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Transforming Growth Factor β Gene Polymorphisms in Different Phenotypes of Sarcoidosis

Stefan Pabst[1], Teresa Fränken[1], Janina Schönau[1], Sebastian Stier[2], Georg Nickenig[1], Rainer Meyer[3], Dirk Skowasch[1], Christian Grohé[4]

[1] Department of Internal Medicine II, Division of Pneumology, University of Bonn, Germany
[2] Medizinische Poliklinik, University of Bonn, Germany
[3] Institute of Physiology II, University of Bonn, Germany
[4] Evangelische Lungenklinik Berlin Buch, Germany

Corresponding author:
Dr. med. Stefan Pabst
Department of Internal Medicine II
Division of Pneumology
University of Bonn
Sigmund-Freud-Str. 25
D-53105 Bonn
Germany
Phone: +49 (0)228 287 15259
Fax: +49 (0)228 287 19017
stefan.pabst@ukb.uni-bonn.de

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Abstract:

The aetiology of sarcoidosis is unclear. Single nucleotide polymorphisms (SNPs) in transforming growth factor beta (TGFβ)-2 and -3 have been reported to be associated with the development of lung fibrosis in patients with sarcoidosis.

SNPs in TGFβ-2 (rs1891467) and TGFβ-3 (rs3917200) were investigated in 296 patients with sarcoidosis (acute/self remitting=70 (including 62 patients with Löfgren´s syndrome), chronic n=168, acute/chronic=58) by real-time-polymerase chain reaction. 32 patients showed radiological signs of lung fibrosis. The genotype frequencies were compared among the sarcoidosis groups as well as to 377 healthy controls.

We found a significant association with the G-allele in rs1891467 in TGFβ-2 and an acute/self remitting course of sarcoidosis compared to a chronic course ($p=0.001$). The results were even more evident for patients with Löfgren´s syndrome ($p<0.001$). Moreover, we could demonstrate a borderline significance between TGFβ-3 (rs3917200) and lung fibrosis ($p=0.050$).

Carriers of the G-allele in rs1891467 might be protected of developing a chronic course. Moreover, there is evidence that rs3917200 is involved in the development of lung fibrosis in sarcoidosis. This study is the first in sarcoidosis patients to suggest a genetic implication of TGFβ-2 as a protective factor in the course of sarcoidosis.

Introduction:

Sarcoidosis is an inflammatory granulomatous multisystem disorder, in > 90% primarily affecting lungs and lymph nodes. Other organs, including skin, heart and liver may also be afflicted. The disease is characterized by noncaseating granulomas and an exaggerated cellular immune response caused by increased inflammatory activity of macrophages [1]. Chest X-rays are used to describe the different stages of pulmonary sarcoidosis. The clinical course of most sarcoidosis patients is short and favourable, but about 20% develop a complications such as lung fibrosis, requiring a long-term treatment with corticosteroids [2,3]. Although, considerable efforts towards understanding the pathogenesis of sarcoidosis have been made, the aetiology remains unclear. Evidence suggests that it is the product of an unknown exogenous antigenic stimulus and an endogenous genetic susceptibility [3]. A recently published registry-based twin study showed an 80-fold increased risk of developing sarcoidosis in co-twins of affected monozygotic brothers or sisters compared with the general population [4]. The increased risk in dizygotic twins was 7-fold. This suggests that the genetic impact in the aetiology of sarcoidosis is important. Moreover, there is evidence that distinct genes have the possibility to alter the course of the disease. Kruit and colleagues could show that single nucleotide polymorphisms (SNPs) in the transforming-growth factor BETA (TGFβ) gene isoforms are associated with the development of lung fibrosis in patients with pulmonary sarcoidosis [5]. TGFβ isoforms regulate components of the adaptive immune system, such as T-cells, and of the innate immune response, such as natural killer cells [6,7]. As there is evidence that SNPs in TGFβ2- and 3 are associated in the development of lung fibrosis in patients with sarcoidosis we tried to analyse these results in a large Caucasian cohort of 296 German sarcoidosis patients with a mean follow-up time of 6.9 years. We hypothesized that SNPs in TGFβ isoforms could be involved in the conversion to a chronic disease.

