Ankylosing spondylitis in Iceland

Clinical studies with reference to epidemiology, heritability and the connection with inflammatory bowel disease

Árni Jón Geirsson

Supervisor:

Dr. Björn Guðbjörnsson, professor

Doctoral committee:

Dr. Bjarni Þjóðleifsson, professor emeritus

Arnór Víkingsson, assistant professor in clinical rheumatology

Kristleifur Kristjánsson, MD

Gunnar Tómasson, research specialist

UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES
FACULTY OF MEDICINE

Thesis for the degree of Philosophiae Doctor

May 2014

Hryggikt á Íslandi

Birtingarmyndir og erfðir, með sérstöku tilliti til tengsla við þarmabólgusjúkdóma

Árni Jón Geirsson

Umsjónarkennari:

Dr. Björn Guðbjörnsson, prófessor

Doktorsnefnd:

Dr. Bjarni Þjóðleifsson, prófessor emeritus

Arnór Víkingsson, klínískur lector

Kristleifur Kristjánsson, MD

Gunnar Tómasson, rannsóknarsérfræðingur

HÁSKÓLI ÍSLANDS

HEILBRIGÐISVÍSINDASVIÐ

LÆKNADEILD

Ritgerð til doktorsgráðu

Maí 2014

ISBN 978-9935-9186-0-4

Printing by Háskólaprent ehf.

Reykjavik, Iceland 2014

ÁGRIP

Hryggikt er bólgusjúkdómur sem leggst á ungt fólk. Höfuðeinkenni sjúkdómsins eru langvinnir verkir og stirðleiki í þjóhnöppum og mjóbaki. Í upphafi myndast bólga í spjaldliðum ásamt smáliðum hryggjar og þar sem liðbönd og liðpokar festast við hryggjarliðboli. Sjúkdómurinn getur þróast upp alla hryggjarsúluna. Beinnýmyndun í miðlægum liðum og í liðböndum hryggjarins, samfara vaxandi hreyfiskerðingu, er dæmigert fyrir framvindu sjúkdómsins. Liðbólgur, bólgur í hælsinafestum, lithimnubólga og blöðruhálskirtilsbólga, eru algeng utan-hryggjar einkenni eða fylgieinkenni þessa sjúkdóms.

Tilgangur rannsóknarinnar var að lýsa og skilgreina hryggikt á Íslandi, kanna erfðaþætti þessa sjúkdóms og skyldleika hans við þarmabólgusjúkdóma. Gerð var kerfisbundin leit að sjúklingum með hryggikt í sjúkraskrárkerfum LSH og FSA. Einnig var leitað til sjálfstætt starfandi gigtarlækna á Íslandi um samstarf. Til þátttöku komu 280 sjúklingar með hryggikt. Einnig var leitað eftir þátttöku nákominna ættmenna sjúklinga. Vefjaflokkun m.t.t. HLA-B27 var framkvæmd og ættir raktar með hjálp Íslendingabókar. Ennfremur voru tengsl hryggiktar við þarmabólgusjúkdóma rannsökuð.

Niðurstöður hafa verið birtar í fjórum sjálfstæðum vísindagreinum í alþjóðlegum fræðiritum (I-IV). Rannsóknin sýnir að sjúkdómurinn birtist með öðrum hætti hér á landi en í nágrannalöndunum. Algengi sjúkdómsins er lægra og kynjamunur er minni hér, en annars staðar, sjúkdómurinn kemur fram á svipaðan hátt í báðum kynjum. Liðagigt sem sjúkdómseinkenni er algengt. Erfðatengsl eru sterk í marga ættliði, auk þess sem það eru sterk erfðatengsl á milli hryggiktar og þarmabólgusjúkdóma. Margar af þeim niðurstöðum sem hér verða birtar eru grunnur að nýrri þekkingu á fræðasviðinu.

Lykilorð:

Hryggikt, birtingarmynd, algengi, ættlægni, þarmabólgusjúkdómar

ABSTRACT

Ankylosing spondylitis (AS) is a chronic inflammatory disease. It is characterized by low back and buttock pain, with morning stiffness of insidious onset, usually beginning in early adulthood. This disease can cause progressive stiffness or ankylosis of the spine the typical feature of the disease, which is syndesmophyte formation or bony ankylosis of the ligaments and joints of the vertebral bodies, costovertebral and sternocostal joints. Peripheral arthritis and enthesitis are common. The main extra-articular manifestations of the disease are recurrent iritis, and recurrent or chronic prostatitis, while other extra-articular manifestations are rare.

The Icelandic AS Study has been ongoing since the year 2000 and its aim has been to recruit all Icelandic patients with AS and to examine them and their relatives in respect to clinical symptoms and their genetic background, as well as to explore the connection to inflammatory bowel diseases. In this way, 280 AS patients were recruited, examined and their diagnosis confirmed. HLA-B27 typing was performed, and the geneology database of DeCode used for assessing heritability.

The main results of this investigation have been published in four independent articles in international journals, which are the basis of this thesis (Papers I-IV). I have shown that patients with AS in Iceland have a different presentation than in neighboring countries. The sex ratio is more even and peripheral arthritis is common. However, presentation of the disease is similar in both sexes. The familiality is exceptionally strong, through several generations, and is cross-linked with patients with inflammatory bowel disease. The present findings reflect new knowledge in several aspects.

Keywords:

Ankylosing Spondylitis, demographics, epidemiology, heritability, inflammatory bowel disease

ACKNOWLEDGEMENTS

There are many people that have contributed to this work, too many for all to be mentioned here.

I would like to express my sincere gratitude to my tutor Professor Björn Guðbjörnsson for his support, encouragement and friendship for many years.

I would like to thank Professor Bjarni Þjóðleifsson for his scientific guidance and support through the years and good friendship.

I would like to thank Professor Kristján Steinsson for his support and friendship for many years.

I would like to thank my dear colleagues Arnór Víkingsson, Gerður Gröndal and Helgi Jónsson for their friendship and for being there all the time.

I would like to thank Helga Eyjólfsdóttir, Kristján Orri Helgason, Ólafur Kjartansson and my other coauthors for their valuable contribution.

I would like to thank Rannveig Ásgeirsdóttir for her secretarial assistance.

I would like to thank the Icelandic Society of Rheumatology for their financial support.

Last but not least I would like to thank my wife Sigríður Þórdís Valtýsdóttir for her invaluable support and love.

CONTENTS

LIST OF ABBREVIATIONS

AS	Ankylosing Spondylitis
CD	Crohn´s disease
CT	Computerized tomography
CU	Colitis ulcerosa
ESSG	European Spondyloarthropathy Study Group
FDR	First degree relative
HLA	Human leucocyte antigen
IBD	Inflammatory bowel disease
IBP	Inflammatory back pain
ICD	International classification of diseases
LBP	Low back pain
MRI	Magnetic Resonance Imaging
NSAID	None steroid anti-inflammatory drug
PCR	Polymerase chain reaction
RR	Relative risk
SDR	Second degree relative
SI	Sacroiliac
SMR	Standardized mortality ratio
SPA	Spondyloarthropathy
TDR	Third degree relative
TNF	Tumor necrosis factor

LIST OF FIGURES

LIST OF TABLES

LIST OF PAPERS

I. Prevalence and clinical characteristics of ankylosing spondylitis in Iceland – A nationwide study.
Arni Jon Geirsson, Helga Eyjolfsdottir, Gyda Bjornsdottir, Kristleifur Kristjansson, Bjorn Gudbjornsson.
Clinical and Experimental Rheumatology 2010;28(3):333-40.

II. A strong familiality of ankylosing spondylitis through several generations.
Arni Jon Geirsson, Kristleifur Kristjansson, Bjorn Gudbjornsson.
Annals of the Rheumatic Diseases 2010;69(7):1346-8.

III. Subclinical intestinal inflammation and sacroiliac changes in relatives of patients with ankylosing spondylitis.
Ingvar Bjarnason, Kristjan Orri Helgason, Arni Jon Geirsson, Gudmundur Sigthorsson, Inga Reynisdottir, Daniel Gudbjartsson, Anna Sigurlin Einarsdottir, Roy Sherwood, Kristleifur Kristjansson, Olafur Kjartansson, Bjarni Thjodleifsson.
Gastroenterology 2003;125(6):1598-605

IV. A common genetic background for inflammatory bowel disease and ankylosing spondylitis: A genealogic study in Iceland.
Bjarni Thjodleifsson, Arni Jon Geirsson, Sigurdur Bjornsson, Ingvar Bjarnason.
Arthritis & Rheumatism 2007;56(8):2633-9

DECLARATION OF CONTRIBUTION

I, Arni Jon Geirsson [Árni Jón Geirsson], declare that I have participated in the planning and execution of all of the research described in this thesis, unless indicated by references to work of others. Specifically, I contributed to the published papers in the following manner:

Paper I: Planning, data collection, statistical analysis, writing, editing

Paper II: Planning, data collection, writing, editing

Paper III: Planning, data collection, writing, editing

Paper IV: Planning, data collection, writing, editing

1 INTRODUCTION

The name of ankylosing spondylitis (AS) is derived from Greek ankylos, meaning stiff or bent, and spondylos, meaning spinal vertebrae, i.e. stiff or bent spine. The earliest descriptions of skeletons with changes typical of AS are attributed to Realdo Colombo in 1559 and Bernando Connor in 1693 (1), who described an ankylosed skeleton with fused pelvis and spine found in a French cemetery. Evidence of AS has also been found in ancient mummies in Egypt from the year 2000 BC (2). The first clinical descriptions of AS, a woman and two of her daughters, appeared in the late 19th century, followed by a series of publications from St. Petersburg by the Russian Vladimir von Bechterew (3). Other pioneers in this field were the German neurologist Adolf Strümpel and the French neurologist Pierre Marie. Therefore, AS was known earlier as Morbus Bechterew or Marie-Strümpel disease, named after these doctors.

1.1 Classification criteria

Changes in the sacroiliac joints (SI joints) have been considered the hallmark of AS and central to the diagnostic confirmation of the disease. Therefore, it was given a central place in the classification criteria of AS in the Rome 1960 criteria (4), and the revised New York criteria from 1984 (5). The modified New York criteria classify a patient as having AS if he or she meets one clinical criterion and has the classical radiological criteria of sacroiliitis. The clinical criteria are: 1) Lower back pain (LBP) for more than three months which improves with exercise; 2) Limitation of motion of the lumbar spine in both the sagittal and frontal planes; 3) Limitation of chest expansion relative to normal values for age and sex (Table 1). The Amor criteria published in 1995 for spondyloarthropathy (SpA) refer to undifferentiated SpA based on clinical manifestation or past history and radiological findings (6). The Amor criteria and the European Spondyloarthropathy Study Group criteria (ESSG) (7) are classification criteria for undifferentiated spondyloarthropathy patients; however, these criteria have not become widely used for classification or diagnosis of AS in the research field or in clinical daily work. Thus, the modified New York criteria have been used in the vast majority of studies for the past 25 years and other classification criteria have not gathered popularity or global acceptance (8).

Table 1: Three different classification criteria for Ankylosing Spondylitis

Rome 1961

Clinical Criteria

1. Low back pain and stiffness for more than 3 months, not relieved by rest
2. Pain and stiffness in the thoracic region
3. Limited motion in the lumbar spine
4. Limited chest expansion
5. History of iritis or its sequelae

Radiological Criteria

6. Roentgenogram showing bilateral sacroiliac changes, characteristic of ankylosing spondylitis.

Definitive AS if:

1. Grade 3-4 bilateral sacroiliitis with at least one clinical criterion.
2. At least 4 clinical criteria.

New York 1966

Clinical Criteria

1. Limitation of motion of the lumbar spine in all three planes: Anterior flexion, lateral flexion and extension.
2. Pain at the dorsolumbar junction or in the lumbar spine.
3. Limited chest expansion to 2.5 cm or less, measured at the 4th intercostal space

Grading of radiographs

Normal 0, suspicious 1, minimal sacroiliitis 2, moderate sacroiliitis 3, ankylosis 4

Definitive AS if:

1. Grade 3-4 sacroiliitis with at least one clinical criterion
2. Grade 3-4 unilateral or grade 2 bilateral sacroiliitis with clinical criterion 1, or with both clinical criterion 2 and 3.

Probable ankylosing spondylitis

Grade 3-4 bilateral sacroiliitis with no clinical criterion

Modified New York 1984

Criteria

1. Low back pain for at least 3 months duration, improved by exercise and not relieved by rest.
2. Limitation of lumbar spine motion in sagittal and frontal planes
3. Chest expansion decreased relative to normal values for age and sex.

4.a Unilateral sacroiliitis grade 3-4

4.b Bilateral sacroiliitis grade 2-4

Definite AS if 4a or 4b and any clinical criterion (5)

Since the introduction of magnetic resonance imaging (MRI) in medicine in the early 90´s, sacroiliitis has become much easier to diagnose with MRI showing early bone oedema and subchondral or chondral changes in the SI joints that are characteristic for AS. It is therefore probably only a matter of time that we see new diagnostic criteria for AS that include MRI instead of plain X-rays for revealing SI abnormalities in AS. In this context, new classification criteria of "non radiology SpA" have recently been introduced (9).

1.2 Epidemiology

The annual incidence of AS per 100 thousand inhabitants has been reported to be 0.5-8.2 (10). AS has a strong association with the HLA-B27 antigen and the prevalence of AS in different cohorts frequently relates to the prevalence of this MHC molecule in the study population. The majority or up to 95% of Caucasians with AS carry the HLA-B27 antigen compared to around 8–14% in the background population. Although the incidence rate has been reported to correlate with the prevalence of HLA-B27 in the population, not more than 1-2% of HLA-B27 positives individuals will develop AS. The highest prevalence of HLA-B27 is around 40% in tribes in Eastern Russia (11, 12), 50% in Western Canada (13), and 53% in New Guinea (14). In Northern Scandinavia in the Sami population it is 24% (15), compared to around 8% in the Caucasian population of Western Europe (16). The prevalence of AS is thought to be highest in the Haida Indians in Western Canada 4.5-10% (17, 18). In Europe, AS has been reported in about 0.14-2.20% of the population, with the highest prevalence in Northern Scandinavia (16). Meanwhile, the incidence has been reported to be low in Greece or 1.5 in 100,000 inhabitants per year (19, 20) while in central Europe and Scandinavia the incidence has been estimated to be 6.4-10 per 100,000 inhabitants per year (10, 21, 22). The sex ratio in Northern Norway has been found to be 5:1 in favour of males (23). Other studies have shown a gender difference of 3-8:1 in favour of males (24), while in the present study the gender differences is much lower or only 1.9:1 , paper I.

1.3 Clinical Manifestation

AS is a disease of young people, with onset of symptoms usually in the early third decade of live and almost never after 45 years of age (25). About 15% of the patients have disease onset before 16 years of age, often presenting with lower extremity oligoarthritis and

enthesitis. AS is a chronic inflammatory disease, characterised by low back and buttock pain with morning stiffness of insidious onset. It takes several years after onset for typical radiological signs of AS to be seen on radiographs of the SI joints and lumbar spine. This is in part the reason for the prolonged diagnostic delay in this disease. The other main reason for diagnostic delay is doctor unawareness of inflammatory back pain in young people. As the back pain in AS results mainly from inflammation of the SI joints and/or the small joints and the ligaments of the spine, it has been termed inflammatory back pain (IBP) (26). The hallmark of IBP is that the pain gets worse with rest and improves with exercise.

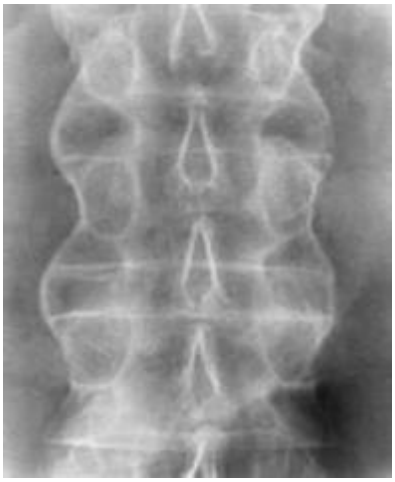


Figure 1: Bamboo spine due to AS

Long-standing inflammation in the SI joints and spine can cause structural damage in the form of erosions of the joint cartilage, joint space narrowing and subsequently ankylosis, all classical imaging signs of AS. Sacroiliitis with syndesmophyte formation can in advanced disease lead to ankylosis of the SI joints and the formation of so-called bamboo spine (figure 1), where the vertebral bodies are joined together by syndesmophytes or bony union of the intervertebral discs with the vertebral bodies and the anterior and posterior longitudinal ligaments with the vertebral bodies and intervertebral discs (27). This can extend up the entire vertebral column in severe cases, which causes the vertebral column to be fixed and immobile.

Arthritis of peripheral joints, costochondritis and enthesopathy, especially of the Achilles tendon insertion, are also commonly described in AS (28). Extra-articular manifestations of AS include iritis (25–40%) (29, 30), prostatitis (13–83%) (31, 32), and asymptomatic gastrointestinal tract involvement (69%) has also frequently been described (33). Meanwhile, cardiac and pulmonary involvement seems to be less commonly reported in patients with AS (34, 35). Thus the clinical features of AS can be divided into musculoskeletal features and extra-articular features.

1.3.1 Musculoskeletal manifestations - Axial involvement

The most common presenting symptoms of AS are low back pain and stiffness, and pain in the buttocks. The buttock pain may be unilateral to begin with, but usually becomes bilateral and symmetric as time passes. These symptoms are of insidious onset and chronic, worse in the early morning and tend to awaken the patient in the early morning hours. These symptoms get worse after rest, but improve with exercise. The symptoms of AS can be confined to the low back region and SI joints for years or permanently, but they can also ascend up the spine to the thoracic and cervical part of the spine, causing pain and stiffness. AS can affect the axial joints of the thoracic cage, the costo-vertebral and costo-transverse as well as the costo-sternal joints; the sterno-clavicular, manubrio-sternal, and sterno-xiphoid joints can also become affected in AS. This may cause pain and stiffness with deep breathing, coughing and sneezing. The thoracic cage can become stiff, thus affecting chest expansion and causing restrictive lung disease. The presenting symptoms of AS may on rare occasions originate from the thoracic spine, the costo-vertebral joints or costo-sternal joints. Sternal joint involvement can also on rare occasion be a presenting symptom of AS, with pain and tenderness over the sternal joints. AS can affect the cervical spine such that movements of the head are restricted and in time the head movements can become fixed due to ankylosis of the cervical spine, causing restriction of head movement which can evolve with time to total fixation of the cervical spine due to ankylosis (27, 36). On rare occasions cervical pain, stiffness and limitation of head movement can be the presenting symptoms of AS (37, 38).

1.3.2 Peripheral arthritis and enthesitis

Approximately half of all patients with AS eventually display significant large joint involvement, especially hip and knee involvement at some stage of the disease, and about 25% of those develop peripheral joint arthropathy (36). Some patients may as well develop small joint arthritis. Hip joint involvement in AS is usually bilateral and is seen at some point in close to 50% of the patients, it can cause erosion of the articular cartilage and joint space narrowing with eventual destruction of the hip joint. The next most frequent joint to be affected in AS is the knee joint, followed by arthritis in the ankle and shoulder joints. Polyarticular involvement has been reported in up to 25% of the patients (39). Achilles tendinitis is commonly seen

in up to 20% of patients and plantar fasciitis in 6-10% of the patients (40). Enthesitis as a part of AS seems to be rare in other places like the trochanter region, ischial tuberosity and the tibial tuberosity (41).

