Screening for Arthrofibrosis After Anterior Cruciate Ligament Reconstruction: Analysis of Association With Human Leukocyte Antigen

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Purpose: Arthrofibrosis represents a severe complication of trauma and reconstructive joint surgery because of generalized connective tissue proliferation resulting in painful joint stiffness. It often appears stereotypical in terms of its clinical and pathologic features, comprising excess deposition of extracellular matrix proteins such as collagen type I, III, and VI and proliferation of fibroblasts. However, trauma and surgery around joints does not always lead to fibrosis, suggesting a genetic predisposition. For a number of autoimmune diseases, strong associations have been described. The objective of the study was to investigate whether an association of HLA (human leukocyte antigen) with primary arthrofibrosis exists. **Type of Study:** Retrospective cohort study. **Methods:** Seventeen patients with primary arthrofibrosis after autologous anterior cruciate ligament (ACL) reconstruction were identified and clinically reviewed. Blood samples were taken, and DNA was isolated by column extraction method. DNA samples were typed for the loci HLA-A, -B, -C, -DRB1, and -DQB1. Results were compared with the frequencies of allelic groups as determined for the caucasoid population. **Results:** HLA-Cw*07 was significantly less often found in the patient group than in the general population ($P = .022$). The opposite effect was seen for Cw*08, which was found in 17.6% of the patient group but only in 3.8% of the reference group ($P = .045$). A significant difference was also seen for DQB1*06, because 23.5% of the patients but 48.6% of the reference group possessed an allelic variant of this group ($P = .048$). However, according to the relatively small number of patients, a statistical bias cannot be excluded. **Conclusions:** A possible link may exist between arthrofibrosis and HLA-Cw*07- and DQB1*06-negative as well as Cw*08-positive individuals. Further investigation is necesessary to confirm or vitiate the possible association. **Level of Evidence:** Level IV. **Key Words:** Arthrofibrosis—ACL reconstruction—HLA-association—Genetic predisposition.

Arthrofibrosis, a severe complication after traumatic injuries and reconstructive surgery, represents a challenge to the attending surgeon in terms of early diagnosis and management. It is often associated with injury of the anterior cruciate ligament (ACL). However, causal pathogenesis is unclear. Clinical appereance often comprises painful loss of motion, including extension and flexion.[1] Restriction of both flexion and extension occurs when diffuse scar tissue or fibrous adhesion form within the joint. A variety of factors have been shown to be associated with loss of motion. However, why the knees of certain patients form an exaggerated pathologic fibrous hyperplasia and others do not is not known. Therefore, we consider arthrofibrosis as "primary" when secondary reasons for motion loss, such as surgical complications, prolonged immobilization, or poor rehabilitation, can be excluded.[2]

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0749-8063/04/2005-3531$30.00/0

doi:10.1016/j.arthro.2004.03.003

Arthrofibrosis is the result of increased cellular proliferation and an enhanced synthesis of matrix proteins (collagen I, III, and VI).[3] The rationale might be a chronic inflammatory reaction caused by activated inflammatory cells and cytokines. The inflammatory cells, in turn, are supposed to activate local cells to proliferate and to synthesize extracellular matrix proteins.[4,5]

One important mediator for induction of fibrillary matrix proteins or fibroblast proliferation are the cytokines platelet-derived growth factor (PDGF)-B and transforming growth factor (TGF)-β.[6,7] These factors were identified by Murakami et al.[8] in elevated concentration in Hoffa's fat pad after reconstruction of the ACL.

One physiologic effect of TGF-β is inhibition of macrophages and activation of neutrophil granulocytes. Additionally, TGF-β positively influences collagen synthesis and proliferation of fibroblasts. The influence of TGF-β1 on induction of adhesions or scarring has been proven experimentally by Ghellai et al.[9] and clinically by Tredget et al.[10] Krotzsch-Gomez et al.[11] confirmed the inductive effect of PDGF and TGF-β on fibroblasts.