Material and Methods

Patient population:

This study was in conformity with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the Ethics Committee of Bonn University School of Medicine (No. 080/05). Written informed consent was obtained from each patient and of all healthy controls prior to their enrolment.

Patients with severe medical disorders including chronic obstructive lung disease, allergic asthma or immunological disorders were excluded from the study. All patients were at least 18 years of age.

Sarcoidosis group:

Two hundred ninety six Caucasian patients with diagnosed sarcoidosis (age: 53.0 ± 12.9 years, 168 female (56.8%), 128 (43.2%) male) were included in the present study. They were recruited from the outpatient clinic of the Department of Internal Medicine, Rheinische-Friedrich-Wilhelms University, Bonn, Germany and from sarcoidosis peer-support groups in Wuppertal, Mainz and Cologne, Germany.

Sarcoidosis was confirmed by biopsy evidence of noncaseating epitheloid cell granulomas in any organ and chest X-ray (posterior-anterior and lateral) abnormalities. Chest radiographs were assessed in consensus by chest physicians and radiologists specialized in diffuse lung diseases to determine disease severity using standard radiographic staging for sarcoidosis, in accordance with ATS/ERS/WASOG-Guidelines and the Scadding criteria: Stage I characterizes bilateral hilar lymphadenopathy, stage II additional parenchymal infiltrates, in stage III merely pulmonary infiltrates can be seen. Stage 0 describes a normal chest radiograph (including extrapulmonary manifestation), stage IV lung fibrosis [3,8].

The diagnosis was further completed by history data, physical examination, lung function tests, chest computed tomography (81 Patients), and bronchoalveolar lavage (BAL) fluid analysis.

For the statistical analysis, patients with chronic course were defined as *either* having symptoms over a time span of two years or more *or* had a minimum of two episodes in a lifetime as described before [9-11]. Acute sarcoidosis was defined as only one episode of a clinically acute sarcoidosis, which has totally resolved and has not relapsed in the follow-up time. Therefore all patients with only one episode of Löfgren´s syndrome and all patients with an otherwise acute form of sarcoidosis were included in this group, whereas patients with two or more episodes of Löfgren´s syndrome or a chronic persistent or a relapsing course were grouped into the ´chronic group´. The mean follow-up time was 6.9 years in all sarcoidosis patients (4.2 years in the ´acute group´). Löfgren´s syndrome was defined according to the ATS/ERS/WASOG-Guidelines [3]. 70 patients presented with an acute course, 62 of these suffered from Löfgren´s syndrome. The remaining eight patients of the ´acute group´ did not show classical Löfgren´s syndrome and had primarily skin and eye involvement. Lung fibrosis was diagnosed by chest x-ray according to the Scadding criteria as described before (stable stage IV or progressive toward this stage) [5,8]. Accordingly, the non-fibrosis group comprises all patients with radiological stage 0,I and II as well as two patients of stage III which presented with no signs of fibrosis in the most recent chest x-ray. The baseline characteristics of the sarcoidosis groups are shown in table 1.

Control group:

Three hundred seventy-seven Caucasian, healthy and unrelated volunteers who were age and gender matched served as the control cohort (age 53.2 ± 17.6 years, 202 (53.6%) female, 175 (46.4%) male). All were residents of Germany and were selected in pre-employment examinations at the University of Bonn, Germany. None had a history of lung disease or

showed any symptoms of lung or other disease. All showed a normal chest X-ray and laboratory results, including complete blood count, urine analysis, hepatic enzyme activities and BUN levels.

Methods:

Peripheral venous blood samples of 9 ml were drawn from each individual by standard venous puncture. DNA was purified from EDTA blood using the salting out protocol described by Miller and colleagues [12].

SNP Selection and Genotyping:

SNPs rs1891467 (TGFβ2) and rs3917200 (TGFβ-3) were selected as both were reported to be associated with lung fibrosis in sarcoidosis [5]. The SNPs, alleles and structures of PCR primers and fluorescence labeled detection probes for the variant TGFβ-2 and -3 alleles are given in table 2. For genotyping the TGFβ2 and -3 polymorphisms, real-time PCR analysis was used (Light-CyclerTM, Roche, Mannheim, Germany), hybridization probes were applied in combination with a Light-CyclerTM DNA Master Hybridization Probes Kit (Roche, Mannheim, Germany). Both, the PCR primers and the fluorescence labeled detection probes were synthesized by TIB MOLBIOL, Berlin, Germany.