1.3.3 Extra-articular manifestation - Anterior uveitis

Uveitis is a disease that affects young people. Anterior uveitis is an inflammation affecting the iris and or the ciliary body in the anterior chamber of the eye It is a common complication of AS, occurring in around 20-40% of AS patients (30). Posterior uveitis, an inflammation affecting the choroid and sometimes by extension also the retina, is hardly ever seen in AS, but can be a part of other systemic inflammatory diseases, e.g. sarcoidosis or juvenile idiopathic arthritis, various infectious conditions, or it may be idiopathic. Anterior uveitis or iritis accounts for around 90% of total uveitis cases. Uveitis is most common in younger people from 20-50 years of age; it is rare in children and in people over 70 years of age (42). In the population with acute anterior uveitis the prevalence of HLA-B27 is 50% (43). The symptoms of uveitis in AS are usually unilateral and acute, with red and painful photophobic eye (44, 45). The visual acuity is usually decreased in the acute episode. The sex ratio is even in respect to uveitis. Acute anterior uveitis is rarely the presenting symptom of AS; however, it is often the first clue to the recognition that LBP may be part of a systemic inflammatory disorder (45).

1.3.4 Prostatitis

Chronic recurrent non-bacterial prostatitis is an integral part of the clinical symptoms of AS in some male patients (32). It presents with perineal pain and urethral burning that is exaggerated by urination and sexual activity. The aetiology behind this is unknown, and although the prostatitis is usually “non-bacterial” it often responds to anti-microbial agents (46), and has been connected with *Klebsiella Pneumonia*, a gram negative bacterium, that has been connected with the aetiology of AS (46, 47). By examining prostatic fluid obtained after prostatic massage the prevalence of prostatitis was found to be very high in a group of AS patients (48).

1.3.5 Inflammatory bowel disease

A relationship between AS and inflammatory bowel disease (IBD) has been known for some time. It has been shown that subclinical intestinal inflammation revealed by endoscopy is present in 25-50% of AS patients (49). Histopathological lesions seen in microscopy are

even more common and increased intestinal permeability as a manifestation of intestinal inflammation in AS patients has been demonstrated in up to 60% of cases (49, 50).

The causal relationship between enteropathogenic infections such as salmonella species, campylobacteria and other related bacteria and reactive arthritis that resembles AS a few weeks later points to an etiological connection between the gut microflora and AS (51). These findings do suggest that the etiological and pathogenic factor for AS may lie in the gut. Thus, the gut mucosal inflammation and increased permeability of the intestinal mucosa may indeed activate the immune system by confronting it with foreign bacteria that may have a resemblance to the HLA-B27 molecule. In AS there is a strong association with HLA-B27; in the IBD population the incidence of HLA-B27 is not increased (52, 53). When IBD and AS go together the incidence of HLA-B27 increases up to 25-80% (54, 55). Some studies have, however, reported a lower incidence of HLA-B27 in this patient population. It seems that in the presence of gut inflammation as seen in IBD and AS the presence of HLA-B27 is less crucial than in idiopathic AS. The subclinical gut inflammation in AS can be distinguished both as acute and chronic (56). The acute form consists of infiltration of polymorphonuclear cells into villi and crypts with preserved mucosal architecture. In the chronic type the mucosal architecture is distorted. The lamina propria and mucosa are infiltrated by mononuclear cells and there are lymphoid follicles to be found in the lamina. The crypts may be distorted and granulomas may be seen (57, 58). It will be shown in this thesis that there is a cross-over increased risk among relatives of both AS and IBD of acquiring these diseases, paper IV.

1.3.6 IgA-nephropathy

IgA nephropathy is the most common condition causing glomerulonephritis in the adult population; however, it is usually a mild form of glomerulonephritis that presents with macro- or microscopic haematuria and sometimes with proteinuria. Occasionally, it can cause nephrotic syndrome with renal failure. The histopathological lesion consists of a mesangial glomerular deposition of IgA. The diagnosis is made by kidney biopsy. This specific type of nephropathy is increased in frequency in the AS population. Earlier, secondary renal amyloidosis was the most common cause of renal disease in AS, but with improved treatment options it is of decreasing importance. IgA nephropathy is the most common cause of renal

affliction in AS and all AS patients should be screened for the presence of nephropathy with urinalysis (59).

1.3.7 Heritability and genetics

The discovery of the genetic link between HLA-B27 and AS in the early seventies showed us that the heritability of AS is very strong. Around 90% of all AS patients carry the HLA-B27 genotype. However, it does not act alone and it is likely that several other genes may play a role in the susceptibility for the disease as well as some unknown environmental factors.

Multiple subtypes of HLA-B27 are now recognized. The overwhelming majority of HLA-B27 in Western Europe is the B2705 or 90% but B2702 accounts for about 10% (60, 61). In other parts of the world the subtypes of HLA-B27 are different (62). In Asia the most common subtype is B2704 among the Chinese and Japanese whereas in others B2706 is the most common subtype (62). In Western Africa AS is a rare disease and the most common HLA-B27 subtype is B2703, which interestingly seems not to be associated with AS. The HLA-B2709 that has been found in Sardinians and B2706 have not been associated with AS (63, 64). Among African Americans, AS patients are only 50% HLA-B27 positive (16). Only a small proportion of HLA-B27 positive individuals will develop AS; it is estimated to be close to 1-5%. On the other hand, 20% of HLA-B27 positive relatives of these African American AS patients will develop AS.

The HLA-B27 gene is thought to account for 50% of the heritability of the disease. Twin studies have shown that there is a 63% concordance rate among monozygotic twins, and 12.5% concordance for dizygotic twins, which increases to 23% if the twins are HLA-B27 positive (39). AS has been known to run in families; the risk ratio for FDR of AS patients for contracting the disease ranges from 50-80% (65). AS does not demonstrate a Mendelian pattern of inheritance, but rather an oligogenic type of inheritance where several genes interact (66). Three genome-wide linkage studies have been performed on AS. A British study demonstrated that the strongest logarithm of odds (LOD) score was in the MHC region due to HLA-B27, while outside the MHC the most significant linkage was noted in chromosome 16q. Suggestive LOD scores were noted for chromosomes 2q, 9q, 10q and 19q (67). In a North American family study on AS, linkage was also noted in the MHC region as well as on chromosomes 6q and 11q (68). In a French study linkage was also

found in the MHC region and suggestive linkage at 5q, 9q,13q, and 17q (69). The French study included patients with spondyloarthropathy defined by the Amor (6), and ESSG criteria, while the British and North American studies included patients with AS that fulfilled the modified New York criteria. The International Genetics of Ankylosing Spondylitis Consortium (IGAS) combined data from the three whole genome linkage scans for AS (70). Besides linkage at the MHC region, suggestive linkage was noted on chromosomes 10q and 16q and nominal linkage was noted on chromosomes 1q, 3q, 5q, 6q, 9q, 17q, and 19q. There is an increasing list of genes and genetic regions identified as being associated with AS beside the MHC region, like IL23R, ERAP1, 2p15 and 21q22 (71).

2 AETIOLOGY AND PATHOGENESIS

The aetiology of AS is unknown. The similarity between AS and Reiter's syndrome (reactive arthritis) suggests that AS may be reactive to an infectious or exogenous antigen entering through the intestinal mucosa in an individual that is genetically susceptible. The most common sites of inflammation in AS are the SI joints and the junction between the intervertebral discs and ligaments with the vertebral body, together with enthesis and the synovial joints. The early lesions contain CD4 positive lymphocytes and to a lesser extent CD8 type together with CD68 positive macrophages and proliferating fibroblasts. TNF-alpha and TGF-beta are overexpressed in the inflammatory lesions of AS at the bone connective tissue junction and the tissue is infiltrated by T lymphocytes, proliferating fibroblasts and osteoclasts. Destroyed bone is replaced by boneforming osteoblasts, a process that results in new bone formation and subsequently ankylosis of ligaments and joints. Rats that are transgenic for HLA-B27, and over-express HLA-B27 and β 2 microglobulin acquire spontaneous disease resembling AS and psoriatic spondyloarthritis. T cells are required, as transgenic HLA-B27 rats that are without a thymus gland and, thus, have no T cells do not get AS. When they are brought up in a germ-free environment they remain disease free. Thus, when the gut is colonized with normal gut flora, it seems to trigger disease (72, 73).

3 TREATMENT AND PROGNOSIS

The main goal of treatment for patients with AS is symptomatic relief, i.e. to eliminate pain, stiffness and fatigue, restore function and prevent complications of the disease. Physical therapy and pharmacological therapy are both very important in the treatment of AS patients. Non-pharmacological therapy includes patient education and regular exercise programs that aim at restoring and preserving function and hinder flexion contractions and posture that limits head and spinal movements. Smoking cessation for those who smoke is very important because pulmonary restriction due to thoracic cage stiffness is common in this group of patients (74, 75). Cardiovascular disease is more common among male AS patients than in the general population, which is an additional reason for smoking cessation. This contributes to increased mortality among this group of patients (76, 77). Compared to the general population, patients with AS are at increased risk for many types of cardiovascular and cerebrovascular diseases and are more likely to be hospitalized for these diseases. The excess risk is greatest in younger patients with AS (78).

Pharmacological treatment includes non-steroidal anti-inflammatory drugs (NSAIDs), sulfasalazine, TNFα inhibitors and intra-articular steroids for some patients. Most patients benefit from NSAIDs and this should be the first line of treatment for all symptomatic AS patients. Which NSAID to choose is a matter of personal preference, as most of them are effective against the symptoms of AS in their maximal dosage (79, 80). There are studies that indicate that both indomethacin and diclofenac are effective in AS treatment and even retard radiographic progression (81). There are also studies that indicate that COX-2 inhibitors like celecoxib and etoricoxib are as effective as other NSAIDs and can even delay radiographic progression of the disease (82, 83). Sulfasalazine has long been the first line of treatment for AS after NSAIDs, and studies indicate that sulfasalazine is more effective than placebo for spinal stiffness and improving peripheral arthritis. It has been concluded that sulfasalazine is more effective in the treatment of arthritis in the extremities than for spinal disease in AS (84-86). Thus, most doctors recommend sulfasalazine only for those AS patients that have peripheral arthritis. Methotrexate and leflunomide are not effective for AS and should not be used (87, 88). It seems that all the anti-TNF

inhibitors are equally potent drugs for usage in AS. Concomitant use of an immunomodulatory drug such as methotrexate seems to be unnecessary, as it brings no additional beneficial effect but increases the risk of adverse effects (89). The response to anti-TNF inhibitors is rapid, with 80% of responders improving within only six weeks. The response is durable, but stopping the treatment after the patients have obtained good clinical response results in a rapid relapse in most cases (90, 91). There are indications that anti-TNF treatment improves bone density in AS, but it is still debated whether the anti-TNFs halt radiological progression of the disease; as yet there is no proof but some indications that it may halt radiological progression of AS (92).

According to a newly published study there is an increased mortality among men with AS (SMR 1.63), while there is no significant increase in mortality in women with AS. A third of the patient population died of cardiovascular diseases. Death due to neoplastic diseases was the second commonest cause of death. The overall mortality of patients with AS were 1.61 (SMR). Variables independently associated with increased mortality in AS were increased C-reactive-protein level, NSAID use and work disability (93).

4 AIMS

To shine a clearer light on Ankylosing Spondylitis in Iceland and study its epidemiology, heritability and interconnection with inflammatory bowel disease:

Firstly, to describe the epidemiology of AS in Iceland with focus on the prevalence and incidence of AS, the symptoms and signs of the disease and the gender difference in a nationwide cohort of AS patients in Iceland.

Secondly, to define the heritability of AS through several generations in Iceland, and to emphasize family history when it comes to diagnosing the disease.

Thirdly, to study the connection between inflammatory bowel disorders (IBD) and AS and examine if subclinical intestinal inflammation can be the first sign of AS.

Lastly, to analyse the common genetic denominator in AS and IBD and examine if there is an increased cross-over risk for developing either disease in close relatives of patients with AS or IBD.

5 MATERIAL AND METHODS

5.1 Paper I

In this study all known patients with AS in Iceland were recruited by looking up hospital files and by personal contact with all rheumatologists in Iceland.From the first database of ongoing studies of AS and IBD, 205 individuals who were alive in 2005 and who had been diagnosed as having AS with the diagnosis verified by a rheumatologist (AJG) were included in the present study, paper IV. The second source was an electronic registry of patients admitted to the two major hospitals in Iceland, which both have rheumatologic specialist services: Landspitali-University Hospital in Reykjavík (LSH) and the Akureyri Hospital in Northern Iceland (FSA). A systematic search of AS and sacroilitis according to the International Classification of Diseases (ICD) 10th diagnostic registry, including the following codes: M45, M45.5, M45.9, M46 and M46.9, was performed in all hospital records. This source yielded 54 patients who had been diagnosed with AS and who were alive in 2005. The third source was a repeat personal call to all private rheumatology services in Iceland to have them report patients to the study. This yielded 64 patients who had been diagnosed with AS and who were alive in 2005. The three sources named above yielded 323 patients, but, since many patients were found in more than one database, the total number of patients diagnosed with AS in Iceland came to 280 individuals. Signed informed consent was obtained from all the participants in the study.

All of these 280 known cases of AS in Iceland were initially contacted by a letter of invitation, followed by a telephone call by our study nurse; 24 individuals (8.6%) could not be reached or did not respond. Of the remaining cases 256 or 91.4% of the original study group agreed to participate in the study. All participants were interviewed and examined by the same rheumatologist (AJG). All participants were also asked to answer an extended questionnaire in connection with the genetic study and 223 patients (87.1%) filled out and returned the questionnaire. Of the 256 patients examined, 33 (12.9%) were excluded from the prevalence analysis.

The inclusion criteria used were the modified New York criteria for classification of AS (5). Patients who did not have active arthritis or inflammatory back pain at evaluation were included if they had been

diagnosed with AS by a rheumatologist and were taking remitting drugs at the time of the study. Meanwhile, patients who reported a diagnosis of a rheumatic disease other than AS when interviewed, or who were observed to have another rheumatic condition when examined, were excluded from the study. Furthermore, patients with AS associated with psoriasis were also excluded from the present study as the group of patients with psoriatic arthritis in Iceland has recently been reported separately (94).

Chest expansion was measured on maximal inspiration after forced maximal expiration, at the level of the fourth intercostal space in males and just below the breasts in females. The normal values were set at ≥6 cm for males and ≥4.5 cm for females, as normal values of chest expansion are sex dependent. Cervical flexion, extension and rotation were evaluated according to standard clinical measurements and lumbar flexion ability was evaluated by the Schober index and measured in centimetres (cm) (95). Extent of extra-spinal symptoms, i.e. peripheral joint involvement, was also assessed according to standard clinical evaluation.

Concerning systemic manifestations of, for example, iritis and prostatitis, the diagnosis was reviewed by one of the study members (AJG) with the requirement that these manifestations were confirmed by a physician at the time of occurrence, e.g. ophthalmologist or rheumatologist in the case of eye inflammation and urologist, general physician or rheumatologist in the case of prostatitis, respectively. All radiographs, computed tomographs and magnetic resonance imaging were re-evaluated by members of the study group.

Whole blood was drawn for later genetic studies and for extended typing of the major histocompatibility complex molecules to estimate the prevalence of HLA-B27 (Dynal HLA-Kit, F. Hoffmann-La Roche Ltd, Basel, Switzerland). A total of 524 randomly chosen healthy volunteers were used as controls and tested for the HLA-B27 antigen, and of those 15.4% turned out to be positive for HLA-B27. The study data were stripped of information allowing identification of individuals before the analysis of data began and the code for this information was kept in a separate, encrypted database. Point prevalence was based on all living individuals in Iceland on the 31st of December in 2005 and on those who were known to have AS according to the study protocol.

5.1.1 Statistical analysis

The occurrence of AS during the study period is expressed with incidence rates and a point prevalence rate at the end of December 2005, among Icelanders 18 years of age and older, with 95% confidence intervals. To evaluate the difference between men and women with respect to demographic factors, clinical presentation, HLA-B27 positivity status and treatments used, p-values were calculated with the Fisher exact test for categorical and continuous variables respectively. All p-values were two-sided and a cut-off of 0.05 was chosen to express statistical signifance.

5.2 Paper II

In paper II the same database of 280 AS patients was used as in paper I. DeCODE Genetics has built a computerised genealogy database of more than 760,000 individuals. The database contains records of all living Icelanders, comprising more than 300,000 individuals and a large proportion of all individuals who have ever lived in the country from the time of the settlement in the late ninth century. The genealogy database is essentially complete from the 18th century to the present day, allowing distant relationships to be traced accurately. Each patient with AS was matched to a single control individual in each control group. The controls were drawn at random from the genealogy database and matched for the year of birth, gender and the number of ancestors recorded in the genealogy database.

An example of clustering of AS in Icelandic families was graphically expressed with a pedigree originating from the deCODE Genetics genealogy database. To protect the anonymity of the families, some of the unaffected relatives are not shown.

5.2.1 Statistical analysis

The risk of getting AS among FDR, SDR, TDR and fourth degree relatives of patients with AS compared to the “average risk” in the Icelandic population is expressed with RR with 95% CI. Obtaining valid estimates of the RR is, however, not straight forward because many sampling schemes may lead to biased or inaccurate estimates (96). We determined the RR for AS in a previously described Icelandic population. To assess the significance of the RR obtained for a given group of relatives, we compared their observed values with the RR computed for up to 1000 independently drawn and matched control individuals (97). Empirical p values can be

calculated using the control groups; thus, a p value of 0.05 for the RR would indicate that 5% of the matched control groups had values as large as or larger than that for the patient's relatives or spouses. The number of control groups required to obtain a fixed accuracy of the empirical p values is inversely proportional to the p value. We therefore selected the number of control groups generated adaptively up to a maximum of 1000. When none of the values computed for the maximum number of control groups were larger than the observed value for the patient's relatives and spouses, we report the p value as being less than 0.001.

5.3 Paper III

In this paper we studied the prevalence and possible mode of inheritance of subclinical intestinal inflammation in FDR of patients with AS. Secondly, we studied the possible consequences of this subclinical intestinal inflammation by assessing the prevalence of skeletal abnormalities that are suggestive of early AS by CT. These findings were also assessed in relation to the subjects HLA-B27 genotype status.

