SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2014; 31; 136-141

Lack of correlation of BTNL2 polymorphism and cancer risk in sarcoidosis. BTNL2 and cancer risk in sarcoidosis

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Abstract. *Background:* Sarcoidosis is a polygenic immune disorder disease with predominant manifestations in the lung. BTNL2 gene polymorphisms were previously linked to susceptibility to sarcoidosis. Relationships between sarcoidosis and cancers have been suspected for a long time but little evidence has been reported until now, and recent works show a link between butyrophilin family proteins and anti tumor immunity. *Methods:* We studied the polymorphism of the rs2076530 SNP of BTNL2 gene in 35 patients with sarcoidosis and cancer compared to 340 sarcoidosis without cancer, 271 controls and 32 cancer matched controls. *Results:* We found no association between BTNL2 genotype and cancer risk in sarcoidosis (OR: 2,34; CI95%: 1,30 – 4,20; p = 0,15). Sarcoidosis is associated with the rs2076530SNP of BTNL2 gene. The AA genotype is significantly associated with a 3 more increased risk of sarcoidosis compared to the GG genotype in a co-dominant model (OR: 3,22; CI95%: 2,58 – 4,06; p < 0.0001). The AG genotype is associated with a 1,8 more increased risk (OR: 1,79; CI95%: 1,42 – 2,26). *Conclusions:* These results confirm the association of BTNL2 rs2076530SNP with the susceptibility to develop sarcoidosis, but not with an increased risk of cancer in these patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2014; 31: 136-141)*

Key words: Sarcoidosis, cancer, BTNL2 gene

Abbreviations:
ACE = angiotensin conversion enzyme
BTNL2 = butyrophilin like 2
FEV = forced expired volume
LT = T lymphocyte
SNP = single-nucleotid Polymorphism
TLC = total lung capacity
VC = vital capacity

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Introduction

Sarcoidosis is a multisystemic immune disorder, of unknown etiology, characterized by noncaseating granulomas and an exaggerated cellular immune response due to increased inflammatory activity of macrophages and CD4 helper T cells. A genome screen of 63 families with sarcoidosis identified linkage of the disease to chromosome 6p21(1). Several studies show an association between sarcoidosis and butyrophilin-like 2 (BTNL2) gene variant, rs2076530SNP. BTNL2 is a member of immunoglobulin superfamily and has been implicated as a costimulatory molecule involved in T-cell activation. The rs2076530SNP, characterized by a

G → A transition, leads to a truncated protein with disturbance membrane localization (2). Coexistence of sarcoidosis and malignant disease has been reported for a long time. The assessment of such reports is difficult and physiopathological links are unknown. The LT CD4+ cells play a central role in Pro- and Anti-tumor immunity, especially Th1 lymphocytes that allow tumor rejection (3). So, we hypothesized that the rs2076530 BTNL2 variant, leading to a non-functional co-stimulation protein disrupting the T lymphocyte activation, increases the cancer risk in sarcoidosis patients.

Material and methods

Among a French national cohort of familial and sporadic sarcoidosis, we have selected patients with cancer.

We performed a case-control association study in 35 patients with "sarcoidosis + cancer", versus:
- 340 sarcoidosis without cancer,
- 271 controls without sarcoidosis,
- 32 cancer matched controls without sarcoidosis named "cancer matched controls".

All sarcoidosis are histologically proven (non-caseating epithelioid granulomas).

The control group derived from subjects taken from other genetic disease by genetic laboratory, Edouard Herriot Hospital, Hospices Civils de Lyon, France. Sarcoidosis was excluded in these patients.

The "cancer matched controls" come from different departments from Lyon-Sud Hospital, Hospices Civils de Lyon, France. They were matched on histological type of cancer, sex and age at cancer diagnosis. Three cases were not matched because we did not found satisfactory control during the time of the study (oesophageal adenocarcinome, osteosarcome, essential thrombocythemia). In total we matched 32 cases with 32 control cancer, summarized in table 1.

The genetic analysis used the primer for amplification and sequencing5-3'CAGATGGCAGAGTACAGAGG and 3'-5'AAGGACCTGTTAAAGAGACT. PCR amplification of genomic DNA was performed by standard PCR protocols. PCR products were sequenced by DNA cycle sequencing by use BigDyeXterminator solution. Sequence delineation and base calling were performed using automating fluorescent DNA sequencer, Applied Biosystem model 3130xl.