For PCR, conditions were 3mM $MgCl_2$, 1 pmol of each hybridization probe, 20 pmol of the two PCR primers each, 2 µl of Light-CyclerTM DNA Master Hybridization Mix (Roche, Mannheim, Germany) and 100 pg to 10 ng DNA in a final volume of 20 µl. After 5 min of denaturation (at 95°C), 40 PCR cycles (TGFβ3) and 45 PCR cycles (TGFβ-2) were performed

with 3 s denaturation at 95°C, 20 s annealing at 58°C (TGFβ-3) and 54C (TGFβ-2) and 25 s extension at 72°C. Differentiation of the TGFβ-2 and -3 alleles was performed by determination of melting curves after PCR. Melting curves were obtained following a denaturation period of 5 s at 95°C at a start temperature of 45°C and a final temperature of 80°C, with a temperature gradient of 0.4°C/s. PCR and melting procedure were detected online with the Light-CyclerTM instrument. As an internal control, in 10 randomly chosen assays sequencing on an automated DNA sequencer was performed (Qiagen), which was compared to the results by the Light-CyclerTM technique. For each sample, melting curves were produced, showing a temperature-dependent decrease in fluorescence intensity (Fig. 1 and 2). The melting curve analysis shows different melting maxima (−dF/dT) for the hybridization probes, depending on the genotype. Concerning TGFβ-2, there was a single melting maximum of 58.0°C in the case of the wild-type and two melting maxima of 56.1° and 64.2°C in heterozygous DNA. Homozygous variant genotypes showed a melting point of 56.1°C. For TGFβ-3, a single melting maximum of 67.0°C was found in the case of the variant genotype and of 58.0°C in the case of reference genotype. In heterozygotes, the two melting maxima were located at 58.0° and 67.0°C, as shown in Fig. 1 and 2.

Thus, the differentiation of genotypes was possible by melting curve analysis. For internal control, 10 randomly selected samples were sequenced and showed 100% concordance with the Light-CyclerTM data.

Statistical Analysis

Descriptive statistical analysis was performed using software SPSS (version 14.0, Chicago, IL, USA). Single marker association was evaluated with Pearson´s chi-square test [13]. Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE). Statistical power was calculated with an on-line tool (case control for discrete traits test), available at

http://pngu.mgh.harvard.edu/~purcell/gpc/. Statistical significance was denoted at $p < 0.05$ for all tests performed.

Results

To determine whether SNPs in the TGFβ-2 and -3 genes influence the susceptibility or the phenotype of sarcoidosis, we performed an association analysis of the markers in our control group against all sarcoidosis, acute course sarcoidosis, chronic course sarcoidosis, fibrosis and non-fibrosis sarcoidosis groups. The results are presented in table 3. The distributions of allele carrier and genotype frequencies of TGFβ-2 and TGF-β3 in the sarcoidosis patients and healthy control subjects did not deviate from the Hardy-Weinberg equilibrium.

We compared patients with an acute course towards a chronic course of sarcoidosis and each group towards the control group. In TGFβ-2 we detected much less mutated G-allele in the cohort with a chronic course of sarcoidosis. We found a significant association of TGFβ-2 rs1891467 with p=0.001 (chronic vs. acute) respectively p = 0.030 (chronic vs. controls). For the Löfgren´s cohort, we could show the same results (Löfgren´s vs. chronic, p<0.001). For TGFβ-3 we could not demonstrate significant results.

In a second calculation we compared 32 fibrotic with 200 non-fibrotic patients. 64 patients of the total sarcoidosis cohort had to be excluded as we could not obtain the current radiological stage. In TGFβ-3, we observed more patients with the mutated C-allele in patients with pulmonary fibrosis with a p-value of 0.050. In TGFβ-2, patients with fibrosis showed less mutated G-allele than patients without fibrosis with a clear statistical trend in Pearson´s chi-square test (p=0.091).

Discussion

We examined two SNPs in the TGFβ-2 and TGFβ-3 gene for the association with the course of sarcoidosis and with the development of lung fibrosis. The G-allele in rs1891467 seems to protect of developing a chronic course. Therefore to the best of our knowledge this study is the first in sarcoidosis patients to suggest a genetic implication of TGFβ-2 as a potential protective factor in the course of sarcoidosis. Moreover, for both SNPs we could observe a clear, for TGFβ-3 even a borderline significant, trend towards the formation of lung fibrosis.

