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Respiratory Medicine (2007) **101**, 1563–1571

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A CHI3L1 gene polymorphism is associated with serum levels of YKL-40, a novel sarcoidosis marker

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Received 26 July 2006; accepted 4 December 2006
Available online 22 January 2007

KEYWORDS
CHI3L1;
Fibrosis;
Genetics;
Sarcoidosis;
Single nucleotide polymorphism;
YKL-40

Summary
Background: YKL-40, a chitinase-like cartilage glycoprotein, has recently shown its potential as a marker for sarcoidosis.
Methods: This study aimed to assess whether YKL-40 at presentation may predict the course of sarcoidosis over a 4-year follow-up period and to investigate whether polymorphisms in the chitinase 3-like 1 (CHI3L1) gene might influence serum YKL-40 levels in sarcoidosis patients ($n = 63$) and controls ($n = 333$).
Results: Patients had significantly higher (mean, 95% CI) serum YKL-40 levels (181.3 ng/ml, 50.7–648.1) compared to controls (36.6 ng/ml, $p < 0.0001$. Serum YKL-40 was elevated in 79% of the patients and was inversely correlated with DLco at presentation ($r^2 = -0.27$, $p = 0.03$), but not after 2–4 years of follow-up ($r^2 = -0.16$, $p = 0.27$). Serum YKL-40 levels in controls were dependent on the CHI3L1 −329 G/A polymorphism (mean, 95% CI): GG ($n = 213$) 48.3 ng/ml, 41.7–56.0; GA ($n = 101$) 31.2 ng/ml, 26.6–36.3; AA ($n = 17$) 17.8 ng/ml, 13.6–23.4, $p < 0.0001$. In patients, this effect was not observed.
Conclusions: YKL-40 may be used as a sarcoidosis disease marker, but it is unsuitable as a marker to predict the course of the disease. The CHI3L1 −329 G/A polymorphism contributes to inter-individual variations of YKL-40 levels, but does not influence sarcoidosis disease susceptibility or severity.
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0954-6111/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2006.12.006

Background

Sarcoidosis is a systemic disease of unknown cause and is characterized by the presence of noncaseating granulomas in one or multiple organs.[1] The majority of patients have pulmonary involvement and although most undergo spontaneous remission, about 10–15% of the cases progress insidiously towards pulmonary fibrosis.[2] Fibrosis of the lung parenchyma is marked by excessive extra-cellular matrix deposition which can lead to end-stage fibrosis with honeycombing of the lungs. This sequela is associated with loss of lung capacity and has a poor prognosis with high mortality.[3]

It is challenging to find reliable sarcoidosis disease markers that are capable of predicting the course of the disease. The human cartilage glycoprotein-39, or YKL-40 (its name derived from the first three N-terminal amino acids and from its molecular weight of 40 kDa) has recently shown its potential merit as a marker for sarcoidosis.[4] Beside the reported overall increase of serum YKL-40 levels in sarcoidosis patients compared to controls, pulmonary carbon monoxide diffusion (DLco) was found to be inversely correlated with serum YKL-40 levels.[4] The authors of this study concluded that YKL-40 may serve as a marker for disease activity as well as fibrosis in sarcoidosis patients.

In addition to sarcoidosis, serum YKL-40 is also elevated in systemic sclerosis,[5,6] rheumatoid arthritis,[7–9] liver fibrosis,[10,11] inflammatory bowel disease,[12] and type 2 diabetes.[13] Moreover, elevated YKL-40 has been reported to predict shorter survival in cancer patients.[14–19]

YKL-40 is a member of the chitinase family and is secreted by articular chondrocytes and synovial cells[20] as well as by disease-associated cells including cancer[21,22] and inflammatory cells.[4,23,24] Unlike its other family members, YKL-40 does not exert chitinase activity.[20] Instead, a single amino acid substitution causes YKL-40 to replace its potential chitinase property to the ability to bind lectin.[25] Although its precise function has not yet been elucidated, the biological properties of YKL-40 suggest that it plays a role in tissue inflammation, remodeling, and the development of fibrosis.[26–28] In fact, in vitro studies by Recklies et al.[29] have shown that YKL-40 is a potent growth factor for connective tissue cells including human fibroblasts.

Another chitinase that has been studied as a potential marker for sarcoidosis is chitotriosidase.[30] Contrary to YKL-40, this protein does exert chitinase activity and is also highly expressed by activated macrophages.[25] Chitotriosidase has even shown to be higher in advanced radiographic stages of the sarcoidosis.[30] The precise role of chitotriosidase in the pathogenesis of sarcoidosis has, like YKL-40, not yet been identified.