5.3.1 Study population

In the study presented in paper III the AS and IBD registries of patients were used. In this study the early database of 205 AS patients and the population-based sample of data from all patients diagnosed as having IBD in Iceland over a 50-year period, i.e.1950–2000, most of whom have been described in detail elsewhere (98-100) were used. Altogether, 53 of 54 patients with AS who were approached for this study by mail and telephone participated. This represented all of the patients with AS under the care of the rheumatologists at the Landspitali-University Hospital in Reykjavik and about 25% of all AS patients in Iceland. The diagnosis of AS was based on the modified New York criteria at least one year before they were included in this part of the study. There was clustering of cases in nine families with two FDR with AS; in one family, there were four FDR with AS. Intestinal studies in patients with AS are complicated by the fact that these patients are treated with a number of drugs that cause or modify intestinal inflammation (101). All of the patients with AS in this study were receiving or had received conventional nonsteroidal anti-inflammatory drugs (NSAIDs) for prolonged periods, which can lead to NSAID enteropathy (101). At

the time of this study, seven were not receiving any treatment, 41 were taking NSAIDs, and 21 were taking sulfasalazine which may reduce the intestinal inflammation associated with AS (102) as well as that due to NSAIDs (103). Furthermore, four were taking prednisolone and three were taking methotrexate, both of which may reduce intestinal inflammation in patients with IBD. The predetermined exclusion criteria for the patients and relatives were age younger than 16 years and older than 80 years; severe neurologic, psychiatric, endocrine (including diabetes mellitus), cardiovascular, pulmonary, hepatic, or renal diseases; malignancy; and pregnancy. Relatives with established gastrointestinal disease were excluded, as were those misusing alcohol (n=14) and those taking NSAIDs (n=8) (104). Low-dose aspirin (<300 mg/day) was not an exclusion criterion (n=5) because, unlike conventional NSAIDs, aspirin does not cause small bowel inflammation (104-106). Seventeen spouses also underwent studies for intestinal inflammation; if they were affected similar to the patients or FDR, this would suggest that environmental rather than genetic factors might be responsible for the inflammatory changes. The couples had been living together for 4–33 years (mean, 8 years). The spouses were subject to the same exclusion criteria as the FDR and were not taking aspirin or NSAIDs.

Table 2: Demographic details of AS patients studied and their first-degree relatives.

	Patients with AS	First degree relatives	Spouses of patients with AS
Number	47	124*	17
Sex: male/female	37/10	51/73	3/14
Age (years)	40±12	45±16	38±11

*A total of 213 first-degree relatives, which excludes those younger than 16 and older than 80 years of age, were potentially available for study.

Table 2 shows the demographic details of the patients, first-degree relatives, and spouses studied. Of the 124 FDR studied, 100 were randomly invited to participate in the CT study. Fifteen declined; therefore, 85 (32 men [38%] and 53 women [62%]) underwent a CT of the sacroiliac joints to assess the possible consequence of the subclinical intestinal inflammation. Participants attended an

investigational unit at the Landspitali, the National University Hospital, for medical evaluation as described.

5.3.2 Study variables

Intestinal inflammation was assessed by measurement of calprotectin in feces. Calprotectin is a neutrophil-selective protein that is also present in small quantities in other polymorphonuclear white cells. Its presence in feces relates quantitatively to the neutrophil flux in the gastrointestinal tract, i.e., it is proportional to the degree of acute inflammation (106-108). Subjects provided a stool sample within three days of visiting the investigational unit. The samples were usually received within eight hours of passing the stool, and 20-g portions were frozen and stored at -20°C. Calprotectin is resistant to bacterial degradation and is stable in feces at room temperature for at least 1 week (107). After thawing, 5-g aliquots were processed for quantitative measurements by a sensitive and specific enzyme-linked immunosorbent assay, as previously described (106). The within-assay coefficient of variation was 1.2%, and the between-assay variation was 15%.

The normal range of fecal calprotectin excretion and concentration was established in 163 healthy Icelandic volunteers during these studies, mostly from health care professionals and their immediate families (88 men and 75 women; mean age, 46±8 years; range, 19–72 years). None of the controls had a FDR with IBD or a chronic arthritic condition (excluding osteoarthritis). Genomic DNA was isolated from whole blood according to standard protocols, and HLA-B27 status was determined by PCR as previously described page 34 (109).

The sacroiliac joints were examined with CT (CT Lightspeed; General Electric, Milwaukee, WI), and 3.75-mm contiguous scans were taken over the sacroiliac joint with the subjects in a supine position and the gantry angled craniocaudally parallel to the sacrum and the sacroiliac joints. The estimated effective radiation dose equivalent received during this procedure was 2.65 millisieverts. Two radiologists without any knowledge of the clinical findings read all images independently. Numeric data were generated as the mean from the two observers. Overall, there was excellent agreement between the two observers, with only a single inter-observer variation that was resolved by discussion. A widely accepted scoring system for the evaluation of sacroiliitis with CT is not available. For the

purpose of this study, a system was devised based on a conventional radiography scoring system that describes six stages of arthritis from grade 0 (normal) to grade 5 (bony ankylosis in the sacroiliac joints) (110). The CT score in the relatives fell within grade 0 (normal) and grade 1 (suspicious for early AS changes), and none had grade 2 (definite AS changes) or higher. Grade 1 included the presence, number, and size of subchondral cysts/erosions and blurring of joint margins. The predetermined grade 1 criteria also included subchondral sclerosis (sacral/iliac), osteitis condensans ilii, joint space narrowing (<2 mm wide), and air streak in the sacroiliac joint, but these were so infrequently encountered that reliable statistical analysis between groups was not possible.

5.3.3 Statistical analysis

To assess for statistical difference in mean calcprotectin levels of AS patients and their first degree relatives vs. controls two sided p-values were calculated with the Welch modified test to test the difference between the relatives of patients and of controls, assuming the variance in the relatives and the controls was unknown and not necessarily the same in the groups. A cut-off of 0.05 was chosen for statistical significance.

To assess inheritance in respect to calprotectin levels, variance component analysis was performed (111) to identify possible factors controlling the calprotectin concentrations in first-degree relatives of patients with AS. This modelling allows for a single major gene affecting the trait as well as the trait being affected by environmental influences or an additive polygenic component. The polygenic and environmental influences are not distinguishable in this type of analysis, and their combined effect will therefore be referred to as polygenic, paper III.

Radigraphic damage is expressed as proportion of subjects with grades 0 to 5, mean number of subchondral cysts and mean cystic diameter in mm. Difference in radiographic features between HLA-B27 positive vs HLA-B27 negative subjects and between subjects with normal vs. elevated calprotectin levels is expressed as difference in proportions and means between the groups. Two-sided p-values were calculated using Fisher exact test and Student´s t test for categorical and continuous variables respectively.

5.4 Paper IV

In this study the familiality of AS and IBD was compared

5.4.1 Study population

In this study the databases of all AS patients and IBD patients in Iceland that were previously described were used. The IBD patients were born in the years 1885–1990. This group consisted of a total of 1,352 patients, including 1,091 with ulcerative colitis and 261 with Crohn's disease; eight patients had indeterminate colitis and were therefore excluded from this study. The diagnosis of these patients were biopsy-confirmed and independently reviewed; the disease in all patients was confirmed to fulfil the accepted diagnostic criteria (112). Each patient was required to have had at least one year of follow-up, and many patients have undergone decades of reevaluation and had received confirmation of their final diagnosis.

5.4.2 Statiscial Analysis

We determined the risk ratio for IBD and the risk ratio for AS, as described above (see section 5.2.1) (97).

The cross risk ratio was used to estimate the relatedness between AS and IBD, as well as the relatedness of the subcategories ulcerative colitis and Crohn's disease. The cross risk ratio is an estimate of the risk of IBD or its subcategories ulcerative colitis and Crohn's disease in relatives of patients with AS compared with the risk in the population as a whole. This was computed in a manner similar to that for the risk ratio, using the patients with AS as the proband and determining the number of IBD cases among their relatives. The cross risk ratio was symmetric, so we did not consider the risk of AS among relatives of patients with IBD separately (113).

The degree of familiality within the patient groups was assessed for both AS and IBD with the kinship coefficient (KC), which is a genealogical index of familiality. It is technically defined as the probability that 2 randomly selected alleles at an autosomal locus, 1 from each individual, are inherited from a common ancestor. For this analysis the mean pairwise kinship coefficient (KC) was calculated for all of the patients with IBD and all of the patients with AS. The KC values were compared with the distribution of the mean KCs among 100,000 sets of control subjects who were matched to the patients in the same manner as described above for the risk analyses.The KC values are expressed as mean KCx100,000 (114).

5.5 Ethics

All subjects, both patients and their relatives, provided written informed consent. To ensure anonymity of the patients in these studies, the social security numbers of participants were encrypted by the Data Protection Commission of Iceland before being used in the analyses (115). These studies were approved by the National Bioethics Committee of Iceland (approval no. 98-059) and by the Icelandic Data Protection Authority (2001/36).

6 RESULTS

The results in this chapter refer to the four papers that have already been published in peer-reviewed international medical journals and are the basis of this thesis. Patients with psoriatic spondyloarthritis and other forms of secondary SPA were excluded from this study. For further details the reader is referred to the four articles at the end of this thesis

6.1 Prevalence and incidence of AS in Iceland

The population of Icelanders, 18 years and older, was 220.441 by the end of 2005, paper I. Prevalence calculations of AS are based on this number. The point prevalence of clinically diagnosed AS in Iceland was 127 (112-141) per 100.000 inhabitants (280 cases of AS). The minimum prevalence of AS in Iceland was 101 (88-114) per 100.000 inhabitants (223 cases of AS), when clinically diagnosed AS patients who were not evaluated and confirmed by the research group were excluted.

The prevalence for males and females was 132 and 71 per 100.000 inhabitants, respectively, giving a male:female ratio of 1.86. The crude annual incidence for the period 1947-2005 ranged from 0.44-5.48 per 100.000 inhabitants (figure 2).

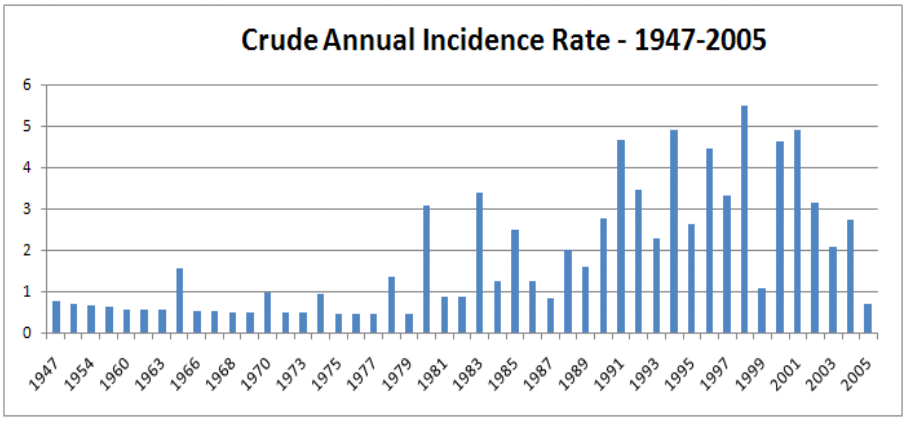
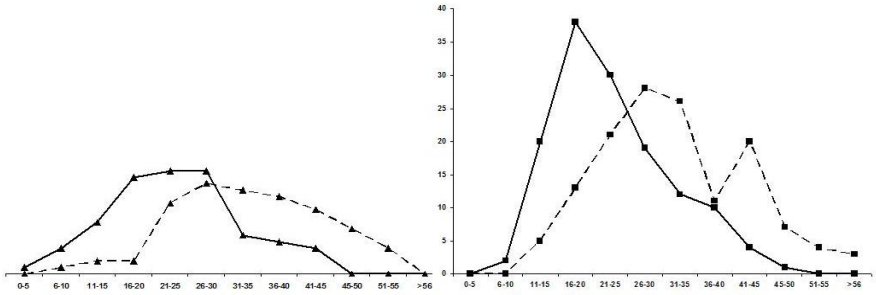


Figure 2: Annual incidence rate of AS

6.2 Demographics of AS

The demographic data are based on the 223 AS patients who fulfilled diagnostic criteria for AS, answered questionnaires and came for a

clinical interview. The data are presented in Table 3. There was no gender difference regarding the age of onset of symptoms, which was at the mean of 24 years for both sexes. In both sexes the onset of symptoms was before 30 years of age in 80% of cases. The diagnostic delay was not statistically different between the sexes, 8 and 10 years for males and females, respectively, although there was a trend for a longer diagnostic delay of correct diagnosis of AS in females (figure 3).



Onset of symptoms related to AS and age of diagnosis in 145 male (right) and 78 female (left) patients with AS. The full line shows the age of onset of symptoms related to AS, while the broken line shows age at diagnosis of AS.

Figure 3: Gender difference in onset of symptoms and diagnosis of AS.

The clinical characteristics were similar for both sexes (Table 3) as well as the prevalence of HLA-B27 haplotype, which was about 84%.

Table 3: Demographic data for 223 patients with AS.

Demographic data	Male n = 145	Female n = 78	p value
Age at onset of symptoms; mean ± SD years	23.6 ± 8.4	24.1 ± 8.9	0.55
Age at diagnosis of AS; mean ± SD years	32.1 ± 10.2	34.2 ± 10.1	0.13
Diagnostic delay; mean ± SD years	8.3 ± 7.7	9.6 ± 10.0	0.87
Clinical characteristics			
Limited chest expansion; n (%)	65 (44.8)	22 (28.2)	0.021
Modified Schober;mean ± SD cm	3.50 ± 1.63	3.84 ± 1.26	0.09
Limited flexion/extension of neck; n (%)	56 (38.6)	30 (38.5)	1
Limited rotation of neck; n (%)	67 (46.2)	35 (44.9)	0.89
Active peripheral arthritis; n (%)	18 (12.4)	15 (19.2)	0.23
HLA-B27 positive; n (%)	124 (85.5)	63 (80.8)	0.34

In more than half of the cases (59%) there was an insidious onset of symptoms of the disease, dominated by pain problems (Table 4).

Table 4: Self-reported symptoms by 223 AS patients

Symptoms of AS	Male N = 145 (%)	Female N =78 (%)	p-value
Pain problem	*133 (91.7)*	*74 (97.4)*	*0.15*
Joint inflammation	*64 (44.1)*	*52 (66.7)*	*0.0013*
Eye inflammation - iritis	*55 (37.9)*	*24 (30.8)*	*0.31*
Prostatitis	*39 (26.9)*	-	-

6.3 Arthritis in AS

Arthritis was seen in aproximately half (53%) of the patients at some time in the course of the disease. It was more common in female AS patients (67%) than in males (44%) (Table 4). The most common joints to be involved in descending order of frequency were the hip, the knee and the shoulder joints. Polyarthritis was reported in a quarter of the patients with no gender difference. Enthesitis was observed on the day of examination in 21% of the Achilles tendon in both sexes and in 6% and 12% of the plantar fascia in males and females, respectively.

6.4 Extra-articular manifestations in AS

Anterior uveitis or iritis was experienced by 38% of the males and 31% of the females in this nationwide cohort. In most case it was unilateral, however, 10% of the male and 2.6% of the female patients suffered from bilateral iritis. The second most common extra-articular manifestation was prostatitis, either chronic or recurrent, reported by 27% of the male AS patients. Cardiac manifestations with conduction disturbances or aortic insufficiency were rare in the present cohort of AS-patients.

Two and nine AS patients were diagnosed with Mb Crohn's or ulcerative colitis, respectively, simultaneously or following the onset of their AS. As previously stated patients with psoriatic SPA were excluded from the present study.

6.5 Familiality and AS risk for relatives

In figure 4 the clustering of AS patients in an extended family tree becomes apparent, when seen in this context. Here, the relationship of 18 patients spanning 10 generations is shown. Disease status is

known only for the later generations (filled squares or circles: Individuals with AS).

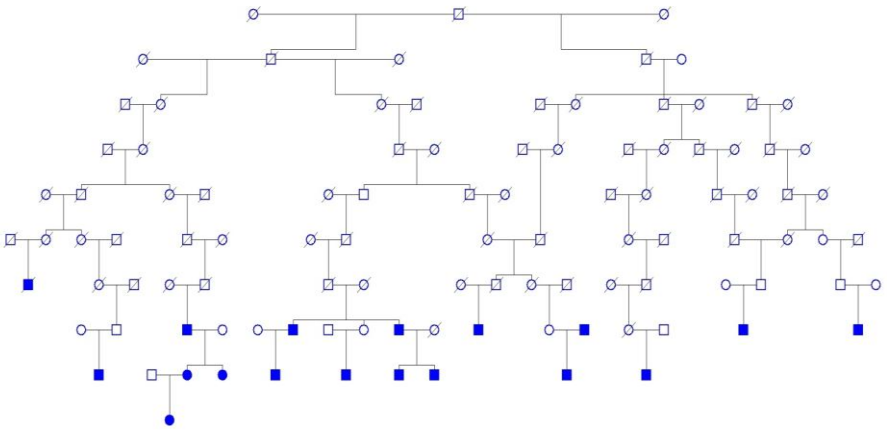


Figure 4: Pedigree of patients with AS extending over 10 generations

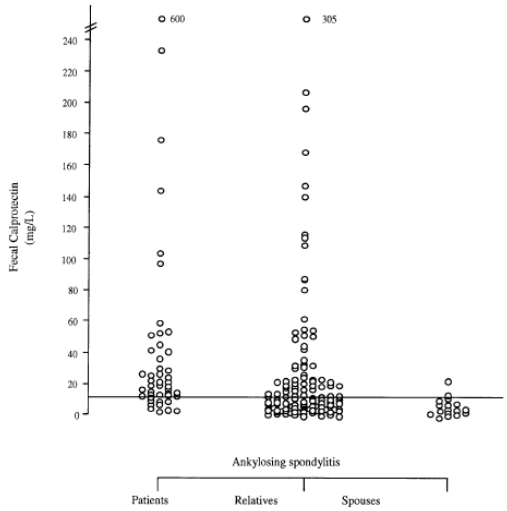
The RR estimates for disease in relatives of affected patients with AS are shown in table 5. The RR for AS were 75.5, 20.2 and 3.5 (all p values<0.0001) among FDRs, SDRs and TDRs of affected patients, respectively. The RR for the fourth-degree relatives did not reach significance levels: RR 1.04 (95% CI 0.58-1.66); p value=0.476.

Table 5: Risk ratio estimates of AS through four generations

The degree of relationship	Risk ratio (95% CI)	P-value	No of affected relatives
1o relatives	75.49 (60.19-93.87)	< 0.001	112
2o relatives	20.21 (15.15-30.20)	< 0.001	50
3o relatives	3.52 (2.20-5.67)	< 0.001	28
4o relatives	1.04 (0.58-1.66)	< 0.476	18

6.6 Intestinal inflammation and sacroilitis

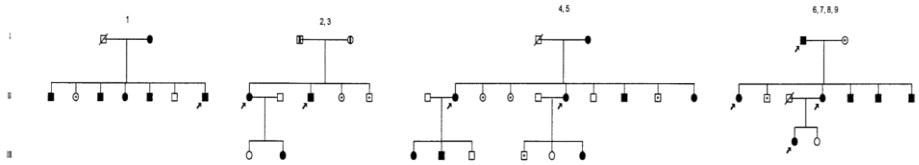
Calprotectin in the stools as an indicator of intestinal inflammation was significantly increased in both AS patients (median 21.5 mg/L) and their FDR (median 8.6 mg/L) compared with controls (p <0.0001). Fecal calprotectin in the spouses of the patients did not however demonstrate any differences from the controls. Fecal calprotectin was above the normal range in 78% of AS patients and 41% of FDR (Figure 5).