There is growing evidence that only genetically susceptible individuals will suffer from sarcoidosis. As the postulated exogenous stimulus leading to a sarcoidosis is still not found yet, a lot of effort has been undertaken in the last decade to find *the* sarcoidosis gene or a pattern of susceptibility genes that may lead to sarcoidosis. Furthermore the finding of disease modifying genes is of growing importance. These genes could be either protective - terminating the disease spontaneously like Löfgren´s syndrome - or may alter the disease towards a chronic course with multiple organ involvement such as lung fibrosis.

Therefore in this study we wanted to answer the question if SNPs in TGFβ-2 (rs1891467) and -3 (rs3917200) may alter the course of sarcoidosis or might have a protective or harming influence towards the development of pulmonary fibrosis in patients with sarcoidosis. Both examined SNPs were chosen as there is a recent publication from Kruit et al [5], where these SNPs were associated with a higher rate of pulmonary fibrosis in a Dutch cohort of patients with sarcoidosis.

The main finding of our study is the diminished amount of mutated G allele of TGFβ-2 59941 A/G in patients with a chronic course of sarcoidosis. Besides the fact that in the chronic group we found less wildtype A-allele, the main reason for the significant finding obviously is the lower number of patients with a homozygous G-mutation. Comparing the chronic to the fibrosis group, interestingly in the chronic group we observed more patients with homozygous mutated alleles. Actually we would have expected that fibrotic patients more frequently tend

to a chronic course and - vice versa – chronic patients will develop more often a pulmonary fibrosis. The most probable reason for this discrepancy is that ´chronic course´ and ´fibrosis´ are two distinct phenotypes which might overlap in only some rare cases. Probably there is more than one genetic factor at play.

As mentioned above we were not able to replicate the results of the Dutch cohort in detail. Moreover, in TGFβ-2 we detected less mutated alleles in the fibrosis group whereas Kruit and colleagues found significantly more mutations in the fibrosis group. Our TGFβ-3 results and the results of the Dutch cohort were pointing into the same direction, with a borderline statistical significance in our cohort. These partially different results might be explainable due to the samples sizes of the fibrosis groups with 32 patients in our study and 24 in the Dutch study. The fibrosis patients of both studies were assigned to the fibrosis cohort based on the radiological findings and not the combination of lung function and radiological observations. We conclude that "lung fibrosis" sarcoidosis patients consist of a heterogenous gruoup of patients with a variable course determined by both genetic and clinical parameters. The genotype distribution in the control groups of both studies is quite similar for TGFβ-3 17369 T/C. But there are evident differences in the genotype distribution of TGFβ-2 59941 A/G. Therefore, it might be possible to reach different results with an enlarged subcohort of fibrosis patients. We have to admit that in both studies, the Dutch and ours, the analysis of the small fibrosis groups are probably underpowered.

However, although functional data is still missing, we think that the TGFβ family is a promising target in the genetics of pulmonary fibrosis in sarcoidosis. The crosstalk between TGFβ isoforms is not fully understood. The three isoforms TGFβ-1, TGFβ-2 and TGFβ-3 have been associated with different functions [14]. Whereas the isoforms 1 and 2 of TGFβ family have been described generally with profibrotic properties, TGFβ-3 appears to have a more diverse nature [15,16]. A recent study showed that TGFβ-3 might only have modulating effects on the expression of TGFβ-1 [17]. A previously published study could demonstrate

that all three isoforms have the capability to force the synthesis of collagen by fibroblast stimulation and thus contribute to fibrosis. The different isoforms varied considerably in their potency. TGFβ-3 was ten-fold potent than the other two isoforms [18]. In another study of the same group TGFβ-3 gene expression also was detected in spontaneous human pulmonary fibrosis [19]. Here, however TGFβ-1 gene expression was highest. In a recent study profibrotic effects in rat lungs could be detected after exogenous addition of TGFβ-3. Nevertheless, also in this case the induced profibrotic effects of TGFβ-1 predominated [20].