Single nucleotide polymorphisms (SNPs) of genes that are involved in the pathogenesis of sarcoidosis have shown to contribute to the vastly different patterns with which this disease can evolve.[31] SNPs may alter the nature of a protein and is reflected by altered physiologic properties or changed expression levels. Functional polymorphisms present in proteins which are used as disease markers may complicate the interpretation of differential protein levels in both healthy and affected individuals. In the ACE gene, for instance, an insertion/deletion (I/D) polymorphism in intron 16 is known to exhibit gross differences of baseline expression levels based on the genotype of the coding gene.[32] As a consequence, the precision needed to differentiate between normal and deviating levels may be compromised.[33]

The YKL-40 coding chitinase 3-like 1 (CHI3L1) gene also bears a large number of polymorphic sites, some of which are potentially functional. The aim of this study was to investigate whether SNPs in the CHI3L1 gene may influence serum YKL-40 levels in Dutch Caucasian sarcoidosis patients and healthy controls. The biallelic polymorphisms which we evaluated included two that are present in the promoter region, three in different intron/exon boundaries and one SNP in exon 5 that causes an amino acid substitution (Arg145Gly).

We also tested the hypothesis by Johansen et al.[4] that YKL-40 levels may predict the course of pulmonary sarcoidosis by comparing the YKL-40 levels at presentation with radiographic evolution and pulmonary function tests over a 4-year follow-up period.

Methods

Healthy volunteers and patients

Venous blood samples were obtained from 333 ostensibly healthy employees of the St Antonius Hospital; 214 women (39 ± 12.0 years) and 119 men (42 ± 10.2 years). By completing a questionnaire, relevant background information was provided by these volunteers which included medication, ethnicity, and hereditary diseases. The over-representation of women who participated in this study is explained by the predominantly female workforce at this hospital. Fifty-five individuals (33 women and 22 men) smoked for at least 5 pack-years. Ethnicity of both parents was used as the criterion for assuming Dutch Caucasian ethnicity of the subject. Exclusion criteria included known pulmonary disease and non-Dutch Caucasian ethnicity. The medical ethical committee of this hospital approved the study conducted and all subjects gave formal written consent.

Seventy-five unrelated Dutch Caucasian sarcoidosis patients (44 males/31 females; age at diagnosis (years) $\pm$ SD/range: 39 ± 11.2 years/17–71) were included in this retrospective study. The diagnosis of sarcoidosis was established in 50 patients when clinical findings were supported by histological evidence and after exclusion of other known causes of granulomatosis in accordance with the consensus of the ATS/ERS/WASOG statement on sarcoidosis.[34]

Chest radiographs were assessed to determine disease severity using standard radiographic staging for sarcoidosis, classified according to the Scadding criteria.[34] One patient presented with radiographic stage 0, 20 patients presented with stage I, 11 patients presented with stage II, 15 patients presented with stage III, and three patients presented with stage IV disease. In 25 patients, the diagnosis was made without biopsy proof because these patients presented with the classic symptoms of Löfgren's syndrome, namely fever, erythema nodosum, arthralgia, and bilateral hilar lymphadenopathy.

No prior treatment for sarcoidosis was given prior to presentation. After presentation, individuals who were treated with corticosteroids for at least 3 months during

Table 1 Primer sequences for the identification of six biallelic single nucleotide polymorphisms of CHI3L1.

Polymorphism and location	dbSNP accession*	Primer	Consensus	Product size (bp)
−329 (G/A) Promoter	rs10399931	5′-CGG CTG AGT CAC ATC TCC G/A (forward)	5′-CAG GCC CTG TAC TTC CTT TA (reverse)	261
−247 (C/T) Promoter	rs10399805	5′- AGA GGA GGG TTG AGA AAC CG/A (reverse)	5′-TGC TGA AGA TGC AAA GGT AGA G (forward)	220
48 (T/A) Intron1/exon1	rs7515776	5′-ATC TGG GCT AGC CAA GGT TAA T/A (forward)	5′-AGA GAA ATC CAG GAT GAG ACC (reverse)	247
1219 (C/T) Intron2/exon3	rs1538372	5′-CCT GAA GGA GAA GTC TGG G G/A (reverse)	5′-TAG CGT ATT CAT CCC CTG CA (forward)	265
2117 (C/G) Intron4/exon4	rs2071579	5′-GAA CAT CCA TAC AGT GGA TG G/C (reverse)	5′-TAA CCC AGC CTC TCA CCC AA (forward)	264
2950 (G/A) Exon 5	rs880633	5′-GGT GGT AAA ATG CTG TTT GTC TC C/T (reverse)	5′-GCA TGC TAC AGG GCT GAT TTT (forward)	171

*The SNP loci were identified using accession numbers according to the SNP database at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp (accessed July 2005).

the 4-year follow-up period, were considered as having received treatment ($n = 28$). No other treatment regimens had been used in the study group.

Radiographic evolution over a 4-year follow-up period was categorized as follows: acute/self-remitting (normalization or improvement towards stage I) ($n = 25$), chronic (persistent stage II/III or progression towards this stage) ($n = 16$), and fibrosis (stable stage IV or progressive towards this stage) ($n = 9$), and patients with Löfgren's syndrome ($n = 25$). Except for one, all patients with Löfgren's disease had received radiographic follow-up. These patients had normalized chest radiography after 2 or 4 years following presentation, except for one patient, who remained at radiographic stage I after 4 years.

