophages is upregulated by interferon-γ, TNF-α, lipopolysaccharide and granulocyte-macrophage colony-stimulating factor (GM-CSF) and downregulated by IL-10 (19, 20). CTO is increased in several diseases (21, 22), including sarcoidosis. High CTO activity was observed in approximately 90% of patients with active sarcoidosis (23) and correlates with clinical, radiological or functional deterioration of the disease (24). This could point at a possibility that it is involved in the etiopathogenesis of sarcoidosis. Recently, positive correlation between CTO values and clinical outcome status (COS) was shown, thus giving CTO a potential prognostic value (24, 25). The level of CTO is influenced by environmental and genetic factors. There is a common 24-base pair duplication polymorphism in the CTO gene (CHIT1) which results in the production of an inactive enzyme that is mostly degraded intracellularly (26). The expected frequency of normal subjects (wt/wt) is 60%, heterozygote (wt/H) 34% and homozygotes (H/H) 6% (26-28). Compared to normal subjects, heterozygotes are expected to have 50% of CTO activity, while in homozygotes there is no CTO activity. It was argued that the observed lower CTO values in patients with favorable COS might be due to clustering of heterozygotes in groups with better outcome. This would support the hypothesis of protective role of CHIT1 polymorphism in sarcoidosis. A previous study on Slovenian sarcoidosis patients did not show different frequency of this CHIT1 polymorphism in patients as compared to general population (29) but in this study CHIT1 polymorphism was not correlated to the course (outcome) of the disease. The aim of this study was to test the correlation between common CHIT1 24-base pair duplication polymorphism, clinical picture and course of the disease as defined by COS.

Methods

Subjects

For this restrospective study we screened all patients (in total 200) included in our sarcoidosis registry with newly diagnosed sarcoidosis between years 1995 and 2010. The diagnosis in all patients was in accordance with ATS/ERS/WASOG criteria (30). 180 patients completed minimal follow-up of 3 years and had sufficient data for COS determination at 3 years. The patients presenting with acute clinical picture consisting of bihilar lymphadenopathy, erythema nodosum and arthalgia were considered to have Loefgren's syndrome. All subjects gave a written consent to participate in the study which was approved by the National Ethics Committee of the Republic of Slovenia.

CHIT1 genotypization

DNA was isolated from blood leucocytes using standard protocols. Genotypization of inactivating mutation (24-base pair long duplication) in CHIT1 gene was done using a polymerase chain reaction (PCR) and two primers (sequences of the primers CHIT1-F: 5'-AGCTATCTGAAGCAGAAG-3' in CHIT1-R: 5'-GGAGAAGCCGGCAAAGTC-3'). In the mutated gene the resulting amplicon was 24 base pair longer (75 bp in normal and 99 in mutated genes). They could be separated by electrophoresis which was done on 3% agar gel colored by SYBR® Safe DNA gel stain (Invitrogen, Carlsbad, USA).

Clinical outcome status

The patients were classified into subgroups according to classification proposed by WASOG task force (31). The recommended period of observation for determining the COS is 5 years. In this study we also determined COS at 3 and 10 years of follow-up. The major groups were no disease, minimal disease (less than 25% of maximal disease) and persistent disease. The patients were further divided according to the need for systemic therapy. The patients currently on therapy (or that received therapy in the last year) were additionally divided into asymptomatic, symptomatic and with functional deterioration in the last year. The groups were: COS 1 – no disease,

never treated; COS 2 – no disease, not treated in last year; COS 3 - minimal disease, never treated; COS 4 – minimal disease, not treated in last year; COS 5 – persistent disease, never treated; COS 6 – persistent disease, not treated in last year; COS 7 – persistent disease, treated in the last year, asymptomatic; COS 8 persistent disease, treated in the last year, symptomatic, COS 9 persistent disease, treated in last year, with functional deterioration.