Concerning sarcoidosis, a study by Zissel et al. found increased TGFβ-1 levels associated with a spontaneous remission [21]. A recent study of the ACCESS group, exploring sarcoidosis susceptibility genes, could demonstrate associations of functional TGFβ-1 haplotypes and a chronic course of sarcoidosis [22]. It is not clear which functional effects are caused by the examined SNPs in our study. Therefore in future studies the function of the TGFβ isoforms, especially TGFβ-2 and -3, and the functional influence of the SNPs lying in these genes are still to be explored.

The exact phenotyping makes or breaks the performance of genetic association studies in complex diseases, especially when performing analyses of subcohorts. In this study the definition of lung fibrosis is difficult. It is usual to classify sarcoidosis patients according to the conventionally radiological classification of Scadding [8]. But nowadays, computed tomography (CT) often is performed in addition to chest-x-ray. Due to the enhanced resolution of CT-scans patients with sarcoidosis often are classified in a radiological stage III or IV, even if no changes in conventional chest-x-ray are evident. We tried to rule this out by regarding only conventional chest-x-ray results, but a bias by knowing CT-scan results might be present. Even though the fibrosis group presents with a lower transfers factor for carbon monoxide (TLCO) in lung function testing not all patients with radiological diagnosed lung fibroses show a functional impairment in TLCO. Interestingly, TLCO in this study is much

higher than in the Dutch study (83 vs. 63%). However, these differences are very hard to interpret as in our study TLCO data was available in 31 of 32 patients whereas in the Dutch study the calculation was based on only 7 of 23 patients with fibrosis. This makes a direct to comparison of both studies difficult.

Furthermore phenotyping in acute and chronic course sarcoidosis is not specific, but has proven to be a useful descriptive tool. This has been demonstrated for BTNL-2 (butyrophilin-like 2), which until now is the gene best associated with sarcoidosis and preferentially with a chronic course [10,23]. The division into the two subgroups ´acute´ and ´chronic´ as we have defined it might have been arbitrary. Of course there are many other possibilities to phenotype sarcoidosis patients. In even larger cohorts than in ours different phenotyping tools might be eligible. At the present moment we would propose for large cohorts the SCAC (sarcoid clinical activity classification) -phenotyping as recently defined by Prasse and colleagues [24]. This classification also makes a cut after two years to define a chronic course. Therefore we think our subclasses are reasonable chosen, and subdivision in the six SCAC-classes would result in too small subcohorts for ours study. Our sample size reached 296 patients, although sarcoidosis is a rare disease; so this study should have enough statistical power to generate valid results. Compared to other genetic studies in sarcoidosis, 296 well phenotyped patients equal or even exceed the number of subjects in previously published studies [25-27].

In summary, we examined two SNPs, one in the TGFβ-2 and on in the TGFβ-3 gene, for the association with the course of sarcoidosis and with the development of lung fibrosis. Carriers of the G-allele in rs1891467 in TGFβ-2 seem to be protected of developing a chronic course. This study is the first in sarcoidosis patients to suggest a genetic implication of TGFβ-2 as a protective factor in the course of sarcoidosis. Moreover, in both SNPs we could observe a clear and for TGFβ-3 nearly significant trend towards the formation of lung fibrosis.

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Table 1: Baseline characteristics of sarcoidosis patients and healthy controls