Pulmonary function tests were performed at presentation and upon 2, and 4 years following diagnosis. Vital capacity (inspiratory) (VC), forced expiratory volume in 1 s (FEV_1), and carbon monoxide diffusing lung capacity (DLco) were used to assess the presence of lung function impairment at presentation and follow-up of disease. All lung function parameters are expressed as percent predicted values. VC and FEV_1 were calculated from volumes in liters and adjusted to BTPS (body temperature, ambient pressure, saturated with water vapor) in accordance with the ERS recommendations.[35]

The medical ethical committee of the St. Antonius Hospital approved the study conducted and all subjects gave formal written consent.

Serum YKL-40 measurements

Serum YKL-40 was measured with an enzyme-linked immunosorbent assay kit (Metra YKL-40, Quidel Corporation, San Diego, CA, USA), in accordance with the manufacturer's instructions.[36] All samples were run in duplicate and mean values were used for analysis. The mean intra-assay coefficient of variance (CV) was calculated from two control samples (low: 40 ng/l and high: 150 ng/l) that were both measured in duplicate on 5 different days. The CV was 3.5%.

Serum YKL-40 levels were measured in patients only at presentation of sarcoidosis.

Genotyping

Biallelic SNPs were determined using sequence-specific primers) and polymerase chain reaction (PCR).

The identification numbers of the SNP loci and the sequences of SNP-specific primers with their complementary consensus primers are shown in Table 1. The PCR conditions were as previously described.[37] The final primer concentrations used were 7.6 ng/μl. DNA was available from 63 patients.

Statistical analysis

The statistical evaluation of our data was performed using SPSS 11 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism v. 4 (Graphpad Software, Inc., San Diego, CA, USA) software packages. Statistical analyses of SNP and haplotype frequency distributions were performed using chi-square contingency table analysis with the appropriate number of degrees of freedom. Fisher's exact test was used if expected cell frequencies were lower than 5. Adjustment for multiple tests was made by multiplying the p-value by the number of SNPs being investigated (Bonferroni method). Genotype frequencies were tested for Hardy–Weinberg equilibrium. Haplotypes were determined using Phase, version 2 (Mac OS X).[38,39]

YKL-40 values were ln-transformed before analysis, since these were ln-normal distributed; antilog geometric mean values are denoted as mean. Analysis of variance was performed and the portion of the serum YKL-40 level variance, explained by the CHI3L1 genotype and other factors, was estimated. Fixed factors in the ANOVA model were gender, smoking history, and the CHI3L1 genotype.

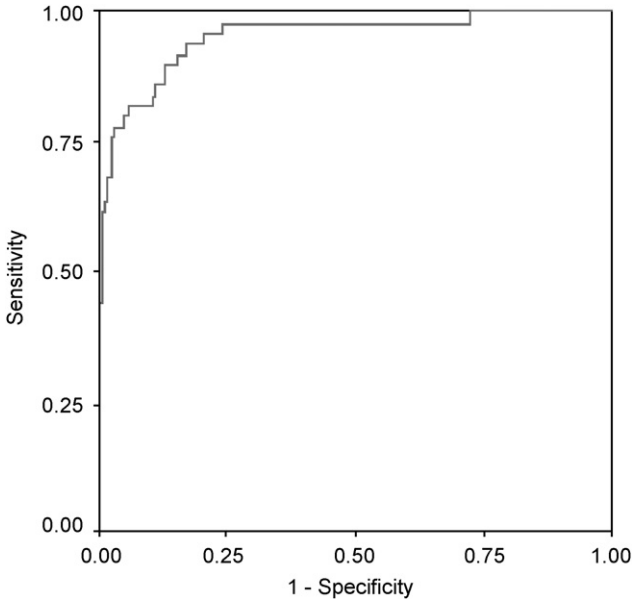

Figure 1 Receiver operating characteristic curve of serum YKL-40 with 333 healthy controls and 75 sarcoidosis patients.

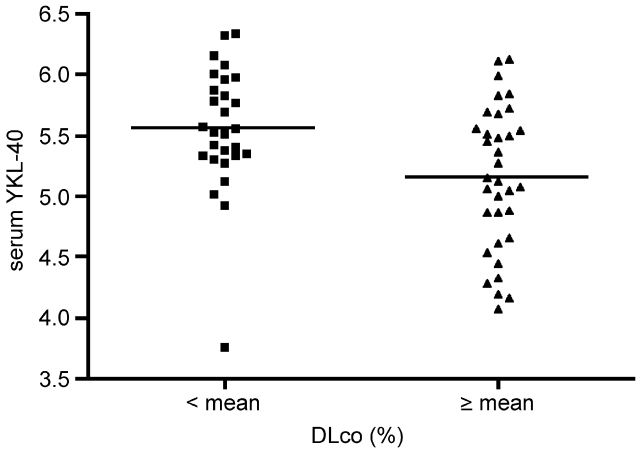

Figure 2 Scatter plot of serum YKL-40 levels (ng/ml) in sarcoidosis patients ($n = 62$) with diffusion capacity (DLco) below and equal/above the mean (85%). Horizontal bars in scatter represent mean values. *Y*-axis represents log-transformed values. Student's *t*-test: $p = 0.004$.

Age was included as a covariate. The ANOVA was followed by the appropriate post-testing for comparisons between groups. Correlation between variables was assessed with Spearman's rho.