The statistical analysis used a non conditional logistic procedure (SAS, windows, V 9.2), and a conditional logistic regression procedure for the comparison between the matched groups.

All cases and controls completed informed consent forms, in accordance with a protocol approved by ethics committee.

Results

375 sarcoidosis patients were included in the French national cohort from November 2004 to August 2011. We found 35 cases with the association "sarcoidosis – cancer" for a total of 36 cancers in the cohort because one patient developed 2 cancers during the follow-up (breast and kidney adenocarcinoma). The clinical characteristics of sarcoidosis are those usually observed in the disease (e.g. table3) except the median age at diagnosis of sarcoidosis, average 51 years. 88% were Caucasian and only 6/35 (17%) had familial history of sarcoidosis. Majority of sarcoidosis are stage I (41%) or II (35%). 20% are stage III and only 3% are stage IV with pulmonary fibrosis. The median rate of ACE is 53UI.

Table 1. Comparison of "sarcoidosis and cancer" cases with cancer group control.

	Sarcoidosis with cancer cases	Cancer matched controls
Total	32	32
Sex (sex ratio W/M)	16W/16M (1,0)	16W/16M (1,0)
Ethnicity		
Caucasian	93,7 %	91%
Black	3,1 %	9%
Carribean	3,1 %	0
Asian	0	0
Smoking		
No	55,1 %	57,6%
Active	17,2%	21,2%
ex smoker	31 %	12,1%
Family history of cancer	31,8%	39,2%
Mean age of cancer	52,9 ± 13,7 (26 – 76)	52,5 ± 15,7 (18 – 90)

Histological types of cancer were mixed with a majority of prostatic and breast adenocarcinoma (16,6%), 5 kidney adenocarcinoma (13,8%), 2 lymphoma and only one lung malignancy (e.g.table 2).

In 13 cases/36 (36%) the cancer preceded the sarcoidosis, 19,4% (7/36) diagnoses were concomitant, and in 44,4% (16/36) sarcoidosis was first diagnosed. Among patients who developed cancer during follow-up of sarcoidosis, only one had received immunosuppressive treatment (azathioprine). A majority had received corticostéroids (87,5%), three had received antimalarials, two methotrexate and one no treatment for sarcoidosis.

In a patient sarcoidosis resolved spontaneously without specific treatment after treatment of her cancer. Both diagnoses were concomitants.

The genotypic distribution of rs2076530SNP of BTNL2 gene in the different groups is summarized in the table 4. The AA mutation is found in almost 30% of controls.

The A mutated allele was found in 33/35 patients in the cases "sarcoidosis + cancer": 57% of AA genotype, 37% of AG genotype. In the sarcoidosis without cancer group 44.4% has de AA genotype, 46% were heterozygous AG, and 9.4% were GG.

The cancer matched controls show much more A mutation than in group control: 40.6% was AA, 46.8% AG, and 12.5% was GG.

In a co-dominant model, the cancer risk in sarcoidosis is 2,34 greater with the AA genotype (CI 95%: 1,30 – 4,20), and 1,53 (CI95%: 0,85 – 2,74) with the AG genotype, but not statistically significant (p = 0,15). Compared to controls, the study confirms the association between rs2076530SNP of BTNL2 gene and sarcoidosis risk. The AA genotype is associated with a 3 times increased risk of sarcoidosis (OR: 3,22; CI95%: 2,55 – 4,06; p < 0,0001) and 7 times increased risk for "sarcoidosis and cancer" (OR: 7,21; CI95%: 4,00 – 12,99, p = 0,001). With the AG genotype the sarcoidosis risk is 1,8 times greater (OR: 1,79; CI95%: 1,42 – 2,26), and 2,68 times greater for the "sarcoidosis and cancer" association (OR: 2,68; CI95%: 1,49 – 4,83).

The comparison with the cancer matched group show a 2,6 increased risk to develop sarcoidosis for the AA genotype (OR: 2,6; CI95%: 1,15 – 5,84) and 1,6 increased risk for AG (OR: 1,61; CI95%: 0,7 –

Table 2. Histological types of cancer in the "sarcoidosis and cancer" group.