Characteristics		**Sarcoidosis n = 296**	**Sarcoidosis acute n=70**	**Löfgren´s syndrome n=62[#]**	**Sarcoidosis chronic n=168**	**Sarcoidosis and lung fibrosis n = 32[#]**	**Controls n = 377**
age		53.0 ± 12.9	49.7 ± 13.0	49.9 ± 13.1	53.8 ± 12.5	56.7 ± 8.8	53.2 ± 17.6
female/male		168 (56.8%) / 128 (43.2%)	39 (55.7%) / 31 (44.3%)	35 (56.5%) / 27 (43.5%)	87 (51.8%) / 81 (48.2%)	14 (43.8%) / 18 (56.3%)	202 (53.6%) / 175 (56.4%)
acute course		70	70	62	0	0	
Löfgren´s syndrome		62	62	62	0	0	
chronic course		168	0	0	168	32	
lung fibrosis		32	0	0	32	32	
course acute/chronic		58*	0	0	0	0	
age at first diagnosis		41.0 ± 12.3	40.2 ± 11.8	40.6 ± 12.1	41.3 ± 12.5	40.8 ± 9.1	
stages at the end of follow-up period							
stage 0		80 (27.02%)	61	54	19	0	
stage I		53 (17.91%)	4	4	49	0	
stage II		65 (21.96%)	4	3	61	0	
stage III		21 (7.10%)	0	0	21	19 (59.37%)	
stage IV		13 (4.39%)	0	0	13	13 (40.63%)	
stage unclear		64 (21.62%)	1	1	4	0	
FEV_1 (l/s)		2.89 ± 0.91	3.29 ± 0.87	3.26 ± 0.88	2.76 ± 0.91	2.55 ± 0.97	2,52 ± 0,94
FEV_1 (% of predicted)		90.70 ± 19.89 (n=268)	98.84 ± 15.66 (n=65)	98.52 ± 15.13 (n=59)	88.06 ± 21.12 (n=146)	81,27 ± 25,40 (n=31)	88,41 ± 25,27 (n=377)
DLco (% of predicted)[§]	$TL_{CO}c$ SB[†]	87.88 ± 16.53 (n=201)	95.33 ± 12.81 (n=54)	94.93 ± 12.94 (n=52)	86.98 ± 17.09 (n=107)	82.99 ± 19.16 (n=31)	n/d
	$TL_{CO}c$ VA[‡]	97.76 ± 15.54	102.43 ± 10.53	101.19 ± 10.26	96.69 ± 16.52	93.22 ± 18.58	n/d

* Mean observation time: 6.9 years, in 51 patients the course is either acute or chronic because the observation time was less than two years, 7 patients were lost in follow-up.
[#] All 62 patients with Löfgren´s syndrome are part of the acute group, 32 patients with lung fibrosis are part of the chronic group.
[§] Transfers factor for carbon monoxide; data was available in 68.0% of all patients, 96.9% of patients with fibrosis, and was not performed in healthy controls
[†] SB = single breath technique. Corrected for haemoglobin.
[‡] Krogh-factor. VA = alveolar volume. Corrected for haemoglobin.
n/d: not done
FEV_1: forced expiratory volume in one second

Table 2: Primer Sequences for the Identification of Biallelic SNPs in the TGFβ-2 and TGFβ-3 Genes

Polymorphism	dbSNP	DNA sequence	primers	sensor / anchor
TGF-β_2 59941 A/G	rs1891467	5' T TGT AAA CAG GCA ACT TAA ATA CAT CCT GAT GCC ATA TGA ATA GTG GTA CTT GCA TAT AGG GTA TAG GCG GGG AAA TTT CAC CAG GGA *GCT GAC ATT TTG **A**TG AGG CCT TGA GAC GTA TCT ATT AAA ACC TGA TGG GGG* ATC ATC ATT CTT GGCA GGA AGG GCA GGC ACT GCA AAG ACA GTC TTG AAT GGG CTT GCT GAG GGT ACC TGA TGC ATA GCG CTC AGT GCC TGG AGG TGA GGA GAG ACT GGG GAG AAG GTG GCC CTC CAA AGA TAG TGT GTA GAG TGA CAC TAC AGA GGA TT '3	forward primer: 5'GTA AAC AGG CAA CTT AAA TAC A '3 reverse primer: 5'CAC TCT ACA CAC TAT CTT TGG A '3	Sensor A : 5'- GCT GAC ATT TTG ATG AGG CC--FL'3 Anchor: 5'-LC640-TGA GAC GTA TCT ATT AAA ACC TGA TGG GGG--PH -'3
TGF-β_3 17369 T/C	rs3917200	5' CA CAC CTC CCT CGC AGA CTG CAC TGC CCC TCC TCC TGG GCA GTG ATG GGG CGT GTG GAG GAG GCA CCC TCC AAG G*GC TCT GCT CTC **T**TC AGA CAG GAG ATT GTC ACT TTC CTT CCC TTC TTC AGG C*GT GGA CAA TGA GGA TGA CCA TGG CCG TGG A '3	forward primer: 5' CAC CTC CCT CGC AGA CT '3 reverse primer: 5' CAT GGT CAT CCT CAT TGT CC '3	Sensor: 5' - GCT CTG CTC TCC TCA GAC AG--FL'3 Anchor: 5' - LC640- GAT TGT CAC TTT CCT TCC CTT CTT CAG GC--PH

The base exchanges are printed in bold and enlarged. The sequence for the Light-Cycler sensor is shown in italics. The sequence for anchor-TGF-$\beta_{2/3}$ is printed in italics and underlined. The insertion for the primers are underlined.