The sensitivity and the specificity were established using the receiver operating characteristic (ROC) curve by plotting the sensitivity against the reverse specificity (1−specificity) at each value.

Statistical significance was denoted by a value of $p < 0.05$ for all tests performed.

Results

Serum YKL-40 levels in controls

Mean (median) serum YKL-40 in controls was 36.6 (37.3) ng/ml. The calculated reference range (mean $\pm$ 1.96 $\times$ SD) was: 11.9–110.0 ng/ml.

Age correlated with YKL-40 levels ($r^2 = 0.23$, $p < 0.0001$) and explained 5% of the variation. Smoking history or gender did not significantly influence serum YKL-40 levels (data not shown).

Serum YKL-40 levels in patients

Sarcoidosis patients had significantly higher (mean (median), 95% CI) serum YKL-40 levels (181.3 (210.0) ng/ml, 50.7–648.1) compared to controls, $p < 0.0001$. Based on the reference interval of controls, 59 sarcoidosis patients (79%) showed serum YKL-40 levels that exceeded the upper reference limit.

An ROC curve revealed that at the value of 72.0 ng/ml, serum YKL-40 had a sensitivity of 90% and a specificity of 72%. The area under the curve was 0.953 (Fig. 1).

YKL-40 levels and clinical parameters in sarcoidosis patients

Radiographic staging at presentation or radiographic evolution were not associated with YKL-40 levels (data not shown). Since treatment in a number of patients ($n = 28$) was not given at the time of presentation, the influence of treatment on serum YKL-40 levels could not be determined for that time point. However, YKL-40 levels at presentation (242.3 ng/ml, 95% CI: 195.7–289.0) were not different between those patients who received subsequent treatment within the 4-year follow-up period and those who did not (232.6 ng/ml, 95% CI: 184.3–280.9).

Serum YKL-40 showed an inverse correlation with DLco (%) at presentation ($r^2 = -0.27$, $p = 0.03$), but not with DLco measured at 2–4 years ($r^2 = -0.16$, $p = 0.27$). Sarcoidosis patients who had DLco levels below the mean (85%) had significantly higher mean serum YKL-40 levels than those with DLco values equal to or above the mean: 259.8 ng/ml, 95% CI: 200.3–317.3 versus 170.7 ng/ml, 95% CI: 134.3–210.6, $p = 0.004$ (Fig. 2).

Neither FEV_1 nor VC (both measured at presentation or after 2–4 years) correlated with serum YKL-40 levels (data not shown).

CHI3L1 single nucleotide polymorphisms

Table 2 summarizes the results of CHI3L1 genotype and allele carrier frequencies in the Dutch sarcoidosis patients and controls. No deviation from Hardy–Weinberg equilibrium was observed for any of the groups studied. No differences were found for genotype, allele or allele carrier frequencies between sarcoidosis patients and controls. None of the polymorphisms were associated with any of the clinical parameters including lung function and radiographic staging (data not shown).

Four major haplotypes with six polymorphic sites in the CHI3L1 gene were established (Table 3). There was no difference in haplotype frequencies between sarcoidosis patients and controls.

Table 2 CHI3L1 allele carrier and genotype frequencies in Dutch Caucasian sarcoidosis patients and controls.

Polymorphism	Allele and genotype	Controls ($n = 333$)	Patients ($n = 63$)
−329 (G/A)	G	0.94 (314)	0.97 (61)
	A	0.35 (118)	0.29 (18)
	GG	0.65 (215)	0.71 (45)
	AG	0.30 (101)	0.25 (16)
	AA	0.05 (17)	0.03 (2)
−247 (C/T)	C	0.98 (328)	0.98 (62)
	T	0.24 (79)	0.25 (16)
	CC	0.76 (254)	0.74 (47)
	CT	0.22 (74)	0.24 (15)
	TT	0.02 (5)	0.02 (1)
48 (T/A)	T	0.98 (328)	0.98 (62)
	A	0.24 (79)	0.25 (16)
	TT	0.76 (254)	0.74 (47)
	TA	0.22 (74)	0.24 (15)
	AA	0.02 (5)	0.02 (1)
1219 (C/T)	C	0.94 (314)	0.98 (62)
	T	0.47 (157)	0.44 (28)
	CC	0.53 (176)	0.56 (35)
	CT	0.41 (138)	0.43 (27)
	TT	0.06 (19)	0.02 (1)
2117 (C/G)	C	0.81 (269)	0.79 (50)
	G	0.70 (233)	0.62 (39)
	CC	0.30 (100)	0.38 (24)
	CG	0.51 (169)	0.41 (26)
	GG	0.19 (64)	0.20 (13)
2950 (G/A)	G	0.83 (277)	0.79 (50)
	A	0.69 (230)	0.62 (39)
	GG	0.31 (103)	0.38 (24)
	GA	0.52 (174)	0.41 (26)
	AA	0.17 (56)	0.20 (13)

Association between genetic variants of CHI3L1 and serum YKL-40 levels in controls and sarcoidosis patients