Histology	Number
Prostate adenocarcinoma	6 (16,6%)
Breast adenocarcinoma	6 (16,6%)
Kidney adenocarcinoma	5 (13,8%)
Colorectal adenocarcinoma	4 (11,1%)
Cervical carcinoma	2 (5,5%)
Testicular seminoma	2 (5,5%)
Lymphoma	2 (5,5%)
Thyroid carcinoma	2 (5,5%)
Stomach adenocarcinoma	1 (2,7%)
Oesophagus adenocarcinoma	1 (2,7%)
Mélanoma	1 (2,7%)
Other skin cancer	1 (2,7%)
Essential thrombocytemia	1 (2,7%)
Non-small cell lung carcinoma	1 (2,7%)
Ostéosarcoma	1 (2,7%)

Table 3. Clinical characteristics of "sarcoidosis and cancer" cases.

Sex	18 women/17 men Sex ratio W/M: 1,05
Ethnicity	white 23/26 (88,4%) black 1/26 (3,8%) carribean 1/26 (3,8%) asia 1/26 (3,8%)
Smoking habits	non smocker: 19/33 (57,5%) smoking (active or weaned): 14/33 (42,4%)
Familial history of sarcoidosis	6/35 (17,1%)
Median age at sarcoidosis diagnosis	51 ± 15,8 ans (24 – 79 ans)
Pulmonary stage	I: 14/34 (41,1%) II: 12/34 (35,2%) III: 7/34 (20%) IV: 1/34 (2,9%)
Respiratory function	FEV (L): 2,39 ± 0,89 (0,99 – 4,83) FEV (%): 80,6 ± 20,7 VC (L): 3,20 ± 1,0 (1,87 – 5,40) VC (%): 90,5 ± 19,6 TLC (L): 5,22 ± 1,46 (3,20 – 7,38) TLC (%): 90,3 ± 16,6 DLCO (%Th): 70,4 ± 22
ACE	median rate 53 UI ± 53,3 (17,8 – 280)
CD4/CD8 ratio	median rate: 2,73 (0,7 – 11,6) > 2,5: 8/14 (57,1%)
Lymphocytes	median rate 1220/mm3 ± 1062 (410 – 6140) < 1000/mm3: 20/31 (64,5%)

Table 4. Genotypic distribution of rs2076530SNP of BTNL2 gene in the different groups.

Genotype	Sarcoidosis + cancer	Sarcoidosis without cancer	Cancer matched controls	controls
AA	20	151	13	78
	57.1%	44.4%	40.6%	28.7%
AG	13	157	15	139
	37.1%	46.2%	46.8%	51.2%
GG	2	32	4	54
	5.7%	9.4%	12.5%	19.9%
Total	35	340	32	271

Table 5. Odds ratio in the different comparisons.

comparison	genotype	OR	CI 95%	p
Sarcoidosis vs control	AA AG	3,22 1,79	2,55 – 4,06 1,42 – 2,26	< 0,0001
"Sarcoidosis + cancer" vs control vs control	AA AG	7,21 2,68	4,00 – 12,99 1,49 – 4,83	0,001
"Sarcoidosis + cancer" vs sarcoidosis without cancer	AA AG	2,34 1,53	1,30 – 4,20 0,85 – 2,74	0,15
"sarcoidosis + cancer" vs cancer matched control	AA AG	2,60 1,61	1,15 – 5,84 0,71 – 3,62	0,25

3,62). But the association between the variant and sarcoidosis in patients with cancer is not statistically significant (p= 0, 25).