Table 3: Statistical Analysis of Variants at the TGFB-2 and -3 loci

Gene/SNP	Sample	Genotypes absolute (%)			P (Pearson´s)			
					vs. Control	vs. Acute	vs. Löfgren	vs. Fibrosis
TGFβ-2 59941 A/G rs1891467	SAR All n=296	AA = 204 (68.9%)	AG = 79 (26.7%)	GG = 13 (4.4%)	0.556			
	SAR Acute n=70	AA = 42 (60.0%)	AG = 20 (28.6%)	GG = 8 (11.4%)	0.173			
	SAR Löfgren´s n=62	AA = 36 (58.1%)	AG = 18 (29.0%)	GG= 8 (12.9%)	0.083			
	SAR Chronic n=168	AA = 117 (69.6%)	AG = 49 (27.4%)	GG = 2 (1.2%)	**0.030**	**0.001**	**<0.001**	
	SAR Fibrosis n =32	AA = 27 (84.4%)	AG = 4 (12.5%)	GG = 1 (3.1%)	0.197			
	SAR Non-Fibrosis n=200	AA = 130 (65.0%)	AG = 60 (30.0%)	GG = 10 (5.0%)				0.091
	Controls n=377	AA = 261 (69.2%)	AG = 93 (24.7%)	GG = 23 (6.1%)				
TGFβ-3 17369 T/C rs3917200	SAR All n=296	TT = 252 (85.1%)	TC = 43 (14.5%)	CC = 1 (0.4%)	0.964			
	SAR Acute n=70	TT = 57 (81.4%)	TC = 12 (17.2%)	CC = 1 (1.4%)	0.370			
	SAR Löfgren´s n=62	TT = 50 (80.7%)	TC = 11 (17.7%)	CC = 1 (1.6%)	0.385			
	SAR Chronic n=168	TT = 142 (84.5%)	TC = 26 (15.5%)	CC = 0 (0.0%)	0.801	0.280	0.238	
	SAR Fibrosis n =32	TT = 23 (71.9%)	TC = 9 (28.1%)	CC = 0 (0.0%)	0.153			
	SAR Non-Fibrosis n=200	TT= 175 (87.5%)	TC = 24 (12.0%)	CC = 1 (0.5%)				0.050
	Controls n=377	TT = 319 (84.6%)	TC = 57 (15.1%)	CC = 1 (0.3%)				

Significant associations are printed in bold
SAR = sarcoidosis, all = all patients