Some diseases such as pulmonary fibrosis and sclerodermia are also characterized by an unbalanced proliferation of fibroblasts. Researchers have hypothesized that lung fibrosis initially begins with influx of inflammatory cells, followed by a cytokine release resulting in fibroblast proliferation and synthesis of matrix proteins. Genetic influence may manipulate this cascade on the immunologic (cellular) level or modulate collagen metabolism.[12]

Murakami et al.[6] suspected a similar pathomechanism in primary arthrofibrosis. Briggs et al.[13] described a correlation of HLA-DR3 or HLA-DR52 and an increased risk of developing lung fibrosis in patients with sclerodermia. Therefore, evidence exists for an association of primary arthrofibrosis to HLA. HLA association has been described in some diseases with immunologic background.[14]

The HLA complex, located on the short arm of chromosome 6, contains over 200 genes. Many of them play an important role in the adaptive immune system. HLA-associated diseases are caused by an interplay of many different genes and environmental factors, in which HLA genes or combination of alleles (haplotypes) often confer the strongest genetic predisposition.

The objective of this study was to investigate whether the frequency of specific allelic groups in patients with manifest arthrofibrosis is different compared with the general healthy Caucasian population. In case of a positive association, this could ease identification of patients at risk and facilitate preoperative screening. For this study, 17 patients with primary arthrofibrosis were identified, and DNA samples were typed for the loci HLA-A, -B, -Cw, -DRB1, and -DQB1 by sequence-specific–primed polymerase chain reaction (PCR). Results were compared with the frequencies of allelic groups as determined for the general Caucasian German population.

TABLE 1. *Level of Arthrofibrosis According to Shelbourne et al.*[15]

	Extension Deficit	Flexion Deficit	Other Criteria
Type I	$<10°$	No	No
Type II	$>10°$	No	No
Type III	$>10°$	$>25°$	Contract patella
Type IV	$>10°$	$>30°$	Patella baja

METHODS

Patients

From all patients who underwent arthroscopy for primary arthrofibrosis of the knee between 1990 and 1998, 18 patients were selected in alphabetical order for further investigation. Only patients were included who had ACL reconstruction before arthrofibrosis developed. All patients had painful restriction of extension or limitation of both extension and flexion caused by intra-articular scarring and without surgical complications such as intra-articular implants. This was considered primary arthrofibrosis. According to Shelbourne et al.,[15] the level of arthrofibrosis comprised type 1 ($n = 0$), type 2 ($n = 7$), type 3 ($n = 4$), and type 4 ($n = 6$) (Table 1). Secondary reasons for arthrofibrosis, such as immobilization, pyarthros, reflexdystrophia, or thrombosis, were excluded. One patient was excluded from the study because he was of another ethnic group than the control group.

Clinic

Of 17 patients who underwent knee surgery (female:male ratio, 11:8; mean age, 35.6 ± 7.3 years), 14 were treated using autologous patellar tendon transplant and 3 using semitendinosus tendon transplant. In 5 patients, additional meniscal repair was performed (Table 2). Initially, the time interval between ACL rupture and reconstruction was 5.7 ± 11.6 weeks. Before arthrolysis, mean extension deficit was 13.9°

TABLE 2. *Postoperative Protocol and Associated Procedures: Duration of Physiotherapy, Immobilization, and Start of CPM*

Patient	Gender	Duration of Physiotherapy (mo)	Start of Physiotherapy After Surgery (wk)	Start of CPM After Surgery (wk)	Meniscal Repair
1	M	>18	0	2	—
2	F	>18	2	2	—
3	M	12	0	0	Yes
4	M	>18	2	0	—
5	M	>18	0	0	Yes
6	M	18	0	0	Yes
7	F	6	0	0	—
8	M	>18	2	2	—
9	F	18	0	0	Yes
10	M	>18	4	3	—
11	F	>18	0	0	—
12	F	>18	0	0	—
13	F	18	2	1	—
14	M	>18	0	0	—
15	F	>18	0	0	Yes
16	F	18	0	0	—
17	M	>18	6	2	—

NOTE. In 5 patients, additional meniscal repairs were performed.