Statistics

For calculations the statistical program SPSS 15.0 was used. Non-parametric tests were used and the data was expressed as median and interquartile range (IQR). A p value <0.05 was considered as significant.

Results

Patients' clinical features and CHIT1 polymorphism

Table 1 summarizes the studied population. CHIT1 polymorphism distribution was not significantly different from general Slovenian population, as it was previously already reported (29). There was no association between CHIT1 polymorphism and age, gender, initial chest X-ray stage, lung function or BAL CD4/CD8 index. There was significantly higher frequency of patients with Loefgren's syndrome in H/H subject (5/10) compared to remaining patients (29/170) (Fisher's exact test, p=0.022).

Table 1. The study patients' characteristics

Number of patients	180
Age in years	
median (range)	43 (21 - 81)
Gender	
female	99 (55%)
male	81 (45%)
Chest X-ray stage	
0	8 (4.4%)
1	84 (46.7%)
2	70 (36.9%)
3	16 (8.9%)
4	2 (1.1%)
Loefgren's syndrome	
yes	34 (18.9%)
no	146 (81.1%)
Lung function	
FVC median % (IQR)	94 (20)
FEV1 median % (IQR)	95 (20)
DLco median % (IQR)	86 (19)
BAL	
CD4/CD8 index (IQR)	4.91 (5.41)
CHIT1	
wt/wt	110 (61.1%)
wt/H	60 (33.3%)
H/H	10 (5.6%)

CHIT1 polymorphisms and clinical outcome status

In the studied group of patients there was no significant correlation between CHIT1 polymorphism and COS at 3, 5 or 10 years (Table 2, data for COS at 3 and 10 years is not shown). However, there was significantly better outcome in H/H sub-

Table 2. Distribution of COS at 5 years and CHIT1 polymorphism

		wt/wt		wt/H		H/H	
		N	%	N	%	N	%
No disease	COS 1	10	11.5	8	16.0	1	12.5
	COS 2	27	31.0	17	34.0	5	62.5
Minimal disease	COS 3	5	5.7	1	2.0	0	0.0
	COS 4	10	11.5	5	10.0	1	12.5
Persistent disease	COS 5	3	3.4	1	2.0	0	0.0
	COS 6	7	8.0	5	10.0	0	0.0
	COS 7	12	13.8	10	20.0	0	0.0
	COS 8	4	4.6	1	2.0	0	0.0
	COS 9	9	10.3	2	4.0	1	12.5

In total COS at 5 years could be determined in 145 patients. There was no significant correlation between CHIT1 and COS at 5 years (Spearman's rho = 0.111, p = 0.184). There was a trend towards better COS for H/H subjects if major COS groups were compared (Spearman's rho = 0.141, p =0.090)

Table 3. COS evaluated at 3, 5 and 10 years of follow-up

		3 years		5 years		10 years	
		N	%	N	%	N	%
No disease	COS 1	24	13.3	19	13.1	4	8.0
	COS 2	48	26.7	49	33.8	19	38.0
Minimal disease	COS 3	8	4.4	6	4.1	0	0.0
	COS 4	23	12.8	16	11.0	8	16.0
Persistent disease	COS 5	5	2.8	4	2.8	1	2.0
	COS 6	17	9.4	12	8.3	4	8.0
	COS 7	26	14.4	22	15.2	14	28.0
	COS 8	13	7.2	5	3.4	0	0.0
	COS 9	16	8.9	12	8.3	0	0.0
Total		180		145		50	

jects compared to all other subjects in major COS groups (no, minimal and persistent disease) at 3 years (Spearman's rho = 0.168, p = 0.025) and almost significant at 5 years (Spearman's rho = 0.141, p = 0.090). This could be accounted for by a higher number of patients with Loefgren's syndrome in H/H genotype group. The patients with Loefgren's syndrome expectedly had better COS at 3 (Spearman's rho 0.371, p < 0.001), 5 (Spearman's rho 0.358, p < 0.001) and 10 years (Spearman's rho 0.329, p = 0.020).