The horizontal line indicates upper normal limit for faecal calprotectin concentrations

Figure 5: Fecal calprotectin concentrations in patients with AS, FDR and spouses.

There was a similar prevalence of HLA-B27 among the AS-patients that had the fecal calprotectin measured as in the AS cohort as a whole. Of the 93 FDRs tested 57% were HLA-B27 positive. The calprotectin level was similar between the two groups of relatives, HLA-B27 positive and negative. Figure 6 shows four examples of pedigrees with relatives having increased or normal faecal calprotectin concentrations**.**



Shaded: subjects with increased fecal calprotectin concentrations.Dots: subjects with normal concentrations. Arrows: index cases of AS. Oblique line: deceased subjects. Vertical lines: subject was outside the age limits for the study. Blank boxes/circles: subject not studied.

Figure 6: Identified relatives having increased or normal fecal calprotectin concentrations

In the FDRs 41% had grade one changes on CT of SI-joints, either subchondral cysts or blurring of joint margins. Table 6 shows the overall results on the background of the HLA-B27 status of the subjects. There was no difference in the prevalence or type of

sacroiliac changes in regards to the HLA-B27 status of the relatives. Those relatives with increased fecal calprotectin were more likely to have increased mean total cystic diameter of subchondral cysts and increased blurring of joint margins compared with those relatives with normal fecal calprotectin. The CT changes of the SI-joints were independent of age and sex. The observed heritability in regards to calprotectin levels was 57%. There was an evidence for major additive gene influencing calprotectin as illustrated in table 7.

Table 6: Sacroiliac CT changes, HLA-B27 genotype and fecal calprotectin

	HLA B27 positive (n = 45)	HLA B27 negative (n = 36)	Raised fecal calprotectin (n = 44)	Normal fecal calprotectin (n = 41)
Subjects with subchondral cysts	19/45 (42%)	13/36 (36%)	21/44 (48%)	12/41 (29%)
Number of subchondral cysts (average number of cysts/relatives)	45 (1.0)	39 (1.1)	59 (1.3)	29 (0.7)
Mean total cystic diameter of sub-chondral cysts (95% CI)	2.0 mm (1.1-2.5)	2.1 mm (1.3-3.0)	2.9 mm (1.7-4.2 mm)	1.2 mm [a] (0.4-2.0 mm)
Blurring of joint margins	5/45 (11%)	5/36 (14%)	9/44 (20%)	1/41[b] (2%)

a Differs significantly (p = 0.026) from relatives with increased fecal calprotectin concentrations

b Differs significantly (p = 0.02) from relatives with increase fecal calprotectin concentrations

Table 7: Results of variance components analysis of the inheritance of calprotectin levels

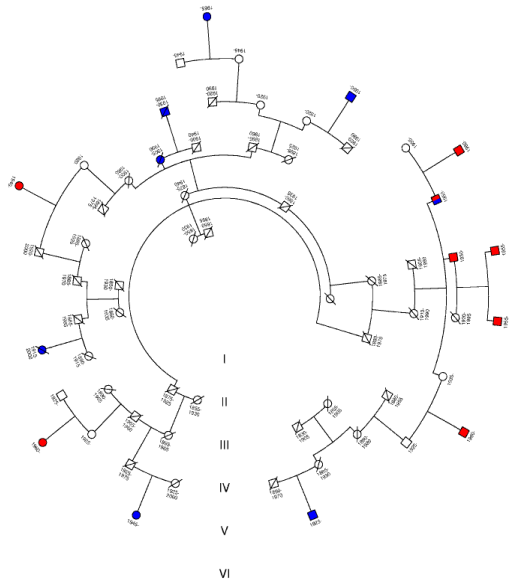
Model	Df	Additive component	Dominance component	Polygenic component	P-val vs H_0	P-val vs H_p	P-val H_{ad} vs
H_p	1			57%	$2x10^{-15}$		
H_a	2	73%		0%	$2x10^{-16}$	0.002	1
H_d	2		42%	55%	$8x10^{-15}$	0.4	0.003
H_{ad}	3	73%	0%	0%	$6x10^{-16}$	0.007	

6.7 Cross risk ratios of IBD and AS, a common genetic factor?

The cross-risk ratios for having IBD, were 3.0 and 2.1 in FDR and SDR of patients with AS, respectively, and they were also the same for having AS in FDR and SDR of patients with IBD. The estimates of cross-risk in relatives of patients with ulcerative colitis and in relatives of patients with Crohn's disease were comparable. For further details see Table 1 in paper IV. Figure 7 demonstrates a representative

pedigree of eight patients with AS and eight patients with IBD (one of these patients had both conditions) extending over six generations.

There was no increased risk for spouses of AS patients to develop AS, Crohn´s disease or colitis ulcerosa. However, there was an increased risk in spouses of patients with Crohn´s disease to develop Crohn´s disease (p=0.003), this did not apply to colitis ulcerosa.



Fourteen of the patients were related within and at a distance of six meioses and could be traced to a common pair of ancestors. The pedigrees demonstrated significant clustering of patients with AS and significant clustering of patients with IBD within families. Pedigree of individuals with AS and IBD extending over 6 generations. The pedigree shows patients with AS (red symbols; n = 8) and IBD (blue symbols; n = 8) where one individual had both conditions. Disease status is known only for the later generations. This pedigree was created with the use of the deCODE Genetics genealogy database. To protect the anonymity of the families, some of the unaffected relatives in the pedigree are not shown.

Figure 7: Individuals with AS and IBD over 6 generations

7 DISCUSSION

7.1 Prevalence and incidence

This study was a nationwide cross-sectional study of AS in Iceland, where the prevalence of HLA-B27 is high (15%) in the population (25). The data were collected systematically and all patients were examined by the same rheumatologist (AJG) by the end of 2005. The reported AS prevalence of 0.13% is lower than in all of the Nordic countries (22, 23,116) and also compared to countries in Southern Europe (20, 117, 118). Intrestingly in Northen Norway the population prevalence of HLA-B27 is high, and the prevalence of AS is high compared to studies from populations with low HLA-B27 prevalence (10). This discrepancy between AS prevalence in Northern Norway and Iceland is not well understood but could be due to other polygenic causes that are not apparent at the moment, or due to the design of the present study.

The crude annual incidence of AS was very low early on in the middle of the 20th century but increased more than ten fold later on during the study period, from 0.44 to 5.48 per 100.000 inhabitants/year. The rise in incidence during the latter part of the study period is probably due to better access to rheumatologic service and better diagnostic imaging methods, in particular the development of and availability of CT and MRI scanners. In spite of this the incidence in Iceland is still lower than reported in Norway (10), Finland (22), Minnesota (119), but higher than reported in Greece (118).

A possible explanation for these differences is that in our study the cohort was exclusively AS patients as psoriasis related SPA and other forms of secondary SPA were excluded. The difference in prevalence could also be due to the fact that our study is nationwide while others are regional studies that might therefore be biased by regional recruitment giving higher figures for prevalence and incidence (10,120). However, there was no difference between rural and urban areas regarding prevalence and incidence in our study. The low gender difference in the current study of less than 2:1 male:female ratio, is different from previously reported. There are studies that have reported 10:1 excess for males (121). The trend has though been lately for a more even distribution of AS between the sexes of 2-3:1 (122).

7.2 Demographic data

The clinical characteristics of the Icelandic AS patients was similar to what others have reported with a majority of around 80% having insidous onset of low back and buttock pain with stiffness of inflammatory character before the age of 30 years, and half of the patients having large joint arthritis of hip, knee, or shoulder joints. There was no significant gender difference between onset of symptoms and diagnostic delay, which is in contrast to what others have found (123). However, female patients had less frequently limited thoracal movement, while they had more often evidence of peripheral arthritis.

7.3 Arthritis in AS

Arthritis of the hip joints was most common in the AS patient even though peripheral arthritis of knees, the shoulder joints and the small joints of the feet are also frequently a part of the clinical picture in AS. Peripheral arthritis is more frequent among female AS patients in our study, indeed peripheral arthritis was more common in our study than conventionally reported (124). Arthritis of the hips can be particularily deleterious for the AS patients, as it often causes flexion contractures of the joints and adds a burden to the already stiff spine of these patients.

7.4 Extra-articular manifestations in AS

Iritis is the best known extra-articular manifestation of AS. In our cohort it was more common in the male population, 38% versus 31% in females. Iritis causes painful, red photophobic eye with blurred vision and because of the severe symptoms the patient usually goes to an ophthalmologist without a delay. Usually the acute attack can be effectively treated with topical steroid and anti-cholinergic agents. Occasionally the inflammation can become more severe and resistant to treatment and result in secondary glaucoma with permanent loss of vision. In our cohort the iritis was usually unilateral, but in 10% of the males and 2.6% of the females it was bilateral.

The second most common extra-articular manifestation was prostatitis either chronic or recurrent in the AS male patients. Recurrent prostatitis has been reported to be a frequent companion of AS and synchronous with flares of the disease (48, 125).

It has been well known that conduction abnormalities of the heart are more common in AS patients than in the general population. A genetically associated HLA-B27 linked cardiac syndrome has been reported, consisting of conduction disturbances and aortic valve insufficiency (126). AS has also been linked with premature ischemic cardiac disease, and increased cardiac mortality like in many other chronic inflammatory diseases (77). Lung fibrosis that has been reported to be of higher prevalence in AS patients was not seen in our cohort.

7.5 Familiality and AS risk for relatives

There seems to be a correlation between the prevalence of HLA-B27 in a given population and the prevalence of AS, which suggests that HLA-B27 mediates an important genetic link that has to be represented in the population in order for the AS disease to occur (125,127). AS patients with HLA-B27 are more likely to present with the disease at a younger age and have more often polyarthritis and iritis than those who are HLA-B27 negative. Being homozygous for HLA-B27 however does not affect the clinical picture of AS (128). The prevalence of AS in our study population was 0.71% for those who were HLA-B27 positive but only 0.03% for those who were negative. In our study we have rather low prevalence of AS in spite of high prevalence of HLA-B27 in the population. We do not have any good explanation for this discrepancy. It is though possible that the recruitment method we employd was at fault and as a consequence we may have missed out on mild cases of AS that did not yet fulfill the diagnostic criteria of AS (129,130).

Employing the genealogy database, we demonstrated that there is a very strong heritability of AS in Iceland over three generations, but it seems to vanish with the fourth generation. The RR for FDR in our cohort is 75. Increased risk of AS in FDR has also been shown in other studies (131-133). The risk of AS in SDR and TDR was increased 20 and 3.5 fold respectively, while De Blecourt and colleques reported 10 and 7 fold increased risk of AS in SDR and TDR, respecively (134). This observation of markedly increased risk of AS in close relatives of AS patients does not apply to any other rheumatic disease that we know of and certainly not for rheumatoid arthritis (135) and psoriatic arthritis (77). This observation suggests that genetic factors play a more important role in the development of AS than in other rheumatic diseases. We looked at if there was an

increased risk for offsprings if the father or mother had AS, but there was no difference in that regard. Still, here seems to be a multigenetic cause for AS that though in majority of cases needs HLA-B27 to be present to develop AS. Thus, multiple genetic components and environmental factors need to interact for this disease to occur.

7.6 Intestinal inflammation and sacroiliitis

In paper III we observed a high prevalence of intestinal inflammation in FDR of AS patients, detected by calprotectin measurements of fecal sample. Furthermore, there was also an association between this intestinal inflammation and CT changes of the sacroiliac joints in relatives of patients with confirmed AS. We conclude that there is a genetic component that makes the relatives of AS patients susceptible to intestinal inflammation. There is a resemblance between the intestinal inflammation of patients with AS and CD, that points to a common pathogenesis in both diseases (105, 136, 137). The intestinal inflammation seems to be genetically determined in both diseases (104, 138). In FDR of patients with CD there is a similar subclinical intestinal inflammation as in FDR of AS pateints (138). The pattern of inheritance in both groups is consistent with the presence of a major additive gen (139). The intestinal inflammation in FDR seems not to be dependent on the HLA-B27 genotype. It can be postulated that FDRs of AS and CD have in common a genetic risk factor. The clinical course of the spondyloarthropathy associated with AS and CD are often very similar (140). There is a transgenic HLA-B27 rat model that when in germ free environment is healthy, but when exposed to certain bacteria, they develop colitis and spondylarthritis (141). This disease takes resemblance from both AS and CD (142), the bacterial flora of the gut seems to play an important role in the pathogenesis of this condition.

7.7 Cross risk ratios of IBD and AS, a common genetic factor?

In paper IV we provide evidence for the postulate that there is a common genetic factor in IBD and AS. It has been shown in our study and by others that the heritability for AS is very strong, even in TDRs, while it is weaker for IBD, paper IV. Furthermore, the AS patients often have subclinical intestinal inflammation that is often indistinguishable from that seen in IBD (137, 143). The methodology used in paper IV where we use the genealogy database of many generations to identify common ancestors and relatives of all patients diagnosed with AS and

IBD and compare it with population based controls is very helpful to discover the common denominator of these diseases (144-146). The important finding in this study is the elevated cross-risk ratio between IBD and AS, found for both FDR´s and SDR´s that is very suggestive for a common genetic factor for AS and IBD. This is supported by the finding of similar micro- and macroscopic findings in ileo-colonoscopic studies and biopsies of patients with AS and CD (147). Twin studies have shown that there is a greater risk for an AS patient to get CD than CU (148-150). This relationship applies also for FDR´s with subclinical intestinal inflammation (138). Relatives of both AS and CD patients have higher prevalence of increased intestinal permeability and inflammation than relatives of patients with CU (151). Until lately IL23R was the only shared genetic loci found for AS and IBD (152), most likely operating through intestinal immunology (153). This thesis provides a strong evidence for a common genetic factor in the pathogenesis of AS and IBD.

8 FUTURE PERSPECTIVES

The important question to answer in the future is: Will we be able to diagnose AS earlier in its course and with greater accuracy than before? The diagnostic delay of 8-10 years is not acceptable, as during that time many patients will develop physical impairment and severe irreversible spinal and joint disease. It is important to increase the awareness of young doctors of the disease and to educate the public about inflammatory back pain and the importance of family history regarding AS. The important tool for diagnostic purposes in AS is magnetic resonance imaging (MRI), which shows the bone marrow oedema adjacent to the sacroiliac joints and at the enthesis in the spine that are the early changes in AS. It is more important now than before to diagnose AS early and accurately because we can now offer patients effective treatment. The recognition of inflammatory back pain by general practitioners that are frequently exposed to young people complaining of back problems is paramount. The difference in mechanical versus inflammatory back pain as presented by Calin and colleagues (154) and later modified by Rudawaleit and colleagues (155) are very important guidelines to doctors in their diagnostic effort to differentiate in a correct way the common complaint of back pain.

The role of bacteria in the aetiology and pathogenesis of both AS and IBD has been investigated by several authors through the years. It has been suspected for a long time that *Klebisella* in the gut might in a susceptible individual be responsible for the initiation of the disease. *Klebsiella* in increased numbers has been recovered from stools of AS patients. There seems to be increased humeral and cellular response to *Klebsiella* in AS and it has also been linked with exacerbation of the disease (156-158). Other investigators have found CD4 positiveT-cells reactive to E-coli in the joint fluid of AS patients (159). Others have found a connection between bacteroides species and both AS and IBD (160).

Even though the TNF∝-inhibitors are very effective in alleviating the symptoms of AS, it is still disputed whether these drugs will change the course of the disease, delay syndesmophyte formation and the development of ankylosis. This is similar to the discussion concerning the effects of DMARDs on RA in the past, whether the

drugs of that time prevented the development of erosions. Lately there seems to be evolving evidence that continuous NSAID use can delay the formation of syndesmophytes in AS. Today we do not know for sure if the TNF∝-inhibiting therapy that leads to a symptomatic cure in AS will at the same time, stop the progression of the bony changes that accompany AS or if the disease progresses in spite of symptomatic remission (161). However, resently there are indications that especially with early initiation of TNF∝-inhibiting therapy there will be a reduction of radiographic progression in AS (162).

9 CONCLUSIONS

In Iceland, the mean age at onset of symptoms in ankylosing spondylitis was 25 years, about 80% of the patients experienced the onset of symptoms before the age of 30 years and almost all had onset of symptoms before the age of 45 years The diagnostic delay was 8-10 years. Back pain was reported to be the presenting symptom in more than 90% of patients. The background population in Iceland carries the HLA-B27 antigen with a prevalence of 15%, which is high in comparison with neighbouring countries. Therefore it is of some surprise to find that the incidence of AS was only 0.44 cases per 100,000 inhabitants in the early period of the study, increasing up to 5.48 later in the study period, which is among the lowest recorded. The prevalence of the disease of 0.13% is compared with other countries low as well. The prevalence of HLA-B27 in the study group was 84%. The male/female ratio of 1.86 is in accordance with recent publications although the sex ratio is more even than in most other studies. History of peripheral arthritis was found in 52% of the cohort and more common in the female population and was mainly in the larger joints of the lower extremities.

The extra-articular manifestations of AS were primarily anterior uveitis, which was more common in males, 38% versus 31% in females, and recurrent or chronic prostatitis was also found in more than a quarter of the male participants.

The pedigrees of AS patients spanning 10 generations shows significant clustering of the disease in families. The relative risk of family members of an affected patient is very high, the highest reported for any given disease to this point. The RR for FDR is 75, and for a SDR it is 20 and lastly for TDR it is still significant at 3.5. However, in fourth degree relatives the risk has vanished. The father of an index case had an increased risk of developing AS. Furthermore there is a high cross over relative risk for relatives of developing AS and IBD and vice versa. For FDR the RR it is 3 and for SDR it is 2, but for TDR the RR is down to is 1. Subclinical intestinal inflammation in asymptomatic relatives of AS patients correlates with changes in the SI joints that can be the first indication of the development of AS in these relatives.

REFERENCES

1. Braun J, Khan MA, Sieper J. Enthesitis and ankylosis in spondyloarthropathy: what is the target of the immune response? Annals of the Rheumatic Diseases. 2000;59(12):985-94.

2. Feldtkeller E, Lemmel E-M, Russell AS. Ankylosing spondylitis in the pharaohs of ancient Egypt. Rheumatology International. 2003;23(1):1-5.

3. Von Bechterew W. Steifigkeit der Wirbelsäule und ihre Verkrümmung als besondere Erkrankungsform. Neurologisches Zentralblatt. 1893;12:426-34.

4. Kellgren JH. The epidemiology of chronic rheumatism: Oxford Blackwell Scientific; 1963.

5. Linden SVD, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. Arthritis & Rheumatism. 1984;27(4):361-8.

6. Amor B, Dougados M, Listrat V, Menkes CJ, Roux H, Benhamou C, et al. Are classification criteria for spondylarthropathy useful as diagnostic-criteria. Revue du rhumatisme. 1995;62(1):10-5.

7. Dougados M, Vanderlinden S, Juhlin R, Huitfeldt B, Amor B, Calin A, et al. The european-spondylarthropathy-study-group preliminary criteria or the classification of spondylarthropathy. Arthritis and Rheumatism. 1991;34(10):1218-27.