Discussion

Links between sarcoidosis and cancer described for the first time by Brincker and Wilbeck in 1974 (4), are still debated. Many epidemiological studies are conflicting. The Swedish study of Ji and al. found a 1,4 relative risk of cancer in a cohort of 10 037 sarcoidosis hospitalized between 1964 and 2004 (5). Immunological disorders present in sarcoidosis as blood lymphopenia, LTCD4+ dysfunction, LT regulator expansion (6), may explain an increased risk of cancer in these disease. In sarcoidosis the LTCD4+ dysfunction seems result from genetic defects of HLA system (7) (8) and costimulation molecule such as BTNL2. A recent work of Swanson and coll. show that BTNL2 promote expression of Foxp3 transcription factir and regulatory T cell allowing control of T CD4+ lymphocyte activation (9). Many studies show an association between sarcoidosis and the rs2076530 SNP variant of BTNL2 gene (2) (10) (11). This variant, characterized by G>A mutation, gives a truncated protein may alter the LTCD4+ activation. Adaptative immunity play a central role against tumor cells (12), so we study the links between the rs2076530 SNP and the risk of cancer in sarcoidosis. Amoung a multicentric French sporadic and familial sarcoidosis we found 35 cases of "sarcoidosis and cancer". We were not able to estimate the relative risk of cancer in our cohort because of a lack of data on the duration of sarcoidosis. The clinical characteristics of sarcoidosis in our cohort are usual only age of diagnosis. The patients with sarcoidosis and cancer are diagnosed on average at age 51, significantly higher than usually observed in the disease. Previous authors had already made this observation (13) (14). Histological types of cancer are various with a majority of breast and prostatic carcinoma, wich are the most common cancers in the women and men in France. We found a lot of renal malignancy (13,8%) and two testis seminoma. Rayson previously found a 100-fold increased prevalence of testicular carcinoma and sarcoidosis association (15). In contrast we found very few lung cancer (1/36) in patients who all had thoracic scanner, and smoking in 40%. According to the literature, it seems that some types of cancer are more common as lymphoma and testis seminoma (16) (17), and

perhaps others less frequent (18). Only one cancer occurred in a patient who received azathioprine. So there is no cancer-induced by immunosuppressive treatment in our series.

Our study has some limitation. First, we have not excluded cancers diagnosed before sarcoidosis (36% of cases). Thus, sarcoid-like reactions could be confused with systemic sarcoidosis, especially in one patient with sarcoidosis spontaneously resolved without specific treatment after treatment of her cancer. Although sarcoidosis was histologically proven in this patient, it is probably a sarcoid-like reaction. Sarcoid-like reactions correspond to granulomatous reaction to tumor antigen in lymph node or remote from tumor site. These reactions have been described in many cancer, frequent with lymphoma, in general resolutive with cancer treatment (19). Secondly controls were not matched on ethnicity witch can influence the genetic. However about 90% are Caucasian in the different groups. Ethnicity is very similar between "sarcoidosis + cancer" cases and "cancer matched controls" with respectively 93,7% and 91% caucasian, no asian patient.

The aim of our work was to study the association between BTNL2 polymorphism and cancer in sarcoidosis. More than 90% of "sarcoidosis and cancer" carried a mutated A allele: 57,1% are homozygous AA, and 37,1% are heterozygous AG. We didn't found signifiant association between the variant rs2076530 of BTNL2 gene and the risk of cancer in sarcoidosis: the risk increases by 2.3 with AA genotype, 1.5 with AG but not statitically signifiant (p=0.15). Samples are small and our study certainly lacks of power. No previous studies have examined this association, making the originality of our works, so we have no comparison possible. We confirmed the association between sarcoidosis and BTNL2 polymorphism, rs2076530, with a 3 time increased risk for the AA genotype and 1,8 time increased for the heterozygotes AG. Our results are slightly higher than the litterature (2) (10) perhaps because of large number of familial sarcoidosis. The AA genotype increases 7 time more the risk for "sarcoidosis + cancer". The variant rs2076530SNP is not associated with risk of sarcoidosis among patients with cancer. Indeed the comparison on the two cancer matched groups shows a 2,6 risk of sarcoidosis for AA genotype and 1,6 for AG genotype, but not statistically signifiant. We noticed that the genotypic distribution in the group control "cancer without sarcoidosis" is close to sarcoidosis with 40,6% of homozygous mutant AA.

We didn't found statistically signifiant association between the variant rs2076530 of BTNL2 gene and the risk of cancer in sarcoidosis. However, the risk of cancer in these patients is twofold higher with the AA genotype. BTNL2 could be involved in cancer development by interfering with LTCD4+ antitumor activity. Recents works show that BTN3, a member of butyrophilin family, occurs in the regulation of anti tumor immunity (20). Further studies are needed to clarify the exact role of BNTL2 in humans and perhaps in anti tumor immunity.

Conclusion

Our study is the first to examine the association BTNL2 rs2076530SNP with cancer risk in sarcoidosis patient. We don't found an association between the BTNL2 variant and the risk of cancer in sarcoidosis but have confirmed the link with sarcoid disease. BTNL2 rs2076530SNP is not associated with risk of sarcoidosis in patient with cancer. Perhaps this variant occurs with risk of cancer in healthy subjects. Further studies are needed to clarify the BTNL2 exact role in humans.

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