± 10.1°. Range of motion was 83.8° ± 20.9°. All patients started with early physiotherapy and continuous passive motion (Table 2). Postoperatively, both values improved significantly (extension deficit, 2.6° ± 3.5°; range of motion, 126.5° ± 16.7°; $P < .001$).

HLA Typing

All patients returned to the clinic for follow-up evaluation and consented for blood samples to be taken (10 mL ethylendiamin tetraacetic acid blood) for further investigation. DNA was isolated by column extraction method. The samples were typed for the loci HLA-A, -B, -Cw, -DRB1, and -DQB1 by sequence-specific–primed PCR (PCR-SSP).[16-18]

Statistical Analysis

The HLA frequencies of the patient group were compared with the distribution of HLA-A, -B, -Cw,[19] Bw4/Bw6 (Lenhard, Biotest AG, Dreieich, Germany, personal communication), -DRB1, 3, 4, 5,[20] and -DQB1 allelic groups[21] in the general Caucasian German population. The statistical analysis was performed on the phenotype level using a 2-tailed Fisher exact test.[22]

RESULTS

In the patient group (n = 17 patients with arthrofibrosis after ACL reconstruction), HLA-Cw*07 was found significantly less often than in the control group ($P = .022$).

That is, 4 of 17 patients (23.5%) possessed this marker compared with in the reference group, of which 53.8% were Cw*07 carriers. The opposite effect was seen for Cw*08, which was found in 3 patients (17.6%), but only in 3.8% of the reference group ($P = .045$; Fig 1).

A significant difference was also seen for HLA-DQB1*06, because only 4 patients (23.5%) were DQB1*06-positive, whereas 48.6% of the reference

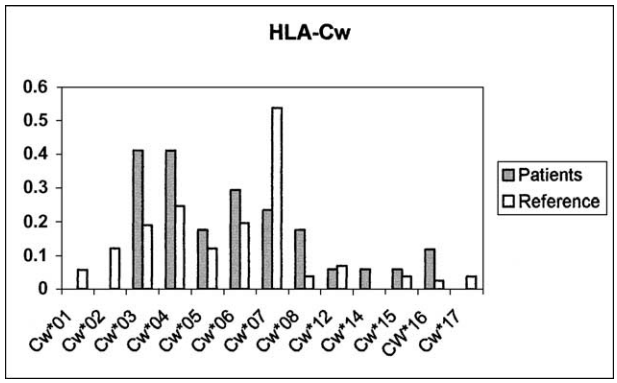

FIGURE 1. Relative frequencies of HLA-C groups at the phenotype level. The difference between the patient and reference groups concerning HLA-Cw*07 ($P = .022$) and HLA-Cw*08 ($P = .045$) is statistically significant. Cw*13 and Cw*18 were represented in neither of the 2 groups.

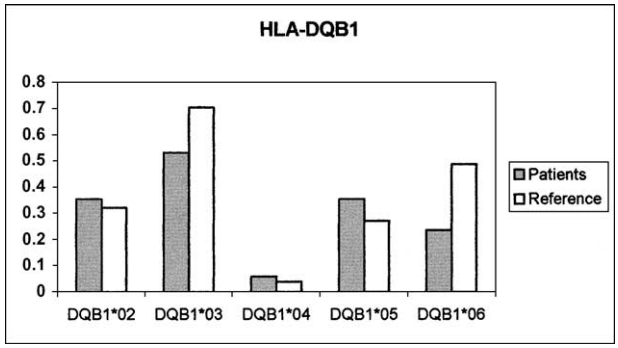

FIGURE 2. Relative frequencies of DQB1 groups at the phenotype level. Only the difference between the patient and reference groups concerning DQB1*06 is statistically significant ($P = .048$).

group possessed an allelic variant of this kind ($P = .048$; Fig 2).