Each SNP in the CHI3L1 gene was analyzed for association with serum YKL-40 levels. All six polymorphic sites were found to influence serum YKL-40 levels in a gene-dose-dependent manner. Figure 3A illustrates the effect of SNP −329 G/A on serum YKL-40 levels in healthy controls. To test whether the variation in YKL-40 levels was influenced independently by the individual SNPs or by linkage disequilibrium between them, a multivariate analysis was performed. In this model, which was adapted from the method used by Zhu et al.,[40] the SNP that explained most of the variation of serum YKL-40 levels was selected as a covariate to identify the influence of the remaining SNPs (analyzed separately). The −329 G/A polymorphism showed the highest variance: 23%, $p<0.0001$). Also, age was included as a covariate and gender and smoking history were included as fixed factors. The analysis revealed that the correction of serum YKL-40 levels by the −329 G/A polymorphism negated the influence of the other polymorphisms on serum YKL-40 levels (Table 4). The isolated effect of the −329 G/A SNP was also illustrated by determining haplotype-specific serum YKL-40 levels. When heterozygous carriers of either of the haplotypes in combination with haplotype 1 were compared with each other in terms of (mean) serum YKL-40 levels, the only significant differences were those observed between haplotypes that differed from the −329 allele: haplotype carrier 1+1 (43.8 ng/ml) versus 1+2 (28.8 ng/ml), $p<0.0001$; haplotype carrier 1+2 (28.8 ng/ml) versus 1+3 (49.4 ng/ml), $p<0.0001$; haplotype carrier 1+2 (28.8 ng/ml) versus 1+4 (44.7 ng/ml), $p<0.05$; haplotype carrier 1+2 (28.8 ng/ml) versus 1+5 (60.3 ng/ml), $p<0.01$; haplotype carrier 1+5 (60.3 ng/ml) versus 1+6 (22.3 ng/ml), $p<0.05$.

The −329 G/A polymorphism did not appear to significantly influence serum YKL-40 levels in sarcoidosis patients (Fig. 3B) despite the trend towards lower mean values associated with the presence of the A allele: genotype AG (153.2 ng/ml) compared to GG (192.7 ng/ml), $p = 0.16$, nor did correcting the effect of the −329 G/A SNP for smoking, gender, and age in a multivariate analysis reveal a genotype-dependent influence on serum YKL-40 levels (data not shown). Age correlated significantly with serum YKL-40 in a

Table 3 CHI3L1 haplotype frequencies in sarcoidosis patients and controls.

Haplotype	−329 (G/A)	−247 (C/T)	48 (T/A)	1219 (C/T)	2117 (C/G)	2950 (G/A)	Frequency in controls ($n = 333$)	Frequency in patients ($n = 63$)
1	G	C	T	C	C	G	0.53	0.58
2	A	C	T	T	G	A	0.17	0.13
3	G	T	A	C	G	A	0.12	0.13
4	G	C	T	T	G	A	0.09	0.10
5	G	C	T	C	G	A	0.02	0.03
6	A	C	T	C	C	G	0.02	0.01
7–10*	—	—	—	—	—	—	0.05	0.02

*One of each haplotype occurred no more than 3 times.