8. Rudwaleit M, Landewe R, van der Heijde D, Listing J, Brandt J, Braun J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. Annals of the Rheumatic Diseases. 2009;68(6):770-6.

9. Rudwaleit M, van der Heijde D, Landewe R, Akkoc N, Brandt J, Chou CT, et al. The Assessment of SpondyloArthritis international Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Annals of the Rheumatic Diseases. 2011;70(1):25-31.

10. Bakland G, Nossent HC, Gran JT. Incidence and prevalence of ankylosing spondylitis in Northern Norway. Arthritis & Rheumatism. 2005;53(6):850-5.

11. Alexeeva L, Krylov M, Vturin V, Mylov N, Erdesz S, Benevolenskaya L. Prevalence of spondyloarthropathies and HLA-B27 in the native population of Chukotka, Russia. Journal of Rheumatology. 1994;21(12):2298-300.

12. Krylov M, Erdesz S, Alexeeva L, Benevolenskaya L, Arnett FC, Reveille JD. HLA class II and HLA-B27 oligotyping in two Siberian native population groups. Tissue Antigens. 1995;46(5):382-6.

13. Gofton JP. Epidemiology, tissue-type antigens and Bechterews syndrome (ankylosing-spondylitis) in various ethnical populations. Scandinavian Journal of Rheumatology. 1980: Suppl 32;166-8.

14. Bhatia K, Prasad ML, Barnish G, Koki G. Antigen and haplotype frequencies at 3 human-leukocyte antigen loci (HLA-A, HLA-B, HLA-C) in the Pawaia of Papua-New-Guinea. American Journal of Physical Anthropology. 1988;75(3):329-40.

15. Johnsen K, Gran JT, Dale K, Husby G. The prevalence of ankylosing-spondylitis among Norwegian Samis (Lapps). Journal of Rheumatology. 1992;19(10):1591-4.

16. Khan MA, Braun WE, Kushner I, Grecek DE, Muir WA, Steinberg AG. HLA-B27 in ankylosing-spondylitis - differences in frequency and relative risk in American blacks and caucasians. Journal of Rheumatology. Suppl 1977;3:39-43.

17. Olivieri I, Barozzi L, Padula A, De Matteis M, Pavlica P. Clinical manifestations of seronegative spondylarthropathies. European Journal of Radiology. 1998;27(Suppl 1):S3-6.

18. Boyer GS, Templin DW, Cornonihuntley JC, Everett DE, Lawrence RC, Heyse SE, et al. Prevalence of spondyloarthropathies in Alaskan eskimos. Journal of Rheumatology. 1994;21(12):2292-7.

19. Anagnostopoulos I, Zinzaras E, Alexiou I, Papathanasiou AA, Davas E, Koutroumpas A, et al. The prevalence of rheumatic diseases in central Greece: a population survey. Bmc Musculoskeletal Disorders. 2010;11:98

20. Trontzas P, Andrianakos A, Miyakis S, Pantelidou K, Vafiadou E, Garantziotou V, et al. Seronegative spondyloarthropathies in Greece: a population-based study of prevalence, clinical pattern, and management. The Esordig study. Clinical Rheumatology. 2005;24(6):583-9.

21. Hanova P, Pavelka K, Holcatova I, Pikhart H. Incidence and prevalence of psoriatic arthritis, ankylosing spondylitis, and reactive arthritis in the first descriptive population-based study in the Czech Republic. Scandinavian Journal of Rheumatology. 2010;39(4):310-7.

22. Kaipianen-Seppanen O, Aho K, Heliovaara M. Incidence and prevalence of ankylosing spondylitis in Finland. Journal of Rheumatology. 1997;24(3):496-9.

23. Gran JT, Husby G, Hordvik M. Prevalence of ankylosing-spondylitis in males and females in a young middle-aged population of Tromso, Northern Norway. Annals of the Rheumatic Diseases. 1985;44(6):359-67.

24. Carter ET, McKenna CH, Brian DD, Kurland LT. Epidemiology of Ankylosing spondylitis in Rochester, Minnesota, 1935-1973. Arthritis & Rheumatism. 1979;22(4):365-70.

25. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Annals of the Rheumatic Diseases. 2002;61(Suppl 3):8-18.

26. Olivieri I, Salvarani C, Cantini F, Ciancio G, Padula A. Ankylosing spondylitis and undifferentiated spondyloarthropathies: a clinical review and description of a disease subset with older age at onset. Current opinion in rheumatology. 2001;13(4):280-4.

27. Gran JT, Husby G. Clinical, epidemiologic, and therapeutic aspects of ankylosing spondylitis. Current opinion in rheumatology. 1998;10(4):292-8.

28. Brown MA, Crane AM, Wordsworth BP. Genetic aspects of susceptibility, severity, and clinical expression in ankylosing spondylitis. Current Opinion in Rheumatology. 2002;14(4):354-60.

29. Ahearn JM, Hochberg MC. Epidemiology and genetics of ankylosing spondylitis. Journal of Rheumatology - Supplement. 1988;16 Sep;16:22-8.

30. Banares A, Hernandez-Garcia C, Fernandez-Gutierrez B, Jover JA. Eye involvement in the spondyloarthropathies. Rheumatic diseases clinics of North America. 1998;24(4):771-84.

31. Young H, Perez N, Martin JC, Goodfellow RM, Rhys-Dillon CC. A study of the clinical relationship between ankylosing spondylitis and prostatitis. Rheumatology. 2008;47:1167.

32. Pollanen R, Sillat T, Pajarinen J, Levon J, Kaivosoja E, Konttinen YT. Microbial antigens mediate HLA-B27 diseases via TLRs. Journal of Autoimmunity. 2009;32(3-4):172-7.

33. Rudwaleit M, Baeten D. Ankylosing spondylitis and bowel disease. Best Practice & Research in Clinical Rheumatology. 2006;20(3):451-71.

34. Arnason JA, Patel AK, Rahko PS, Sundstrom WR. Transthoracic and transesophageal echocardiographic evaluation of the aortic root and subvalvular structures in ankylosing spondylitis. Journal of Rheumatology. 1996;23(1):120-3.

35. Casserly IP, Fenlon HM, Breatnach E, Sant SM. Lung findings on high-resolution computed tomography in idiopathic ankylosing spondylitis - Correlation with clinical findings, pulmonary function testing and plain radiography. British Journal of Rheumatology. 1997;36(6):677-82.

36. Forouzesh S, Bluestone R. Clinical spectrum of ankylosing-spondylitis. Clinical Orthopaedics and Related Research. 1979(143):53-8.

37. Hamilton MG, Macrae ME. Atlantoaxial dislocation as the presenting symptom of ankylosing-spondylitis. Spine. 1993;18(15):2344-6.

38. Roussou E, Sultana S. Spondyloarthritis in women: differences in disease onset, clinical presentation, and Bath Ankylosing Spondylitis Disease Activity and Functional indices (BASDAI and BASFI) between men and women with spondyloarthritides. Clinical Rheumatology. 2011;30(1):121-7.

39. van der Linden S, van der Heijde D. Ankylosing spondylitis. Clinical features. Rheumatic diseases clinics of North America. 1998;24(4):663-76.

40. Colbert RA, Deodhar AA, Fox D, Gravallese EM, Khan MA, McGonagle D, et al. Entheses and bones in spondyloarthritis: 2008 Annual Research and Education Meeting of the Spondyloarthritis Research and Therapy Network (SPARTAN). Journal of Rheumatology. 2009;36(7):1527-31.

41. D'Agostino MA, Palazzi C, Olivieri I. Entheseal involvement. Clinical and Experimental Rheumatology. 2009;27(4):S50-S5.

42. Bodaghi B, Cassoux N, Wechsler B, Hannouche D, Fardeau C, Papo T, et al. Chronic severe uveitis - Etiology and visual outcome in 927 patients from a single center. Medicine. 2001;80(4):263-70.

43. Chang JH, McCluskey PJ, Wakefield D. Acute anterior uveitis and HLA-B27. Survey of Ophthalmology. 2005;50(4):364-88.

44. Wakefield D, Chang JH, Amjadi S, Maconochie Z, Abu el-Asrar A, McCluskey P. What Is New HLA-B27 Acute Anterior Uveitis? Ocular Immunology and Inflammation. 2011;19(2):139-44.

45. Khan MA, Kushner I, Braun WE. Comparison of clinical features in HLA-B27 positive and negative patients with ankylosing-spondylitis. Arthritis and Rheumatism. 1977;20(4):909-12.

46. Rashid T, Ebringer A. Ankylosing spondylitis is linked to Klebsiella--the evidence. Clinical rheumatology. 2007;26(6):858-64.

47. Ahmadi K, Wilson C, Tiwana H, Binder A, Ebringer A. Antibodies to Klebsiella pneumoniae lipopolysaccharide in patients with ankylosing spondylitis. British Journal of Rheumatology. 1998;37(12):1330-3.

48. Mason RM, Murray RS, Oates JK, Young AC. Prostatitis and ankylosing spondylitis. British Medical Journal. 1958;29;1(5073):748-51.

49. De Keyser F, Elewaut D, De Vos M, De Vlam K, Cuvelier C, Mielants H, et al. Bowel inflammation and the spondyloarthropathies. Rheumatic Disease Clinics of North America. 1998;24(4):785-813.

50. Leirisalo-Repo M, Turunen U, Stenman S, Helenius P, Seppala K. High-frequency of silent inflammatory bowel-disease in spondylarthropathy. Arthritis and Rheumatism. 1994;37(1):23-31.

51. Leirisalo-Repo M. Prognosis, course of disease, and treatment of the spondyloarthropathies. Rheumatic Disease Clinics of North America. 1998;24(4):737-51.

52. Palm O, Moum B, Ongre A, Gran JT. Prevalence of ankylosing spondylitis and other spondyloarthropathies among patients with inflammatory bowel disease: A population study (The IBSEN study). Journal of Rheumatology. 2002;29(3):511-5.

53. Peeters H, Vander Cruyssen B, Laukens D, Coucke P, Marichal D, Van den Berghe M, et al. Radiological sacroiliitis, a hallmark of spondylitis, is linked with CARD15 gene polymorphisms in patients with Crohn's disease. Annals of the Rheumatic Diseases. 2004;63(9):1131-4.

54. Purrmann J, Zeidler H, Bertrams J, Juli E, Cleveland S, Berges W, et al. HLA antigens in ankylosing-spondylitis associated with Crohns-disease - increased frequency of the HLA phenotype B27,B44. Journal of Rheumatology. 1988;15(11):1658-61.

55. de Vlam K, Mielants H, Cuvelier C, De Keyser F, Veys EM, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease: Prevalence and HLA association. Journal of Rheumatology. 2000;27(12):2860-5.

56. Demetter P, Van Huysse JA, De Keyser F, Van Damme N, Verbruggen G, Mielants H, et al. Increase in lymphoid follicles and leukocyte adhesion molecules emphasizes a role for the gut in spondyloarthropathy pathogenesis. Journal of Pathology. 2002;198(4):517-22.

57. Cuvelier C, Barbatis C, Mielants H, Devos M, Roels H, Veys E. Histopathology of intestinal inflammation related to reactive arthritis. Gut. 1987;28(4):394-401.

58. Mielants H, Veys EM, Cuvelier C, Devos M, Goemaere S, Declercq L, et al. The evolution of spondyloarthropathies in relation to gut histology .2. Histological aspects. Journal of Rheumatology. 1995;22(12):2273-8.

59. Strobel ES, Fritschka E. Renal diseases in ankylosing spondylitis: review of the literature illustrated by case reports. Clinical rheumatology. 1998;17(6):524-30.

60. Braun J, Breban M, Maksymowych WP. Therapy for ankylosing spondylitis: new treatment modalities. Best Practice & Research in Clinical Rheumatology. 2002;16(4):631-51.

61. Maksymowych WP, Brown MA. Genetics of ankylosing spondylitis and rheumatoid arthritis: where are we at currently, and how do they compare? Clinical & Experimental Rheumatology. 2009;27(4 Suppl 55):S20-5.

62. Khan MA, Mathieu A, Sorrentino R, Akkoc N. The pathogenetic role of HLA-B27 and its subtypes. Autoimmunity Reviews. 2007;6(3):183-9.

63. Gonzalez Roces S, Alvarez MV, Gonzalez S, Dieye A, Makni H, Woodfield DG, et al. HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis. Tissue Antigens. 1997;49(2):116-23.

64. Damato M, Fiorillo MT, Carcassi C, Mathieu A, Zuccarelli A, Bitti PP, et al. Relevance of residue-116 of HLA-B27 in determining susceptibility to ankylosing-spondylitis. European Journal of Immunology. 1995;25(11):3199-201.

65. Brown MA, Wordsworth BP, Reveille JD. Genetics of ankylosing spondylitis. Clinical & Experimental Rheumatology. 2002;20(6 Suppl 28):S43-9.

66. Reveille JD. The genetic basis of ankylosing spondylitis. Current opinion in rheumatology. 2006;18(4):332-41.

67. Laval SH, Timms A, Edwards S, Bradbury L, Brophy S, Milicic A, et al. Whole-genome screening in ankylosing spondylitis: Evidence of non-MHC genetic-susceptibility loci. American Journal of Human Genetics. 2001;68(4):918-26.

68. Zhang G, Luo JC, Bruckel J, Weisman MA, Schumacher HR, Khan MA, et al. Genetic studies in familial ankylosing spondylitis susceptibility. Arthritis and Rheumatism. 2004;50(7):2246-54.

69. Miceli-Richard C, Zouali H, Said-Nahal R, Lesage S, Merlin F, de Toma C, et al. Significant linkage to spondyloarthropathy on 9q31-34. Human Molecular Genetics. 2004;13(15):1641-8.

70. Carter KW, Pluzhnikov A, Timms AE, Miceli-Richard C, Bourgain C, Wordsworth BP, et al. Combined analysis of three whole genome linkage scans for Ankylosing Spondylitis. Rheumatology. 2007;46(5):763-71.

71. Brown MA. Genetics of ankylosing spondylitis. Current Opinion in Rheumatology. 2010;22(2):126-32.

72. Breban M. Animal models and in vitro models for the study of aetiopathogenesis of spondyloarthropathies. Baillieres Clinical Rheumatology. 1998;12(4):611-26.

73. Breban M, Hacquard-Bouder C, Falgarone G. Animal models of HLA-B27-associated diseases. Current Molecular Medicine. 2004;4(1):31-40.

74. Braun J, van den Berg R, Baraliakos X, Boehm H, Burgos-Vargas R, Collantes-Estevez E, et al. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. Annals of the Rheumatic Diseases. 2011;70(6):896-904.

75. Zochling J, van der Heijde D, Burgos-Vargas R, Collantes E, Davis JC, Dijkmans B, et al. ASAS/EULAR recommendations for the management of ankylosing spondylitis. Annals of the Rheumatic Diseases. 2006;65(4):442-52.

76. Lehtinen K. Mortality and causes of death in 398 patients admitted to hospital with ankylosing-spondylitis. Annals of the Rheumatic Diseases. 1993;52(3):174-6.

77. Zochling J, Braun J. Mortality in ankylosing spondylitis. Clinical & Experimental Rheumatology. 2008;26(5 Suppl 51):S80-4.

78. Szabo SM, Levy AR, Rao SR, Kirbach SE, Lacaille D, Cifaldi M, et al. Increased Risk of Cardiovascular and Cerebrovascular Diseases in Individuals With Ankylosing Spondylitis A Population-Based Study. Arthritis and Rheumatism. 2011;63(11):3294-304.

79. Song IH, Poddubnyy DA, Rudwaleit M, Sieper J. Benefits and risks of ankylosing spondylitis treatment with nonsteroidal antiinflammatory drugs. Arthritis & Rheumatism. 2008;58(4):929-38.

80. Calin A, Elswood J. A prospective nationwide cross-sectional study of nsaid usage in 1331 patients with ankylosing-spondylitis. Journal of Rheumatology. 1990;17(6):801-3.

81. Poddubnyy D, Rudwaleit M, Haibel H, Listing J, Marker-Hermann E, Zeidler H, et al. Effect of non-steroidal anti-inflammatory drugs on radiographic spinal progression in patients with axial spondyloarthritis: results from the German Spondyloarthritis Inception Cohort. Annals of the Rheumatic Diseases. 2012;71(10):1616-22.

82. van der Heijde D, Baraf HSB, Ramos-Remus C, Calin A, Weaver AL, Schiff M, et al. Evaluation of the efficacy of etoricoxib in ankylosing spondylitis - Results of a fifty-two-week, randomized, controlled study. Arthritis and Rheumatism. 2005;52(4):1205-15.

83. Dougados M, Behier JM, Jolchine I, Calin A, van der Heijde D, Olivieri I, et al. Efficacy of celecoxib, acyclooxygenase 2-specific inhibitor, in the treatment of ankylosing spondylitis - A six-week controlled study with comparison against placebo and against a conventional nonsteroidal antiinflammatory drug. Arthritis and Rheumatism. 2001;44(1):180-5.

84. Chen JM, Liu C. Is Sulfasalazine effective in ankylosing spondylitis? A systematic review of randomized controlled trials. Journal of Rheumatology. 2006;33(4):722-31.

85. Dougados M, Vanderlinden S, Leirisalorepo M, Huitfeldt B, Juhlin R, Veys E, et al. Sulfasalazine in the treatment of spondylarthropathy - a randomized, multicenter, double-blind, placebo-controlled study. Arthritis and Rheumatism. 1995;38(5):618-27.

86. Clegg DO, Reda DJ, Abdellatif M. Comparison of sulfasalazine and placebo for the treatment of axial and peripheral articular manifestations of the seronegative spondylarthropathies - A department of veterans affairs cooperative study. Arthritis and Rheumatism. 1999;42(11):2325-9.

87. Gonzalez-Lopez L, Garcia-Gonzalez A, Vazquez-Del-Mercado M, Munoz-Valle JF, Gamez-Nava JI. Efficacy of methotrexate in ankylosing spondylitis: A randomized, double blind, placebo controlled trial. Journal of Rheumatology. 2004;31(8):1568-74.

88. van Denderen JC, van der Paardt M, Nurmohamed MT, de Ryck Y, Dijkmans BAC, van der Horst-Bruinsma IE. Double blind, randomised, placebo controlled study of leflunomide in the treatment of active ankylosing spondylitis. Annals of the Rheumatic Diseases. 2005;64(12):1761-4.

89. Sieper J. Developments in the scientific and clinical understanding of the spondyloarthritides. Arthritis Research & Therapy. 2009;11(1):208

90. Baraliakos X, Brandt J, Listing J, Haibel H, Sorensen H, Rudwaleit M, et al. Outcome of patients with active ankylosing spondylitis after two years of therapy with etanercept: Clinical and magnetic resonance imaging data. Arthritis & Rheumatism-Arthritis Care & Research. 2005;53(6):856-63.

91. McLeod C, Bagust A, Boland A, Dagenais P, Dickson R, Dundar Y, et al. Adalimumab, etanercept and infliximab for the treatment of ankylosing spondylitis: A systematic review and economic evaluation. Health Technology Assessment. 2007;11(28):1-158, III-IV.