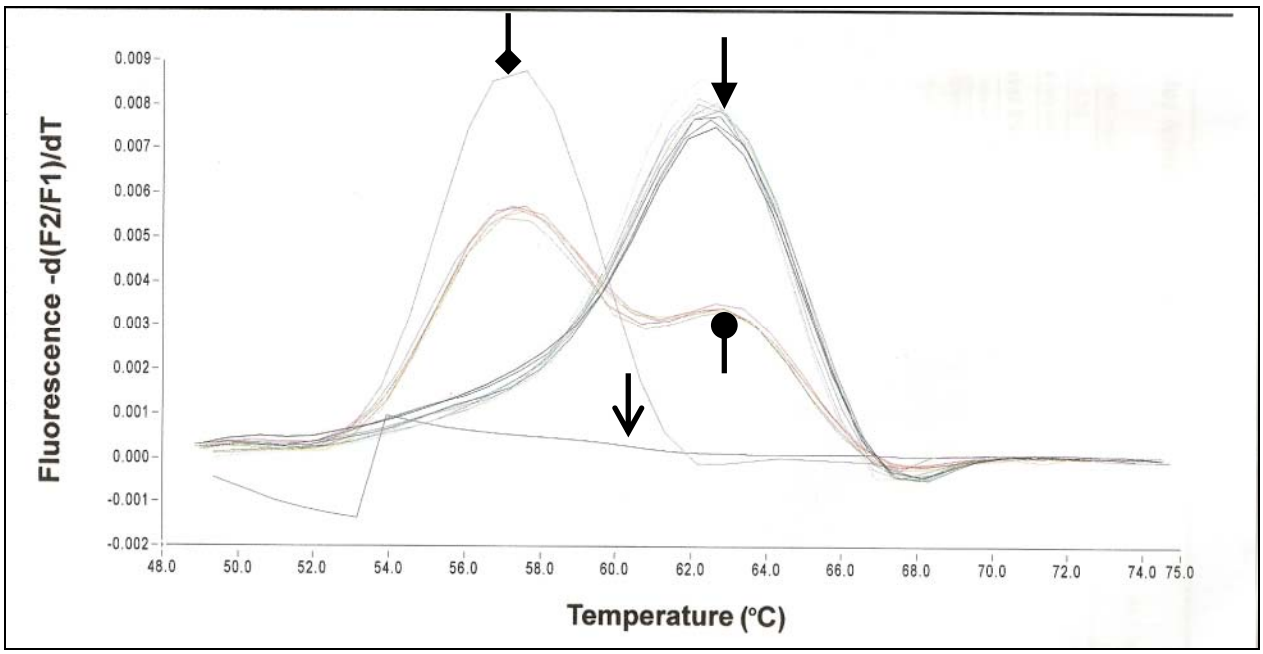

Fig 1: Melting curve of **TGF-β2** 59941 A/G (rs1891467).

homozygous A/A (wildtype), homozygous G/G, heterozygous A/G, control

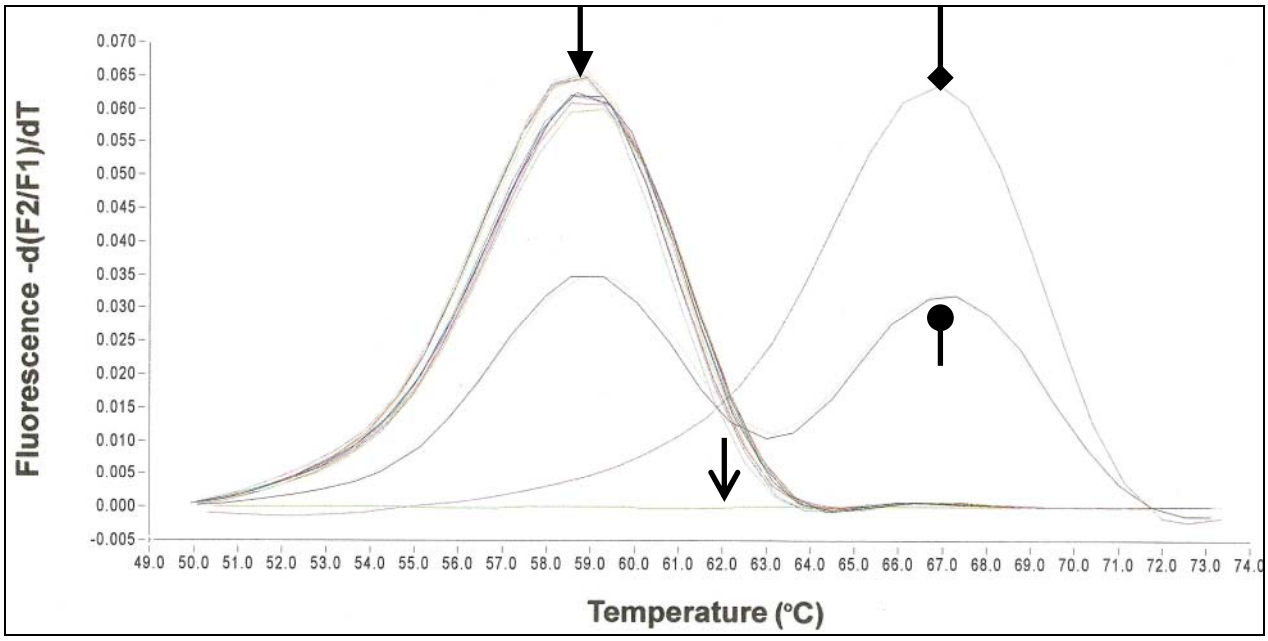

Fig 2: Melting curve of **TGF-β3** 17369 T/C (rs3917200).

homozygous T/T (wildtype), homozygous C/C, heterozygous T/C, control