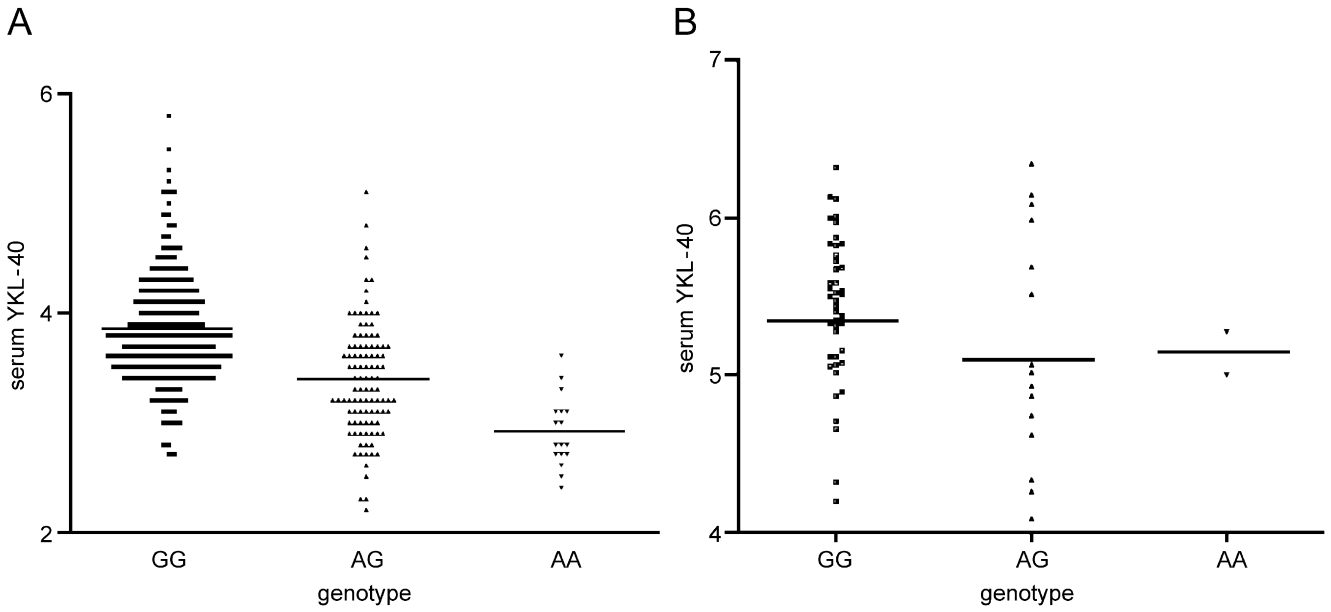

Figure 3 (A) Scatter plot illustrating the association between the −329 G/A polymorphism in the CHI3L1 gene and serum YKL-40 levels in healthy controls ($n = 333$). Horizontal bars in scatters represent mean values. *Y*-axis represents log-transformed values. ANOVA: $p < 0.0001$. (B) Scatter plot illustrating the association between the −329 G/A polymorphism in the CHI3L1 gene and serum YKL-40 levels in sarcoidosis patients ($n = 63$). Horizontal bars in scatters represent mean values. *Y*-axis represents log-transformed values. ANOVA: $p = 0.29$.

Table 4 CHI3L1 gene polymorphisms and the influence on serum YKL-40 levels in controls.

Polymorphism	Genotype-specific YKL-40 levels (ng/ml) (mean, 95% CI)*	Differences between genotype-specific YKL-40 serum levels
−329 (G/A)	GG ($n = 213$) 48.3, 41.7–56.0 GA ($n = 101$) 31.2, 26.6–36.3 AA ($n = 17$) 17.8, 13.6–23.4	$p < 0.00001$
−247 (C/T)	CC ($n = 254$) 39.4, 34.2–45.5 CT ($n = 72$) 43.1, 36.4–51.0 TT ($n = 5$) 50.1, 32.0–78.4	$p = 0.25^{\dagger}$
48 (T/A)	TT ($n = 252$) 39.4, 34.1–45.4 TA ($n = 72$) 43.1, 36.4–51.2 AA ($n = 7$) 47.7, 31.6–72.1	$p = 0.27^{\dagger}$
1219 (C/T)	CC ($n = 176$) 40.5, 34.7–47.3 CT ($n = 138$) 40.0, 34.2–46.8 TT ($n = 19$) 42.2, 32.0–55.5	$p = 0.07^{\dagger}$
2117 (C/G)	CC ($n = 100$) 36.9, 31.2–43.6 CG ($n = 168$) 41.0, 35.5–47.5 GG ($n = 63$) 43.1, 35.9–51.8	$p = 0.14^{\dagger}$
2950 (G/A)	GG ($n = 103$) 39.5, 34.2–45.6 GA ($n = 173$) 42.4, 35.7–50.6 AA ($n = 55$) 49.2, 35.3–68.6	$p = 0.43^{\dagger}$

*Corrected for smoking history, gender, and age.

†SNP −329 G/A was used as a covariate to determine the effects of the remaining five SNPs on serum YKL-40 levels. Smoking history, gender and age were also included as fixed factor or covariate. Mean YKL-40 and 95% CI were calculated from log-transformed data.

similar pattern as that which was observed in the control population ($r^2 = 0.24$, $p = 0.04$). No association was found between the remaining five polymorphisms and serum YKL-40 levels in sarcoidosis patients (data not shown).

The observed association between DLco and serum YKL-40 levels was not attributed to the −329 G/A genotype distribution between patients below and equal/above the mean DLco (85%) (data not shown).

Age and genotype-specific reference intervals

In order to calculate the expected serum YKL-40 levels of an individual that is corrected for age and the −329 G/A genotype, the following formulas were deduced from the multivariate analysis:

−329 GG genotype: ln (serum YKL-40 (ng/ml)) = 3.348+0.012 × age (years) ± 0.944,
−329 AG genotype: ln (serum YKL-40 (ng/ml)) = 2.916+0.012 × age (years) ± 0.944,
−329 AA genotype: ln (serum YKL-40 (ng/ml)) = 2.362+0.012 × age (years) ± 0.944.

When the expected serum YKL-40 levels were calculated for the sarcoidosis patients, the number of patients with either elevated or normal serum YKL-40 levels remained unchanged.

Discussion

This study supports the merits of serum YKL-40 as a disease marker for sarcoidosis, as previously shown by Johansen et al.[4] Serum YKL-40 levels were found to be elevated in 79% of the sarcoidosis patients. However, the low specificity and the previously described elevations of YKL-40 associated with various other diseases[5–12] may limit the use of YKL-40 as a diagnostic marker for sarcoidosis.

Serum YKL-40 levels were found to increase with age, although the extent to which serum YKL-40 was elevated in sarcoidosis patients was too high to allow age to influence the accuracy of serum YKL-40 as a sarcoidosis marker.