92. Sieper J. Developments in therapies for spondyloarthritis. Nature Reviews Rheumatology. 2012;8(5):280-7.

93. Bakland G, Gran JT, Nossent JC. Increased mortality in ankylosing spondylitis is related to disease activity. Annals of the Rheumatic Diseases. 2011;70(11):1921-5.

94. Love TJ, Gudbjornsson B, Gudjonsson JE, Valdimarsson H. Psoriatic arthritis in Reykjavik, Iceland: Prevalence, demographics, and disease course. Journal of Rheumatology. 2007;34(10):2082-8.

95. Moll JMH, Wright V. Objective clinical study of chest expansion. Annals of the Rheumatic Diseases. 1972;31(1):1-8.

96. Guo SW. Inflation of sibling recurrence-risk ratio, due to ascertainment bias and/or overreporting. American Journal of Human Genetics. 1998;63(1):252-8.

97. Reynisdottir I, Gudbjartsson DF, Johannsson JH, Manolescu I, Kristjansson K, Stefansson K, et al. A genetic contribution to inflammatory bowel disease in Iceland: a genealogic approach. Clinical Gastroenterology & Hepatology.2(9):806-12.

98. Bjornsson S. Inflammatory bowel-disease in Iceland during a 30-year period, 1950-1979. Scandinavian Journal of Gastroenterology. 1989;170:47-9.

99. Bjornsson S, Johannsson JH, Oddsson E. Inflammatory bowel disease in Iceland, 1980-89 - A retrospective nationwide epidemiologic study. Scandinavian Journal of Gastroenterology. 1998;33(1):71-7.

100. Bjornsson S, Johannsson JH. Inflammatory bowel disease in Iceland, 1990-1994: a prospective, nationwide, epidemiological study. European Journal of Gastroenterology & Hepatology. 2000;12(1):31-8.

101. Bjarnason I, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology.104(6):1832-47.

102. De Vos M. Spondyloarthropathy and gut inflammation. Journal of Pediatric Gastroenterology and Nutrition. 2004;39:S736-S7.

103. Hayllar J, Smith T, Macpherson A, Price AB, Gumpel M, Bjarnason I. Nonsteroidal antiinflammatory drug-induced small-intestinal inflammation and blood-loss - effects of sulfasalazine and other disease-modifying antirheumatic drugs. Arthritis and Rheumatism. 1994;37(8):1146-50.

104. Bjarnason I, Macpherson A, Hollander D. Intestinal permeability - an overview. Gastroenterology. 1995;108(5):1566-81.

105. Smale S, Natt RS, Orchard TR, Russell AS, Bjarnason I. Inflammatory bowel disease and spondylarthropathy. Arthritis and Rheumatism. 2001;44(12):2728-36.

106. Tibble JA, Foster R, Sigthorsson G, Scott D, Roseth A, Bjarnason I. Faecal calprotectin: A simple method for the diagnosis of NSAID-induced enteropathy. Gastroenterology. 1998;114(4):G1269.

107. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating protein calprotectin in feces - a methodologic study. Scandinavian Journal of Gastroenterology. 1992;27(9):793-8.

108. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut. 2000;47(4):506-13.

109. Calvert GT, Shore EM. Human leukocyte antigen B27 allele is not correlated with fibrodysplasia ossificans progressiva. Clinical Orthopaedics and Related Research. 1998(346):66-70.

110. Vinje O, Dale K, Moller P. Radiographic evaluation of patients with Bechterew's syndrome (ankylosing spondylitis) and their first-degree relatives. Findings in the spine and sacro-iliac joints and relations to non-radiographic findings. Scandinavian Journal of Rheumatology. 1985;14(2):119-32.

111. Abney M, McPeek MS, Ober C. Estimation of variance components of quantitative traits in inbred populations. American Journal of Human Genetics. 2000;66(2):629-50.

112. Lennard-Jones JE. Classification of inflammatory bowel disease. Scandinavian Journal of Gastroenterology - Supplement. 1989;170:2-6.

113. Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, Arnason S, et al. Cancer as a complex phenotype: Pattern of cancer distribution within and beyond the nuclear family. Plos Medicine. 2004;1(3):229-36.

114. Cannon Albright LA, Thomas A, Goldgar DE, Gholami K, Rowe K, Jacobsen M, et al. Familiality of cancer in Utah. Cancer Research. 1994;54(9):2378-85.

115. Gulcher JR, Kristjansson K, Gudbjartsson H, Stefansson K. Protection of privacy by third-party encryption in genetic research in Iceland. European Journal of Human Genetics. 2000;8(10):739-42.

116. Vanderlinden SM, Valkenburg HA, Dejongh BM, Cats A. The risk of developing ankylosing-spondylitis in HLA-B27 positive individuals - a comparison of relatives of spondylitis patients with the general-population. Arthritis and Rheumatism. 1984;27(3):241-9.

117. De Angelis R, Salaffi F, Grassi W. Prevalence of spondyloarthropathies in an Italian population sample: a regional community-based study. Scandinavian journal of rheumatology. 2007;36(1):14-21.

118. Alamanos Y, Papadopoulos NG, Voulgari PV, Karakatsanis A, Siozos C, Drosos AA. Epidemiology of ankylosing spondylitis in northwestern Greece, 1983-2002. Annals of the Rheumatic Diseases. 2004;63:497.

119. Carbone LD, Cooper C, Michet CJ, Atkinson EJ, O'Fallon WM, Melton LJ, 3rd. Ankylosing spondylitis in Rochester, Minnesota, 1935-1989. Is the epidemiology changing? Arthritis & Rheumatism. 1992;35(12):1476-82.

120. Adomaviciute D, Pileckyte M, Norkuviene E, Dadoniene J, Guillemin F. Prevalence survey of rheumatoid arthritis and spondyloarthropathy in Lithuania. Annals of the Rheumatic Diseases. 2005;64:550-.

121. Hill HFH, Hill AGS, Bodmer JG. Clinical diagnosis of ankylosing-spondylitis in women and relation to presence of HLA-B27. Annals of the Rheumatic Diseases. 1976;35(3):267-70.

122. Zink A, Braun J, Listing J, Wollenhaupt J, German Collaborative Arthrit C. Disability and handicap in rheumatoid arthritis and ankylosing spondylitis - Results from the German rheumatological database. Journal of Rheumatology. 2000;27(3):613-22.

123. Dincer U, Cakar E, Kiralp MZ, Dursun H. Diagnosis delay in patients with ankylosing spondylitis: possible reasons and proposals for new diagnostic criteria. Clinical Rheumatology. 2008;27(4):457-62.

124. Lee W, Reveille JD, Davis JC, Jr., Learch TJ, Ward MM, Weisman MH. Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. Annals of the Rheumatic Diseases. 2007;66(5):633-8.

125. Khan MA. Update on spondyloarthropathies. Annals of Internal Medicine. 2002;136(12):896-907.

126. Bergfeldt L. HLA-B27-associated cardiac disease. Annals of Internal Medicine. 1997;127(8):621-9.

127. Smith JA, Marker-Hermann E, Colbert RA. Pathogenesis of ankylosing spondylitis: current concepts. Best Practice & Research in Clinical Rheumatology. 2006;20(3):571-91.

128. Kim TJ, Na KS, Lee HJ, Lee B, Kim TH. HLA-B27 homozygosity has no influence on clinical manifestations and functional disability in ankylosing spondylitis. Clinical and Experimental Rheumatology. 2009;27(4):574-9.

129. Bakland G, Nossent HC, Gran JT. Incidence and prevalence of ankylosing spondylitis in Northern Norway. Arthritis & Rheumatism-Arthritis Care & Research. 2005;53(6):850-5.

130. Pedersen OB, Svendsen AJ, Ejstrup L, Skytthe A, Harris JR, Junker P. Ankylosing spondylitis in Danish and Norwegian twins: occurrence and the relative importance of genetic vs. environmental effectors in disease causation. Scandinavian Journal of Rheumatology. 2008;37(2):120-6.

131. Leclercq SA, Chaput L, Russell AS. Ankylosing-spondylitis - a family study. Journal of Rheumatology. 1983;10(4):629-32.

132. Bedendo A, Glorioso S, Pasini CV, Fabiano F, Casara D, Cavallo A, et al. A family study of ankylosing-spondylitis. Rheumatology International. 1984;5(1):29-32.

133. Brown MA, Laval SH, Brophy S, Calin A. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. Annals of the Rheumatic Diseases. 2000;59(11):883-6.

134. De Blecourt JJ. [Epidemiological and genetic studies on rheumatic diseases, especially rheumatoid arthritis and spondylitis ankylo-poetica.]. Zeitschrift fur Rheumaforschung. 1963;Dec22:413-23.

135. Grant SF, Thorleifsson G, Frigge ML, Thorsteinsson J, Gunnlaugsdottir B, Geirsson AJ, et al. The inheritance of rheumatoid arthritis in Iceland. Arthritis & Rheumatism. 2001;44(10):2247-54.

136. Mielants H, Veys EM, Cuvelier C, Devos M, Joos R. Significance of gut inflammation in the seronegative spondyloarthropathies. Clinical and Experimental Rheumatology. 1987;5:S81-S7.

137. Mielants H, Veys EM, Cuvelier C, Devos M. Ileocolonoscopic findings in seronegative spondylarthropathies. British Journal of Rheumatology. 1988;27 suppl 2:95-105.

138. Thjodleifsson B, Sigthorsson G, Cariglia N, Reynisdottir I, Gudbjartsson DF, Kristjansson K, et al. Subclinical intestinal inflammation: An inherited abnormality in Crohn's disease relatives? Gastroenterology. 2003;124(7):1728-37.

139. Cardon LR, Garner C, Bennett ST, MacKay IJ, Edwards RM, Cornish J, et al. Evidence for a major gene for bone mineral density in idiopathic osteoporotic families. Journal of Bone and Mineral Research. 2000;15(6):1132-7.

140. Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. Gut. 1998;42(3):387-91.

141. Rath HC, Ikeda JS, Linde HJ, Scholmerich J, Wilson KH, Sartor RB. Varying cecal bacterial loads influences colitis and gastritis in HLA-B27 transgenic rats. Gastroenterology. 1999;116(2):310-9.

142. Farrell RJ, LaMont JT. Microbial factors in inflammatory bowel disease. Gastroenterology Clinics of North America. 2002;31(1):41-62.

143. Mielants H, Veys EM, Cuvelier C, Devos M. Subclinical involvement of the gut in undifferentiated spondylarthropathies. Clinical and Experimental Rheumatology. 1989;7(5):499-504.

144. Halme L, Turunen U, Helio T, Paavola P, Walle T, Miettinen A, et al. Familial and sporadic inflammatory bowel disease - Comparison of clinical features and serological markers in a genetically homogeneous population. Scandinavian Journal of Gastroenterology. 2002;37(6):692-8.

145. Binder V. Genetic epidemiology in inflammatory bowel disease. Digestive Diseases. 1998;16(6):351-5.

146. Brophy S, Taylor G, Blake D, Calin A. The interrelationship between sex, susceptibility factors, and outcome in ankylosing spondylitis and its associated disorders including inflammatory bowel disease, psoriasis, and iritis. Journal of Rheumatology. 2003;30(9):2054-8.

147. Vaile JH, Meddings JB, Yacyshyn BR, Russell AS, Maksymowych WP. Bowel permeability and CD45RO expression on circulating CD20+ B cells in patients with ankylosing spondylitis and their relatives. Journal of Rheumatology. 1999;26(1):128-35.

148. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, et al. Susceptibility to ankylosing spondylitis in twins - The role of genes, HLA, and the environment. Arthritis and Rheumatism. 1997;40(10):1823-8.

149. Tysk C, Lindberg E, Jarnerot G, Floderusmyrhed B. Ulcerative-colitis and Crohns-disease in an unselected population of monozygotic and dizygotic twins - a study of heritability and the influence of smoking. Gut. 1988;29(7):990-6.

150. Orholm M, Binder V, Sorensen TIA, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins - Results of a nationwide study. Scandinavian Journal of Gastroenterology. 2000;35(10):1075-81.

151. Resnick RH, Royal H, Marshall W, Barron R, Werth T. Intestinal permeability in gastrointestinal disorders - use of oral tc-99m DTPA. Digestive Diseases and Sciences. 1990;35(2):205-11.

152. Colombo E, Latiano A, Palmieri O, Bossa F, Adriulli A, Annesi V. Enteropathic spondyloarthropathy: A common genetic background with inflammatory bowel disease. Worlf j Gastroenterl 2009; 15(20) 2456-62.

153. Cortes A, Hadler J, Pointon JP, et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotypingof immune-related loci. Nat Genet 2013;45(7):730-38

154. Calin A, Porta J, Fries JF, Schurman DJ. Clinical history as a screening-test for ankylosing-spondylitis. Jama-Journal of the American Medical Association. 1977;237(24):2613-4.

155. Rudwaleit M, Metter A, Listing J, Sieper J, Braun J. Inflammatory back pain in ankylosing spondylitis - A reassessment of the clinical history for application as classification and diagnostic criteria. Arthritis and Rheumatism. 2006;54(2):569-78.

156. Ebringer R, Cooke D, Cawdell DR, Cowling P, Ebringer A. Ankylosing-spondylitis - klebsiella and HL-A B27. Rheumatology and Rehabilitation. 1977;16(3):190-6.

157. Rashid T, Ebringer A. Ankylosing spondylitis is linked to Klebsiella - the evidence. Clinical Rheumatology. 2007;26(6):858-64.

158. Zambrano-Zaragoza F, Garcia-Latorre E, Dominguez-Lopez ML, Cancino-Diaz ME, Burgos-Vargas R, Jimenez-Zamudio L. CD4 and CD8 T cell response to the rHSP60 from Klebsiella pneumoniae in peripheral blood mononuclear cells from patients with ankylosing spondylitis. Revista De Investigacion Clinica. 2005;57(4):555-62.

159. Syrbe U, Scheer R, Wu PH, Sieper J. Differential synovial Th1 cell reactivity towards Escherichia coli antigens in patients with ankylosing spondylitis and rheumatoid arthritis. Annals of the Rheumatic Diseases. 2012;71(9):1573-6.

160. Rhee KJ, Wu SG, Wu XQ, Huso DL, Karim B, Franco AA, et al. Induction of Persistent Colitis by a Human Commensal, Enterotoxigenic Bacteroides fragilis, in Wild-Type C57BL/6 Mice. Infection and Immunity. 2009;77(4):1708-18.

161. Van den Bosch F, Devinck M, Kruithof E, et al. Preliminary evidence for a retardation of structural damage in patients with ankylosing spondylitis continuously treated with infliximab for 4 years. Arthritis and Rheumatism. 2005;52(9):635-35.

162. Haroon N, Inman RD, Learch TJ et al. The impact of Tumor Necrosis Factor α inhibitors on radiographic progression in ankylosing spondylitis. Arthritis and Rheumatism. 2013;65(10):2645-54.

Paper I

Prevalence and clinical characteristics of ankylosing spondylitis in Iceland – a nationwide study

A.J. Geirsson [1], H. Eyjolfsdottir[1], G. Bjornsdottir[4], K. Kristjansson[4], B. Gudbjornsson[2,3]

[1]Department of Rheumatology and [2]Centre for Rheumatology Research, The National Hospital of Iceland; [3]University of Iceland and [4]deCODE Genetics, Reykjavik, Iceland.

Abstract

Objective

To determine the prevalence and clinical characteristics of ankylosing spondylitis (AS) in the Icelandic population, which carries a high prevalence of HLA-B27.

Methods

A nationwide search was performed by screening hospital records and private rheumatology services for cases of AS in association with an on-going genetic study. Individuals diagnosed with AS according to the modified New York criteria were asked to participate in the study by answering a standardised questionnaire and to undergo an interview and clinical evaluation.

Results

A total of 256 individuals fulfilled the modified New York classification criteria for AS (169 male, 87 female); 84% of these individuals were HLA-B27 positive vs. 15% in the population ($p<10^{-16}$). Of those contacted 223 patients (87.1%) answered the standardised questionnaire and were included in the study. The prevalence of AS in Iceland was 0.13% (CI 0.11–0.14%). A highly conservative prevalence number, based only on clinically evaluated patients, gave prevalence of 0.10% (CI 0.09–0.11%). Mean age at onset of symptoms was 24±8 years and at diagnosis 32.1±10.2 for male and 34.2±10.1 for female patients (not significant). Female patients more often had arthritis in peripheral joints and male patients were more often diagnosed with iritis. Prostatitis was experienced by 27% of male patients.

Conclusion

AS is less common in the Icelandic population than reported in various Caucasian populations with a similar prevalence of HLA-B27.

Key words

Ankylosing spondylitis, prevalence, demographics, nationwide

Clinical and Experimental Rheumatology 2010; **28**: 333-340.

Arni Jon Geirsson, MD,
Helga Eyjolfsdottir, MD,
Gyda Bjornsdottir, MSc,
Kristleifur Kristjansson, MD
Bjorn Gudbjornsson, MD, PhD

The study was funded by the research foundations of the University Hospital in Iceland, the Society for Rheumatology in Iceland and the Wyeth Rheumatology Foundation in Iceland.

Please address correspondence and reprint requests to:
Bjorn Gudbjornsson, MD, PhD,
Centre for Rheumatology Research,
University Hospital,
Reykjavik, Iceland.
E-mail: bjorngu@landspitali.is

Received on October 1, 2009; accepted in revised form on December 10, 2009.

Competing interests: none declared.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease, characterised by low back and buttock pain with morning stiffness of insidious onset, usually beginning in adolescence or early adulthood (1-3). Sacroiliitis with syndesmophyte formation can in advanced disease lead to spinal ankylosis or formation of so-called bamboo spine (4). Arthritis of peripheral joints, costochondritis and enthesopathy are also commonly described (1-4). Extra-articular manifestations of AS include iritis (25–40%) (5), prostatitis (13–83%) (6, 7) and asymptomatic gastrointestinal tract involvement (69%) has also frequently been described (8). Cardiac and pulmonary involvement seems to be less common (9, 10). The diagnostic criteria most often used are the modified New York criteria published in 1984 (11, 12). These criteria involve both clinical and radiological aspects.

AS has a strong association with HLA-B27 and the prevalence of AS in different cohorts frequently relates to the prevalence of this MHC molecule in the study population (13). The majority or up to 95% of Caucasians with AS carry the HLA-B27 antigen compared to around 8–14% in the background population (14, 15). Population-based studies have previously reported considerable variability in the prevalence of AS, ranging from 0.1–1.4% (2, 16-19) and up to 6% in Canadian Haida Indians (20). A study from Tromsö, Norway, which represents a population with a high frequency of HLA-B27, reported a prevalence of 0.31% for AS, including both primary and secondary forms (21). However, to our knowledge no nationwide study on the prevalence and clinical presentation of AS has up to now been published.

Our goal in this study was to determine the prevalence of AS on a nationwide basis in Iceland and to describe the demographics and clinical features of AS in the Icelandic population, which is characterised by a high prevalence (15%) of the HLA-B27 molecule.

Materials and methods

Study group

The study involved all known patients with AS in Iceland. Patients were recruited from three main sources, first, from a database of 1557 patients participating in ongoing genetic studies of AS and inflammatory bowel diseases (22). This database included not only clinical data on the index cases but also of all available relatives and other family members of these patients, regardless of whether they were reported to have AS or not. From this database, 205 individuals who were alive in 2005 and who had been diagnosed as having AS with the diagnosis verified by a rheumatologist (AJG) were included in the present study.