Clinical Results

At follow-up evaluation, mean extension deficit and range of motion were significantly improved compared with preoperative findings. The extension deficit was reduced to 2.6° ± 3.5°. Overall range of motion was 126.5° ± 16.7° ($P < .001$).

DISCUSSION

The objective of the study was to investigate whether the frequency of specific allelic groups in patients with manifest arthrofibrosis after ACL reconstruction is different from in the general Caucasian population. In case of a positive association, this could ease identification of at-risk patients and facilitate preoperative screening.

The significantly increased number of HLA-Cw*08 and significantly decreased HLA-Cw*07 and DQB1*06 indicate a possible association to primary arthrofibrosis. However, a statistical bias cannot be excluded because of the small number of patients. Secondary reasons for arthrofibrosis were excluded, resulting in a homogenous patient collective.

The HLA complex, located on the short arm of chromosome 6, contains over 200 genes. Many of them play an important role in the adaptive immune system, whereas other genes are not linked with HLA and have nothing to do with immunity. HLA-associated diseases are caused by an interplay of many different genes and environmental factors, where HLA genes or combination of alleles (haplotypes) often confer the strongest genetic predisposition. HLA-associated diseases include narcolepsy, hemochromatosis, susceptibility to microbial agents, autoimmune diseases, and cancer. HLA class I and II genes may be directly involved in pathogenesis or may be in close proximity to the disease-causing genes on the chromosome and thus simulate causality because of linkage dysequilibrium. For many diseases, HLA associations are weak and may be fortuitous.[10,11,21]

A review by Marshall et al.[12] on lung fibrosis lists 12 publications that focused on HLA associations of the hereditary form of lung fibrosis. These studies found no clear association between HLA type and disease. However, for a number of autoimmune diseases, strong associations have been described, in particular for ankylosing spondylitis and reactive arthropathy (HLA-B27), rheumatoid arthritis (HLA-DR4), and insulin-dependent (type 1) diabetes mellitus (DQB1*0201, DR4, DQB1*0302).

In our study, beside analysis of association with HLA-A, -B, -Cw, -DRB1, and -DQB1, patients were compared with the HLA-B control group with regard to the marker Bw4/Bw6. This marker exists in the 2 serologic variants Bw4 and Bw6 and represents a sequence motif within the α1-domain of HLA-B, which strongly influences the presentation of antigenic peptides[23,24] and which (variant Bw4) may act as an inhibitory receptor for natural killer (NK) cells.[24-26]

In the study presented here, significant associations with primary arthrofibrosis were detected for Cw*07, Cw*08, and DQB1*06. Cw*07 and DQB1*06 were less frequently found in the patient group and thus might confer some kind of protection against the development of primary arthrofibrosis. In contrast, Cw*08 was found significantly more often in the patient group, so that one might hypothesize that this allelic group represents a risk factor for primary arthrofibrosis.

Further studies that include larger numbers of patients and a control group of patients after ACL reconstruction without a clinical diagnosis of arthrofibrosis are necessary to confirm or vitiate the possible association with HLA-Cw* and DQB1. These studies should include high resolution of Cw*07, Cw*08, and DQB1*06 at the allelic level by molecular methods to detect possible associations with distinct allelic variants.

CLINICAL RELEVANCE

The results of the study represent important basic knowledge in terms of HLA association to primary arthrofibrosis. A potential link exists between arthro-

fibrosis and having HLA-Cw*08 as well as not having HLA-Cw*07 or DQB1*06. Further investigation in terms of association with Cw* and DQB1 is necessary. Those studies should be based on larger numbers of patients, using a control group of patients without a clinical diagnosis of arthrofibrosis after ACL reconstruction. If association to certain Cw* and DQB1 alleles could be proven, preoperative screening could be helpful regarding the decision between surgical and nonsurgical therapies.

Acknowledgment: The authors thank H. Hecker, Director of the Department of Biometry, Hannover Medical School, for statistical analyses. Clinical aspects of this study were investigated and evaluated by T. Weig and are part of his thesis.

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