The inverse correlation between serum YKL-40 levels and DLco seemed to suggest that YKL-40 is related to the development of pulmonary fibrosis. This notion was also reported by Johansen et al.[4] and is well supported by the fact that YKL-40 is a strong growth factor for human fibroblasts.[29] However, since the development of pulmonary fibrosis is usually irreversible,[3] the lack of correlation between DLco measured at later time points during follow-up and serum YKL-40 levels measured at presentation does therefore suggest against the presence of fibrosis. Vascular involvement in pulmonary sarcoidosis is often seen[41] and is, like fibrosis, often reflected by a decreased DLco.[42] The observed correlation between YKL-40 and DLco may therefore be attributed to events that remain yet to be identified. Contrary to what Johansen et al.[4] have described, YKL-40 levels above the median were not associated with decreased DLco values. Instead, sarcoidosis patients with DLco values below the mean had higher YKL-40 levels. An observation similar to ours has been reported in systemic sclerosis patients with pulmonary fibrosis by chest X-ray. In this report, patients with decreased DLco showed higher serum YKL-40 levels in serum.[5] Although our findings emerged from assessing a larger group of patients of whom DLco as well as YKL-40 data were available ($n = 44$ versus $n = 27$), even larger groups should be used to resolve the inconsistent findings between Johansen's and our study.

This study is the first to describe a genotypic influence of the CHI3L1 −329 G/A polymorphism on serum YKL-40 levels in healthy individuals. This polymorphism was shown to explain as much as 23% of the variation in serum YKL-40 levels. In the sarcoidosis patients, the genotype-dependent effect of the −329 G/A polymorphism was not observed. Possibly, in a disease state, pathologic factors involved in sarcoidosis which contribute to an increase of YKL-40 levels may over-rule the variance attributed to the −329 G/A polymorphism in CHL3L2. Just as what was argued for age, there may not be a diagnostic merit in correcting serum YKL-40 levels for the −329 G/A genotype.

To test the hypothesis that the −329 G/A genotype might predispose to pulmonary fibrosis, significantly more patients with fibrotic involvement as well as complete lung function, radiographic data, and genotype are required.

Using an online tool to check for functional motifs at position −329 did not reveal any changes in the motif found for the wild-type nucleotide (http://motif.genome.jp/). The actual position of the SNP is therefore unlikely to be the quantitative trait locus (QTL) itself. Instead, it may be in linkage with another, yet to be identified QTL. Like ACE, in which the I/D polymorphism explains as much as 50% of the variance of ACE activity through linkage with the actual QTL,[32,43] additional sequencing of the promoter region of the CHI3L1 gene may indicate a site that is responsible for an even higher variation of YKL-40 levels.

Conclusion

This study shows that YKL-40 can be used as a disease marker for sarcoidosis with the stipulation that other diseases associated with elevated serum YKL-40 levels can be excluded upon assessment and during follow-up. Serum YKL-40 levels could not predict the course of pulmonary disease phenotypes according to radiographic evolution over a 4-year follow-up period. YKL-40 may be a useful marker in combination with clinical parameters such as chest X-ray and lung function and could aid in the follow-up monitoring of disease activity. Furthermore, we delivered in vivo evidence that the CHI3L1 −329 G/A polymorphism contributes to inter-individual variations of YKL-40 levels. However, the CHI3L1 −329 G/A polymorphism does not seem to influence sarcoidosis disease susceptibility or severity.

The observed influence of CHI3L1 −329 G/A on serum YKL-40 levels may also be of interest for investigations involving YKL-40 in other diseases.

Acknowledgements

The authors are indebted to Natalie Pot, Hatice Alpar, and Jan Broess for their technical assistance and to Dr. Pieter Zanen for his expert advice on the statistical methodologies.