The second source was an electronic registry of patients admitted to the two major hospitals in Iceland, who both have rheumatologic specialist services: the Landspitali-University Hospital in Reykjavík (LSH) and the University Hospital in Akureyri (FSA). The LSH serves as a primary hospital for Reykjavik and its suburbs and it is the only secondary and tertiary care hospital in Iceland. The FSA serves as a primary hospital for the northern and eastern part of Iceland. A systematic search of AS and sacroiliitis according to the International Classification of Diseases (ICD) 10th diagnostic registry, including the following codes: M 45, M 45.5, M45.9, M 46 and M 46.9, was performed in all hospital records. This source yielded an additional 54 patients who had been diagnosed with AS and who were alive in 2005.

The third source was a personal call to all private rheumatology services (six rheumatologists were working partly and four solely with private praxis; thus ten rheumatologists are running a private clinic in Iceland at the time of the study) in Iceland to report patients to the study. This yielded 64 additional patients who had been diagnosed with AS and who were alive in 2005.

In Iceland every resident is issued a specific security number which allows a combination of individualised information from different sources without mixing data between individuals. The three sources named above yielded 323 patients, but, since many patients were found in more than one database, the total number of patients with AS in Iceland came to 280 individuals.

Clinical examination
All of these 280 known cases of AS in Iceland were initially contacted by a letter of invitation, followed by a telephone call by our study nurse; 24 individuals (8.6%) could not be reached or did not respond. Of the remaining cases 256 or 91.4% of the original study group agreed to participate in the study. All participants were interviewed and examined by the same rheumatologist (AJG). All participants also were asked to answer an extended questionnaire in connection with the genetic study and 223 patients (87.1%) filled out and returned the questionnaire.

Inclusion criteria
The inclusion criteria used for this study were the modified New York criteria for classification of spondylitis ankylopoetica or AS (12), requiring that patients had to have a radiological criterion of sacroiliitis grade >2 bilaterally or grade 3 or 4 unilaterally and at least one of the three following clinical signs: 1) Low back pain and stiffness for more than three months that improve with exercise but are not relieved by rest; 2) Limitation of motion of the lumbar spine in both the sagittal and the frontal plane; 3) Limitation of chest expansion relative to normal values correlated for age and sex.
Patients who did not have active arthritis or inflammatory back pain at evaluation were included if they had been diagnosed with AS by a rheumatologist and were taking remitting drugs at the time of the study. Meanwhile, patients who reported a diagnosis of a rheumatic disease other than AS when interviewed, or who were observed to have another rheumatic condition when examined, were excluded from the study. Furthermore, patients with AS associated with psoriasis were also excluded from the present study as the group of patients with psoriatic arthritis in Iceland has recently been reported separately (23). Of the 256 patients examined, 33 (12.9%) were excluded from the prevalence analysis.

Disease assessment
Chest expansion was measured on maximal inspiration after forced maximal expiration, at the level of the fourth intercostal space in males and just below the breasts in females. The normal values were set at ≥6 cm for males and ≥4.5 cm for females, as normal values of chest expansion are sex dependent. Cervical flexion, extension and rotation were evaluated according to standard clinical measurements and lumbar flexion ability was evaluated by the Schober index and measured in centimetres (cm) (24). Extent of extraspinal symptoms, *i.e.* peripheral joint involvement, was also assessed according to standard clinical evaluation.
Concerning systemic manifestastions of, for example, iritis and prostatitis, the diagnosis was reviewed by one of the study members (AJG) with the requirement that these manifestations were confirmed by physicians at the time of occurrence, *e.g.* ophthalmologist or rheumatologist in the case of eye inflammation and urologist, general physician or rheumatologist in the case of prostatitis, respectively.
All radiographs, computed tomographs and magnetic resonance imaging were re-evaluated by members of the study group.
Whole blood was drawn for later genetic studies and for extended typing of the major histocompatibility complex molecules to estimate the prevalence of HLA-B27 (Dynal HLA-Kit, F. Hoffmann-La Roche Ltd, Basel, Switzerland). A total of 524 randomly chosen healthy volunteers were used as controls and tested for the HLA-B27 antigen, and of those 15.41% turned out to be positive for HLA-B27 (25).

Data analysis
The study data were stripped of information allowing identification of individuals before the analysis of data began and the code for this information was kept in a separate, encrypted database. Point prevalence was based on all living individuals in Iceland on the 31st of December in 2005 and on those who were known to have AS according to the study protocol. The crude annual incidence rate was also calculated based on the year of diagnosis of AS and expressed per 100,000 of the total midyear population for each year (26).
Informed consent was obtained from all the participants in the study. The study was approved by the National Bioethics Committee of Iceland (approval no. 98-059) and by the Icelandic Data Protection Authority (2001/36).
Data were analysed using R-statistical software. Fisher exact tests were used for comparisons. We calculated 95% confidence intervals for prevalence rates using binomial distributions. All reported *p*-values were based on 2-tailed analyses.

Results
Prevalence
According to Statistics Iceland there were 220,441 individuals living in Iceland aged 18 years and older at the end of December 2005 (26). This means that the point prevalence of AS in Iceland when calculated from the 280 individuals with hospital or outpatient clinic diagnoses of AS was 127 per 100,000 (95% CI 112–142). Of the 256 individuals who were invited to participate in the study for clinical evaluation, AS was confirmed according to the inclusion criteria in 223 or 87.1%. Assuming that no case of AS would have been confirmed among the 34 individuals who did not come for re-evaluation of their disease, a highly conservative prevalence estimate of 101 per 100,000 (95% CI 88–114) can be calculated. Conversely, if all these individuals had AS the prevalence estimate would be 112 per 100,000 (95% CI 98–126). However, as these patients had all been previously diagnosed with AS, and there was no obvious selection bias regarding the individuals who could not be reached for clinical re-evaluation, we extrapolated the inclusion ratio for the patients who were examined clinically to all the 280 patients in the original study group, correcting for age and sex, resulting in an adjusted prevalence ratio of 104 per 100,000 (95% CI 91–117).
The prevalence of AS was significantly higher in males than in females; 132 per 100,000 male inhabitants (95% CI 110-153) *vs.* 71 per 100,000 female inhabitants (95% CI 56-88), $p>0.0001$. Thus, the male *vs.* female ratio was 1.85.

Demographics

Demographic data on the 223 patients who satisfied the inclusion criteria and delivered completed questionnaires on their past medical history, use of medication and clinical symptoms of their AS disease are presented in Tables I and II. The male patients had a similar age of onset of symptoms related to their AS, but were diagnosed around two years younger than the female patients (32 vs. 34 years); thus they had about a year shorter diagnostic delay, but these differences were not significant (Table I).

Table I. Demographic data for 223 patients with AS.

Demographic data	Male n=145	Female n=78	*p*-value
Age at onset of symptoms; mean±SD years	23.6 ± 8.4	24.1 ± 8.9	0.55
Age at diagnosis of AS, mean±SD years	32.1 ± 10.2	34.2 ± 10.1	0.13
Diagnostic delay, mean±SD years	8.3 ± 7.7	9.6 ± 10.0	0.87
Clinical characteristics			
Limited chest expansion; n (%)	65 (44.8%)	22 (28.2%)	0.021
Modified Schober; mean±SD cm	3.50 ± 1.63	3.84 ± 1.26	0.09
Limited flexion/extension of neck; n (%)	56 (38.6%)	30 (38.5%)	1
Limited rotation of neck; n (%)	67 (46.2%)	35 (44.9%)	0.89
Active peripheral arthritis; n (%)	18 (12.4%)	15 (19.2%)	0.23
HLA-B27 positive; no (%)	124 (85.5%)	63 (80.8%)	0.34

Table II. Self-reported pain and history of joint inflammation in 145 male and 78 female AS patients.

Symptoms of AS	Male n=145 (%)	Female n=78 (%)	*p*-value
Pain problem	**133 (91.7)**	**74 (97.4)**	**0.15**
Lumbar spine	84.9%	77.0%	0.69
Buttock	53.4%	52.7%	1.00
Thoracic spine	31.6%	27.1%	0.44
Sternum	25.6%	22.9%	0.74
Joint inflammation	**64 (44.1)**	**52 (66.7)**	**0.0013**
Hip	53.1%	48.1%	0.485
Knee	25.0%	30.8%	0.429
Shoulder	20.3%	25.0%	0.737
Eye inflammation - iritis	**55 (37.9)**	**24 (30.8)**	**0.31**
Prostatitis	**39 (26.9%)**	–	–

Onset of AS and annual incidence

The mean age at onset of symptoms related to AS was 24±8 years, attesting that around 80% of both male and female patients suffered onset of symptoms before thirty years of age; the peak incidence was 16 to 20 years for male patients whereas for female patients it was during their late thirties (Fig. 1). The mean age of diagnosis was 32.1±10.2 for male patients and 34.2±10.1 for female patients.

Of the 223 individuals included in the study, 214 or 96%, reported the year of diagnosis. The crude annual incidence rate for the period 1947 to 2005 ranged from 0.44 to 5.48 per 100,000 inhabitants (Fig. 2).

The majority of patients or 58.7% reported an insidious onset of the disease. Juvenile onset of symptoms (<16 years) was reported by 35 patients (15.7%); 22 (15.2%) male and 13 (16.7%) female patients. Pain was reported as the presenting symptom in 92.8% of both male and female patients; 91.7% vs. 94.9%, respectively. Lower back pain was the most common pain location in both sexes (84.9% *vs*. 77.0 %) and buttock pain was the second most common pain location (male 53.4%; female 52.7%). Table II shows location of self-reported pain and inflammation in both males and females.

Limited flexion and extension, and rotation of the head were the same in both sexes (Table I). Lumbar flexion measure by modified Schober index was also similar in male and female patients, but a significantly higher number of male patients had limited thoracic expansion (44.8% vs 28.2%; p=0.02) (Table I).

HLA-B27

The prevalence of HLA-B27 positive individuals was 84%, with no significant difference observed between the sexes. Analysis by a multiplicative model showed the prevalence of AS among HLA-B27 positive individuals to be 0.71% (CI 0.64%–0.84%), while we found a prevalence of only 0.028% (CI 0.020%–0.036%) in those without the HLA-B27 molecule, *i.e*. HLA-B27 negative individuals.

Patterns of peripheral joint involvement

A total of 116 patients (52%) had a history of peripheral arthritis; 44.1% of the male patients and 66.7% of the female patients (p=0.0013). The most frequent arthritis conditions were reported by the patients themselves in the hip joints (53% in males and 48% in females) and knee joints (25% in males and 31% in females), followed by arthritis in the ankle and the shoulder joints. Polyarticular involvement was reported by 35 out of 145 male patients (24%) and in 19 out of 78 (24%) female patients. On the day of examination 18 male patients (23%) and 15 female patients (19%) had signs of arthritis in their peripheral joints. Achilles tendonitis was observed in 21% of both males and females, while plantar fasciitis was observed in 6% of the male patients and 12% of the female patients (p=0.19).

Extra-articular involvement

The most commonly reported extra-articular sign associated with AS in both males and females was iritis; 38% *vs*. 30.8% (difference between sexes: p=0.31). In most cases the iritis was unilateral, but 15 out of 55 male (10.3%) patients with a history of iritis and two out of 24 (2.6%) female patients gave a history of bilateral involvement of the eyes (p=0.08). The second most common extra-articular manifestation reported by male patients was prostatitis, which 27% of the male patients reported. Other commonly reported extra-articular manifestations were cardiac arrhythmias in males and females, 14.5%

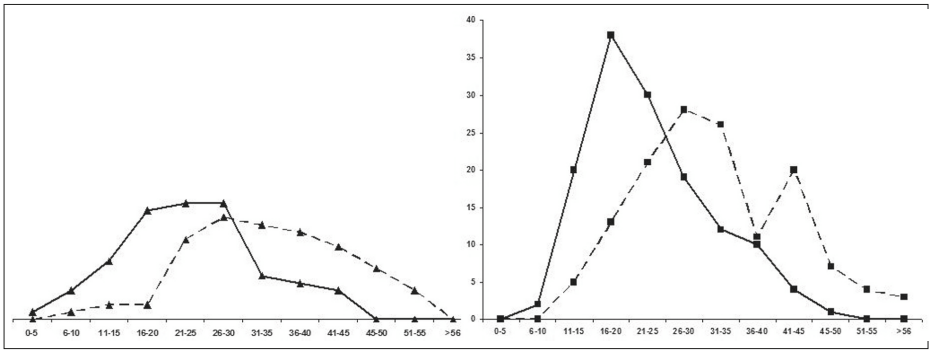


Fig. 1. Onset of symptoms related to ankylosing spondylitis (AS) and age of diagnosis in 145 male (left) and 78 female (right) patients with AS. The full line shows the age of onset of symptoms related to AS, while the broken line shows age at diagnosis of AS.

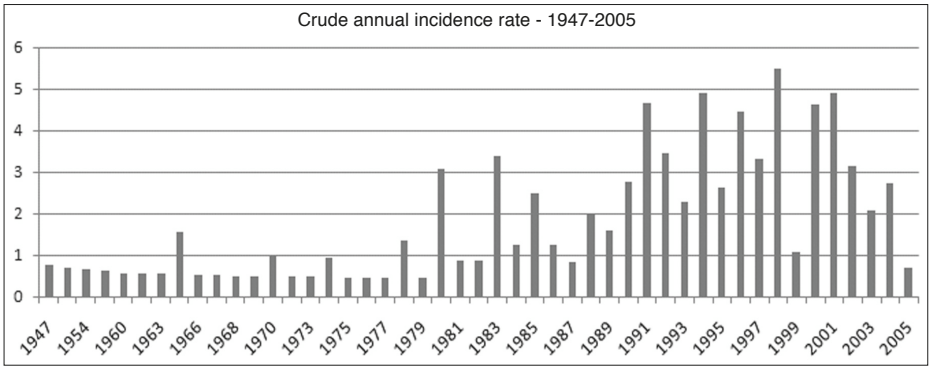


Fig. 2.

and 17.9%, respectively. However, only 1.4% of the male patients reported having a cardiac pacemaker implanted due to atrio-ventricular block, while none of the female patients had a pacemaker. One male patient was found to have aortic valve insufficiency. No information was collected as to whether any patient suffered from pulmonary fibrosis.

Primary vs. secondary AS

Two female patients (2.6%), but no male patients, had been diagnosed with Mb Crohn's after the onset of their AS, while nine of the male and six of the female participants had a history of colitis ulcerosa that had been diagnosed simultaneously or after the onset of their AS, respectively. Patients with psoriatic spondylarthritis were excluded from the present study, as those have been reported separately for the same population of interest (23).

Treatment

Sixty-four percent of male patients were on some specific anti-rheumatic treatment for their AS, compared to 50% of the female patients (p=0.0013). More female patients received regular physiotherapy treatment than male patients; 50% *vs.* 24% (p=0.00015), respectively. Most frequently the patients were treated with infliximab (n=77), sulfasalazine (n=60), or methotrexate (n=25), while 79 patients were using NSAIDs and 10 celecoxib on a regular basis.

Discussion

This cross-sectional study was based on a nationwide cohort of patients with AS in a population with a high prevalence of the MCH molecule HLA-B27. We recruited patients not only through hospital records but also from specialist out-patient clinics and from an extended database focusing on the genetics of AS in Iceland. Thus, our patient group represent a clinically relevant population of AS patients. All patients were re-evaluated by the same experienced rheumatologist and we systematically collected clinical data from 280 cases with AS in an adult population of around 220,000 individuals. Our results demonstrated a disease prevalence of 0.13%, which was in the lower range of previous reported

prevalence of 0.1–1.4% (2, 16-18), independently of whether we compare our result with other Nordic countries (2, 16, 17) or with data from the Mediterranean (18, 19, 27), our prevalence data especially were much lower than reports from areas with a high prevalence of the HLA-B27 molecule, *e.g.* of 0.31% (21).

The crude annual incidence of AS in Iceland was retrospectively found to be 0.44 per 100,000 inhabitants in the last mid-century, which increased up to as high as 5.48 in 1998, but was around 3 per 100,000 the latter decades of the study period. In the early eighties the incidence seemed to increase, probably due to the increased number of practising rheumatologists in Iceland and better access to computed tomography and later to magnetic resonance imaging for diagnostic purposes. The incidence in the later period is lower than what has been reported in Norway (21), Finland (2) and Minnesota, USA (28), but higher than in Greece (19).

One of the possible explanations for these differences in prevalence in the present study and those from northern Norway (21) and Lithuania (29) is that our study extended over the whole of Iceland and might therefore reflect regional differences in recruitment as all participants were invited to our research centre in Reykjavik. However, we did not find any differences in disease prevalence of AS depending on rural or urban areas of Iceland (data not shown). Another explanation of this discordance is that the present study did not include patients with AS associated with psoriatic arthritis, as this has recently been reported for Iceland (23). If we add those patients with AS in our previous study on psoriatic arthritis to the present cohort, the prevalence of AS migth increase up to 0.19%. Thus our prevalence is still lower than reported in high HLA-B27 geographic areas. Other explanations for this discordance might be that the HLA-B27 subclasses in Iceland are different or Icelanders may have some other genetic combination than in the other countries, which may play a role in the pathogenesis of AS. Lastly and even more important, various environmental factors in different populations may have strong influences on the pathogenic processes of AS.

Our observation of a male to female ratio of a little less than 2:1 was clearly lower than previously reported. Earlier studies have reported a male to female ratio of AS up to 10:1 (30), but more recent studies have reported lower male to female ratios or approaching 2-3:1 (31). In this context, we found female dominance in our psoriatic arthritis cohort of a male *vs.* female ratio of 1:2 (23) while most other studies report a male *vs.* female ratio closer to 1:1 for psoriatic arthritis. Thus, this difference may be a true regional difference. However, other factors like excellent access to diagnostic tools, *e.g.* computed tomography and magnetic resonance imaging, may have improved the diagnosis of AS in female patients in Iceland compared to other study areas.

The present study cohort seems to represent a traditional patient group of AS patients in respect to clinical symptoms of insidious onset of low back and buttock pain with morning stiffness, as well as the prevalence of oligoarthritis in large joints in the lower extremities, *i.e.* hip and knee. As expected, female patients had their onset of AS-related symptoms some years later than what male patients reported. However, to our surprise and in contrast to clinical experience and the report by Dincer *et al.* (32) our female patients had just around one year of diagnostic delay compared to our male patients, *i.e.* 9.6 *vs.* 8.3 years. This may reflect active intervention of diagnostic procedures in Iceland, as previously discussed.