Clinical outcome status (COS) development during observational period

COS at different observational periods were compared (Table 3). Excluding patients where 5 or 10 years of follow-up was not yet possible, 88.4% (145 patients) of initially included patients completed 5 years and 75.6% (50 patients) 10 years of follow-up. 61.4% of patients had the same COS at 3 and 5 years, 28.9% had lower COS at 5 years and 9.7% had higher COS at 5 years. This was statistically significant (Wilcoxon signed rank test, p = 0.009). Similarly, 58.4% patients had the same COS at 5 and 10 years, 35.4% had lower COS at 10 years and 6.3% had higher COS at 10 years (Wilcoxon signed rank test, p = 0.005).

Discussion

The primary finding of this study was no correlation between CHIT1 polymorphism and COS evaluated at 3, 5 or 10 years. Previously it was shown that CTO values correlate with COS at 5 years (24, 25). In particular, patients in groups COS 1 and 2 (no disease) had lower values compared to others. The extrapolation of the data from this study means that there was no clustering of specific genotypes in specific COS groups. CHIT1 polymorphism is one of the reasons for variability of CTO values in these studies but since the distribution is even in different COS groups it should not affect the final correlation. Reduced CTO activity as seen in wt/H subjects thus does not appear to have effect on sarcoidosis course. The hypothesis that CTO could have a role in process of fibrogenesis in sarcoidosis (32) was derived from observation of high CTO activity in patients with nonalcoholic fatty liver disease who developed hepatic fibrosis in contrast to those who did not (33). Moreover, increased levels of CTO were also seen in fibrotic lung diseases (34) and in BAL of patients with idiopathic pulmonary fibrosis (35). This is further supported by a study performed by Lee at al (36) who observed higher levels of CTO in patients with severe systemic sclerosis (SSc) associated interstitial lung disease. Additionally, it was demonstrated in a murine model that CTO has a role in bleomycin and IL-13 induced pulmonary fibrosis (36). Specifically, CHIT1 knockout mice had attenuated fibrotic response as compared to wild type mice and CHIT1 hyper-producing mice had exaggerated response. In this model CTO functioned as a co-factor for TGF-β1 which is thought to be central in fibrogenesis in SSc (37). A potential explanation why protective role of CTO was not observed in this study is that CTO levels in wt/H sub-

jects are still high enough to take full effect. Moreover, this study was not designed to quantify the extent of fibrosis.

A possible exception to this is a group of H/H subjects who lack CTO in blood or other tissues. In this group increased frequency of patients with Loefgren's syndrome and better outcome was observed. Loefgren's syndrome is known to have different genetic background, being strongly linked with certain HLA alleles (5, 6). Additionally, there is data that CTO and ACE levels are lower in this group of patients as compared to other phenotypes (24, 38), which suggests lesser macrophage activation. A total absence of CTO could attenuate the exaggerated granulomatous response to yet undefined antigens in sarcoidosis. One of candidate antigens are fungal particles (39-41), which could in susceptible subjects trigger excessive immune response, including CTO secretion. This hypothesis is attractive because CTO is a chitinolytic enzyme and chitin is abundant in fungal cell wall. Nevertheless, conclusions should be drawn carefully as the group of H/H subjects consisted of only 10 patiens (only 6% of patients are homozygous for 24-base pair duplication polymorphism). Larger pool of patients is required to confirm this finding. Moreover, other clinical phenotypes were also observed in H/H patients, including 1 patient with chronic-fibrotising course of the disease.

The minor part of this study was to observe the development of COS through observational period. The patients had statistically significant higher COS at 3 as compared to 5 or 10 years of follow-up (in this respective order). This is consistent with original report by Baughmann et al (31).

In conclusion, in this study it was shown that wt/wt and wt/H subjects have the same clinical outcome status of sarcoidosis. A possible protective role of homozygous condition for CHIT1 24-base pair duplication polymorphism is suggested but additional data will be required for definite conclusions.

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