References

1. Newman LS, Rose CS, Maier LA. Sarcoidosis. *N Engl J Med* 1997; **336**(17):1224–34.
2. Romer FK. Presentation of sarcoidosis and outcome of pulmonary changes. *Dan Med Bull* 1982;**29**(1):27–32.
3. Viskum K, Vestbo J. Vital prognosis in intrathoracic sarcoidosis with special reference to pulmonary function and radiological stage. *Eur Respir J* 1993;**6**(3):349–53.
4. Johansen JS, Milman N, Hansen M, Garbarsch C, Price PA, Graudal N. Increased serum YKL-40 in patients with pulmonary sarcoidosis—a potential marker of disease activity? *Respir Med* 2005;**99**(4):396–402.
5. Nordenbaek C, Johansen JS, Halberg P, et al. High serum levels of YKL-40 in patients with systemic sclerosis are associated with pulmonary involvement. *Scand J Rheumatol* 2005;**34**(4):293–7.
6. La Montagna G, D'Angelo S, Valentini G. Cross-sectional evaluation of YKL-40 serum concentrations in patients with systemic sclerosis. Relationship with clinical and serological aspects of disease. *J Rheumatol* 2003;**30**(10):2147–51.
7. Johansen JS, Stoltenberg M, Hansen M, et al. Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. *Rheumatology (Oxford)* 1999;**38**(7):618–26.
8. Matsumoto T, Tsurumoto T. Serum YKL-40 levels in rheumatoid arthritis: correlations between clinical and laborarory parameters. *Clin Exp Rheumatol* 2001;**19**(6):655–60.
9. Harvey S, Whaley J, Eberhardt K. The relationship between serum levels of YKL-40 and disease progression in patients with early rheumatoid arthritis. *Scand J Rheumatol* 2000;**29**(6): 391–3.
10. Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol* 2003;**39**(2):179–86.
11. Johansen JS, Christoffersen P, Moller S, et al. Serum YKL-40 is increased in patients with hepatic fibrosis. *J Hepatol* 2000; **32**(6):911–20.
12. Koutroubakis IE, Petinaki E, Dimoulios P, et al. Increased serum levels of YKL-40 in patients with inflammatory bowel disease. *Int J Colorectal Dis* 2003;**18**(3):254–9.
13. Rathcke CN, Johansen JS, Vestergaard H. YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance. *Inflamm Res* 2006;**55**(2): 53–9.
14. Pelloski CE, Mahajan A, Maor M, et al. YKL-40 expression is associated with poorer response to radiation and shorter overall survival in glioblastoma. *Clin Cancer Res* 2005;**11**(9): 3326–34.
15. Dupont J, Tanwar MK, Thaler HT, et al. Early detection and prognosis of ovarian cancer using serum YKL-40. *J Clin Oncol* 2004;**22**(16):3330–9.
16. Johansen JS, Drivsholm L, Price PA, Christensen IJ. High serum YKL-40 level in patients with small cell lung cancer is related to early death. *Lung Cancer* 2004;**46**(3):333–40.
17. Hogdall EV, Johansen JS, Kjaer SK, et al. High plasma YKL-40 level in patients with ovarian cancer stage III is related to shorter survival. *Oncol Rep* 2003;**10**(5):1535–8.
18. Johansen JS, Christensen IJ, Riisbro R, et al. High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. *Breast Cancer Res Treat* 2003;**80**(1): 15–21.
19. Cintin C, Johansen JS, Christensen IJ, Price PA, Sorensen S, Nielsen HJ. High serum YKL-40 level after surgery for colorectal carcinoma is related to short survival. *Cancer* 2002;**95**(2): 267–74.
20. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;**268**(34):25803–10.
21. Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. *J Bone Miner Res* 1992;**7**(5):501–12.
22. Morrison BW, Leder P. neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-initiated tumors. *Oncogene* 1994;**9**(12):3417–26.
23. Krause SW, Rehli M, Kreutz M, Schwarzfischer L, Paulauskis JD, Andreesen R. Differential screening identifies genetic markers of monocyte to macrophage maturation. *J Leukoc Biol* 1996; **60**(4):540–5.
24. Volck B, Price PA, Johansen JS, et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. *Proc Assoc Am Physicians* 1998; **110**(4):351–60.
25. Renkema GH, Boot RG, Au FL, et al. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *Eur J Biochem* 1998;**251**(1–2):504–9.
26. Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJ. Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. *Exp Cell Res* 1999;**250**(1):168–73.
27. Recklies AD, Ling H, White C, Bernier SM. Inflammatory cytokines induce production of chitinase-3-like protein 1 by articular chondrocytes. *J Biol Chem*, 2005.
28. Bigg HF, Wait R, Rowan AD, Cawston TE. The mammalian chitinase-like lectin, YKL-40, binds specifically to type I collagen and modulates the rate of type I collagen fibril formation. *J Biol Chem*, 2006.
29. Recklies AD, White C, Ling H. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. *Biochem J* 2002;**365**(Part 1): 119–26.
30. Grosso S, Margollicci MA, Bargagli E, et al. Serum levels of chitotriosidase as a marker of disease activity and clinical stage in sarcoidosis. *Scand J Clin Lab Invest* 2004;**64**(1):57–62.
31. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. *Lancet* 2003;**361**(9363):1111–8.
32. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;**86**(4):1343–6.
33. Tomita H, Ina Y, Sugiura Y, et al. Polymorphism in the angiotensin-converting enzyme (ACE) gene and sarcoidosis. *Am J Respir Crit Care Med* 1997;**156**(1):255–9.
34. Hunninghake GW, Costabel U, Ando M, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis* 1999;**16**(2):149–73.
35. Standardized lung function testing. Official statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;**16**: 1–100.
36. Harvey S, Weisman M, O'Dell J, et al. Chondrex: new marker of joint disease. *Clin Chem* 1998;**44**(3):509–16.
37. Bunce M, O'Neill CM, Barnardo MC, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens* 1995;**46**(5):355–67.
38. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;**68**(4):978–89.

39. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;**73**(6):1162–9.
40. Zhu X, Bouzekri N, Southam L, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. *Am J Hum Genet* 2001;**68**(5):1139–48.
41. Takemura T, Matsui Y, Oritsu M, et al. Pulmonary vascular involvement in sarcoidosis: granulomatous angiitis and microangiopathy in transbronchial lung biopsies. *Virchows Arch A Pathol Anat Histopathol* 1991;**418**(4):361–8.
42. Phansalkar AR, Hanson CM, Shakir AR, Johnson Jr. RL, Hsia CC. Nitric oxide diffusing capacity and alveolar microvascular recruitment in sarcoidosis. *Am J Respir Crit Care Med* 2004;**169**(9):1034–40.
43. McKenzie CA, Julier C, Forrester T, et al. Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: evidence for two quantitative-trait loci. *Am J Hum Genet* 1995;**57**(6):1426–35.