Male AS patients more often reported a history of iritis than female patients, *i.e.* 38% of our male patients had a history of iritis, most frequently unilateral, while only 31% of our female patients had a history of iritis. Other differences between male and female patients in our cohort were that female patients seem to have more frequently had arthritis problems in their peripheral joints than did the males. These findings conform to other reports (33). However, a much higher percent reported a history of involvement of peripheral joints. This may reflect differences in collecting data. In the case of prostatitis, an earlier study reported prostatitis as a frequent systemic manifestation of AS (6, 7). Lately not much particular attention has been paid to this problem. The present study suggests that prostatitis might be a bigger issue in AS than rheumatologists are currently aware of. Unfortunately we did not register active symptoms of urethritis or balanitis at the time of inclusion to the study. Many AS patients of both sexes reported a history of unspecific cardiac arrhythmias and two male patients had an implanted cardiac pacemaker due to an atrioventricular block (AV-block), and another 60 year old male patient had a history of aortic valve insufficiency. A genetically HLA-B27 linked cardiac syndrome has been defined, *i.e.* the combination of conduction system abnormalities and aortic regurgitation (9), and AS is also reported to be associated with a greater risk than expected of cardiac lesions (34). Meanwhile, no patient reported a lung disorder, *i.e.* pulmonary fibrosis; thus, this pulmonary complication of AS seems to be very infrequent or under-diagnosed in our patient cohort. Our study was not designed to investigate the mortality rate of our AS patient population, which has been reported to be greater than for the normal population (34).

The prevalence of HLA-B27 in various populations seems to correlate to some extent with the population prevalence of AS in the same cohort, suggesting that HLA-B27 mediates important antigen presentation which has a role in the pathogenesis of AS (5, 35), More than 90% of Caucasians in Western Europe with AS are HLA-B27 positive, compared to around 8% (5) in the general background population, although the prevalence of HLA-B27 varies in different populations, which may reflect the importance of other pathogenetic factors, including various environmental factors. HLA-B27 positive patients seems to experience disease symptoms at a younger age and they also more frequently have iritis and arthritis in peripheral joints, though homozygosity for HLA-B27 does not effect the clinical presentation of AS (36). Interestingly, in our study population the prevalence of AS in HLA-B27 positive individuals

was 0.71%, while only 0.03% in those who were HLA-B27 negative.

The main strength of our study is that all available patients from both community and hospital based data sources with a verified diagnosis of AS were recruited. Each source has different selection biases that complement the other, as only 43 cases of 280 were harvested from the same source. All these individuals were re-evaluated according to a predefined routine by an experienced rheumatologist. This is important, as most previous studies of the prevalence and demographics of AS have relied either on patient records (2, 19, 21) or self-report by questionnaires (37). In contrast, the main shortcoming of our study is that the participants were not recruited randomly from the population living in Iceland, but such a strategy is hardly realistic for complex diseases with a prevalence as high as for AS. It is likely that relatively mild cases of AS are not diagnosed according to international classification criteria, especially in patients who only attend health centres. However, the majority of patients with inflammatory joint diseases in Iceland attend out-patient specialist clinics. Thus, our adjusted AS prevalence of 0.1% is still probably an underestimate. Further studies are clearly needed to refine epidemiological information on AS, including sex ratios, as well as prospective studies with multiple follow-up visits to monitor the disease course over long periods.

In conclusion, AS seems to be less common in the Icelandic population than reported in other Scandinavian countries and Minnesota, USA, despite the higher population prevalence of HLA-B27 in Iceland. However, the prevalence is similar to that in northern Norway, where the prevalence of HLA-B27 is also high.

Acknowledgments

We would like to thank Valdimar B. Hauksson and Bjarni V. Halldórsson at the statistics department at deCODE Genetics.

References

1. GRAN JT, HUSBY G: The epidemiology of ankylosing spondylitis. *Semin Arthritis Rheum* 1993; 22: 319-34.
2. KAIPIAINEN-SEPPANEN O, AHO K, HELIOVAARA M: Incidence and prevalence of ankylosing spondylitis in Finland. *J Rheumatol* 1997; 24: 496-9.
3. RUDWALEIT M, METTER A, LISTING J, SIEPER J, BRAUN J: Inflammatory back pain in ankylosing spondylitis: A reassessment of the clinical history for application as classification and diagnostic criteria. *Arthritis Rheum* 2006; 54: 569-78.
4. KHAN MA: Clinical features of ankylosing spondylitis. *In*: HOCHBERG M, SILMAN A, SMOLEN J, WEINBLATT M (Eds.) *Rheumatology*. London: Mosby: A Division of Harcourt Health Sciences Ltd. 2003:1161-81.
5. KHAN MA: Update on spondyloarthropathies. *Ann Intern Med* 2002; 136: 896-907.
6. MASON RM, MURRAY RS, OATES JK, YOUNG AC: Prostatitis and ankylosing spondylitis. *BMJ* 1958; 1: 748-51.
7. LANGE U, BERLINER M, WEIDNER W, SCHIEFER HG, SCHMIDT KL, FEDERLIN K: Ankylosing spondylitis and urogenital infection: Diagnosis of urologic infection and correlation with rheumatologic findings. *Z Rheumatol* 1996; 55: 249-55.
8. SMALE S, NATT RS, ORCHARD TR, RUSSELL AS, BJARNASON I: Inflammatory bowel disease and spondylarthropathy. *Arthritis Rheum* 2001; 44: 2728-36.
9. BERGFELDT L: HLA-B27-associated cardiac disease. *Ann Intern Med* 1997; 127: 621-9.
10. ROSENOW E, STRIMLAN CV, MUHM JR, FERGUSON RH: Pleuropulmonary manifestations of ankylosing spondylitis. *Mayo Clin Proc* 1977; 52: 641-9.
11. MOLL JM, WRIGHT V: New York clinical criteria for ankylosing spondylitis: A statistical evaluation. *Ann Rheum Dis* 1973; 32: 354-63.
12. VAN DER LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis: A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; 27: 361-8.
13. BREWERTON DA, HART FD, NICHOLLS A, CAFFREY M, JAMES DC, STURROCK RD: Ankylosing spondylitis and HL-A 27. *Lancet* 1973; 1: 904-7.
14. SIEPER J, RUDWALEIT M, KHAN MA, BRAUN J: Concepts and epidemiology of spondyloarthritis. *Best Pract Res Clin Rheumatol* 2006; 20: 401-17.
15. FERNÁNDEZ-SUEIRO JL, ALONSO C, BLANCO FJ, RODRÍGUEZ- GÓMEZ M, GALDO F, GONZÁLEZ-GAY M: Prevalence of HLA-B27 and subtypes of HLA-B27 associated with ankylosing spondylitis in Galicia, Spain. *Clin Exp Rheumatol* 2004; 22: 465-8.
16. VAN DER LINDEN SM, VALKENBURG HA, DE JONGH BM, CATS A: The risk of developing ankylosing spondylitis in HLA-B27 positive individuals: A comparison of relatives of spondylitis patients with the general population. *Arthritis Rheum* 1984; 27: 241-9.
17. GRAN JT, HUSBY G, HORDVIK M: Prevalence of ankylosing spondylitis in males and females in a young middle-aged population of Tromso, northern Norway. *Ann Rheum Dis* 1985; 44: 359-67.
18. DE ANGELIS R, SALAFFI F, GRASSI W: Prevalence of spondylarthropathies in an Italian population sample: A regional community-based study. *Scand J Rheumatol* 2007; 36: 14-21.
19. ALAMAMNOS Y, PAPADOPOULOS NG, VOULGARI PV, KARAKATSANIS A, SIOZOS C, DROSOS AA: Epidemiology of ankylosing spondylitis in Northwest Greece, 1983-2002. *Rheumatology* (Oxford) 2004; 43: 615-8.
20. GOFTON JP, ROBINSON HS, TRUEMAN GE: Ankylosing spondylitis in a Canadian Indian population. *Ann Rheum Dis* 1966; 25: 525-7.
21. BAKLAND G, NOSSENT HC, GRAN JT: Incidence and prevalence of ankylosing spondylitis in Northern Norway. *Arthritis Rheum* 2005; 53: 850-5.
22. THJODLEIFSSON B, GEIRSSON AJ, BJORNSSON S, BJARNASON I: A common genetic background for inflammatory bowel disease and ankylosing spondylitis: A genealogic study in Iceland. *Arthritis Rheum* 2007; 56: 2633-9.
23. LOVE TJ, GUDBJORNSSON B, GUDJONSSON JE, VALDIMARSSON H: Psoriatic arthritis in Reykjavik, Iceland: Prevalence, demographics, and disease course. *J Rheumatol* 2007; 34: 2082-8.
24. MOLL JMH, WRIGHT V: An objective study of chest expansion. *Ann Rheum Dis* 1972; 31: 1-8.
25. Personal communication; Data on file, deCode Genetics Ltd, Iceland.
26. Statistics Iceland. Population by sex and age. 2009; Available at: ww.statice.is. Accessed December 10, 2008.
27. TRONTZAS P, ANDRIANAKOS A, MIYAKIS S *et al.*: Seronegative spondyloarthropathies in Greece: A population-based study of prevalence, clinical pattern, and management. The ESORDIG study. *Clin Rheumatol* 2005; 24: 583-9.
28. CARBONE LD, COOPER C, MICHET CJ, ATKINSON EJ, O'FALLON WM, MELTON LJ 3RD: Ankylosing spondylitis in Rochester, Minnesota, 1935-1989: Is the epidemiology changing? *Arthritis Rheum* 1992; 35: 1476-82.
29. ADOMAVICIUTE D, PILECKYTE M, BARANAUSKAITE A, MORVAN J, DADONIENE J, GUILLEMIN F: Prevalence survey of rheumatoid arthritis and spondyloarthropathy in Lithuania. *Scand J Rheumatol* 2008; 37: 113-9.
30. HART FD, ROBINSON KC: Ankylosing spondylitis in women. *Ann Rheum Dis* 1959; 18: 15-23.
31. ZINK A, BRAUN J, LISTING J, WOLLENHAUPT J: Disability and handicap in rheumatoid arthritis and ankylosing spondylitis: Results from the German rheumatological database. German Collaborative Arthritis Centers. *J Rheumatol* 2000; 27: 613-22.
32. DINCER U, CAKAR E, KIRALP MZ, DURSUN H: Diagnosis delay in patients with ankylosing spondylitis: Possible reasons and proposals for new diagnostic criteria. *Clin Rheumatol* 2008; 27: 457-62.
33. LEE W, REVEILLE JD, DAVIS JC JR, LEARCH TJ, WARD MM, WEISMAN MH: Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. *Ann Rheum Dis* 2007; 66: 633-8.
34. ZOCHLING J, BRAUN J: Mortality in ankylo-

sing spondylitis. *Clin Exp Rheumatol* 2008; 26 (Suppl. 51): S80-4.
35. SMITH JA, MARKER-HERMANN E, COLBERT RA: Pathogenesis of ankylosing spondylitis: current concepts. *Best Pract Res Clin Rheumatol* 2006; 20: 571-91.
36. KIM TJ, NA KS, LEE HJ, LEE B, KIM TH: HLA-B27 homozygosity has no influence on clinical manifestations and functional disability in ankylosing spondylitis. *Clin Exp Rheumatol* 2009; 27: 574-9
37. PEDERSEN OB, SVENDSEN AJ, EJSTRUP L, SKYTTHE A, HARRIS JR, JUNKER P: Ankylosing spondylitis in Danish and Norwegian twins: Occurrence and the relative importance of genetic vs. environmental effectors in disease causation. *Scand J Rheumatol* 2008; 37: 120-6.

Paper II

A strong familiality of ankylosing spondylitis through several generations

Arni Jon Geirsson, Kristleifur Kristjansson and Bjorn Gudbjornsson

Ann Rheum Dis published online May 6, 2010
doi: 10.1136/ard.2009.125914

ARD Online First, published on May 6, 2010 as 10.1136/ard.2009.125914

Concise report

A strong familiality of ankylosing spondylitis through several generations

Arni Jon Geirsson,[1] Kristleifur Kristjansson,[2] Bjorn Gudbjornsson[3,4]

[1]Department of Rheumatology, University Hospital, Reyjavik, Iceland
[2]deCODE Genetics, Reykjavik, Iceland
[3]Centre for Rheumatology Research, University Hospital, Reyjavik, Iceland
[4]University of Iceland, Reykjavik, Iceland

Correspondence to
Bjorn Gudbjornsson, Centre for Rheumatology Research, Landspitali, Hringbraut – University Hospital, Reykjavik 101, Iceland; bjorngu@landspitali.is

Accepted 6 February 2010

ABSTRACT

Objective To elucidate the familiality of ankylosing spondylitis (AS) in Iceland.

Methods The Icelandic genealogy database and population-wide data on all living Icelanders diagnosed as having AS (n=280), who previously had taken part in an epidemiological study on the prevalence of AS in Iceland, were included in the study. Identification of all interpatient relationships in the genealogy database allowed calculation of estimates of the RR for AS in the first-degree relatives (FDRs) to fourth-degree relatives of patients. For each AS proband, 1000 sets of matched Icelandic subjects in the genealogy database were used as controls.

Results FDRs, second-degree and third-degree relatives had RRs of 75.5, 20.2 and 3.5, respectively (all p values <0.0001), indicating a significantly increased risk for relatives of the patients with AS to develop AS, suggesting a strong heritable factor, while the fourth-degree relatives had a RR of 1.04 ($p=0.476$) for having AS.

Conclusions Patients with AS in Iceland are significantly more related to each other than to randomly sampled control subjects. This is in agreement with previous reports on the familiality of AS, but the present study has more power and extends over larger familiar cohorts than previously reported.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease, characterised by low back and buttock pain with morning stiffness of insidious onset.[1] Population-based studies suggest that the prevalence of AS is between 0.1% and 1.4%[1–3] and we have found the prevalence of AS in Iceland to be close to 0.13%.[4] Clinicians are well aware of the increased prevalence of AS among siblings of patients with AS, an observation confirmed in a number of studies.[5–7]

Several studies demonstrate that 6% to 8% of close relatives of patients with AS have the disease[5–7] in comparison to 0.1% to 1.4% in the general population.[1–4] The prevalence of AS among relatives to human leucocyte antigen (HLA)-B27 positive patients with AS is even higher or up to 20%.[8] Studies of twins have shown the concordance rate in monozygotic twins to be 40% to 75%,[9–11] but 4.3% to 12.5% in dizygotic twins,[11] reflecting that genetic and environmental factors are important in the pathogenesis of AS. Only two reports have reported the prevalence of AS among second-degree relatives (SDRs) and third-degree relatives (TDRs) of patients with AS, respectively.[12 13] Several of these studies have had various methodological limitations, for example, limited cohort size, index cases frequently selected from specialty clinics, lack of well documented prevalence of AS in the background population and the ascertainment bias of looking for first-degree relative (FDR) cases within a family after identifying the index case, which can result in inflated λ_S values, as suggested by Guo.[14]

To overcome some of these methodological problems, we have recently identified all known cases with AS in Iceland[4] and by using the Icelandic genealogy database of deCODE Genetics (Reykjavík, Iceland), we are able to calculate the RR for family members spanning several generations of individuals with AS.

MATERIALS AND METHODS

The study group

The study involved all known patients with AS in Iceland in 2005. Patients were recruited from three main sources. The first source was from a database of individuals participating in genetic studies of AS and inflammatory bowel diseases.[13] From this database, 205 individuals were included in the present study. The second source was an electronic registry of patients admitted to the two major hospitals in Iceland (WHO International Classification of Diseases, 10th edition (ICD-10) codes: M 45, M 45.5, M45.9, M 46 and M 46.9), Landspitali – University Hospital in Reykjavík and the University Hospital in Akureyri. This source yielded an additional 54 patients with AS. The third source was a personal call to all private outpatient rheumatology services in Iceland to report patients to the study, yielding 64 additional patients with AS.

The above-mentioned 3 sources yielded in total 280 individuals with AS, of which 256 were alive in December 2005. A detailed description of the inclusion criteria for verified AS according to the modified New York criteria for classification of AS[15] can be found in our previous work on the prevalence of AS in Iceland.[4]

Genealogy database

deCODE Genetics has built a computerised genealogy database of more than 760 000 individuals.[16] The database contains records of all living Icelanders, comprising more than 300 000 individuals and a large proportion of all individuals who have ever lived in the country from the time of the settlement in the late ninth century. The genealogy database is essentially complete from the 18th century to the present day, allowing distant relationships to be traced accurately.

Use of this database allows the definition of all the relationships between patients with AS, as well as the degree of the relatedness. It also allows the

creation of matched control groups for calculation of the relative risks and their statistical significance. To ensure anonymity of the patients in the present study, the social security numbers of participants were encrypted by the Data Protection Commission of Iceland before being used in the analyses.[17] The study protocol was approved by the Icelandic Bioethics Committee and the Icelandic Data Protection Commission.

Assessment of inheritance

The RR for disease in relatives is a measure of the risk of disease in a relative of an affected person as compared with the risk in the population as a whole. Obtaining valid estimates of the RR is, however, not straightforward because many sampling schemes may lead to biased or inaccurate estimates.[14] The use of a population-based group of patients eliminates some of this potential sampling bias. In addition, a near-complete genealogy database facilitates identification of virtually all relationships between patients. It is important to note that only probands were used in our analysis, and no attempt was made to recruit relatives of cases of AS. This design avoids the potential overestimation of familiarity when secondary cases are recruited through probands, as described by Guo.[14]

In the present study, we determined the RR for AS in a previously described Icelandic population.[4] To assess the significance of the RR obtained for a given group of patients, we compared their observed values with the RR computed for up to 1000 independently drawn and matched groups of control individuals.[18] Each patient was matched to a single control individual in each control group. The controls were drawn at random from the genealogy database and matched for the year of birth, gender and the number of ancestors recorded in the genealogy database. Empirical p values can be calculated using the control groups; thus, a p value of 0.05 for the RR would indicate that 5% of the matched control groups had values as large as or larger than that for the patient's relatives or spouses. The number of control groups required to obtain a fixed accuracy of the empirical p values is inversely proportional to the p value. We therefore selected the number of control groups generated adaptively up to a maximum of 1000. When none of the values computed for the maximum number of control groups were larger than the observed value for the patient's relatives and spouses, we report the p value as being less than 0.001. Using a variance stabilising square root transform, an approximate 95% CI may be constructed based on the distribution of RR for control groups.

RESULTS

Pedigrees

Figure 1 shows an example of an ancestral pedigree with 18 patients with AS (in black) spanning 10 generations. In this case, 102 patients were related at or within the distance of only 4 meioses in a total of 45 families. The pedigrees demonstrated significant clustering of patients with AS. Additional pedigrees can be obtained from the authors on request.

RR in relatives

The RR estimates for disease in relatives of affected patients with AS are shown in table 1. The RR for AS were 75.5, 20.2 and 3.5 (all p values<0.0001) among FDRs, SDRs and TDRs of affected patients, respectively. The RR for the fourth-degree relatives did not reach significance levels: RR 1.04 (95% CI 0.58 to 1.66), p value=0.476.

If the degree of relatedness includes aunts and uncles, nephews and nieces, or cousins, the RR is similar to the above-presented data (further details are presented in table 2).

Sex differences

Calculation of the RR for AS in siblings of affected parents with AS in relation to whether the they were paternal or maternal index cases demonstrated a similar RR; paternal siblings had a RR of 48.2 (95% CI 36.22 to 63.19) and maternal siblings a RR of 43.1 (95% CI 31.52 to 57.24). In contrast, the father of a case with AS had a much higher RR than did the mother of the index case: 167 (95% CI 131 to 194) versus 48.9 (95% CI 24.31 to 63.90).

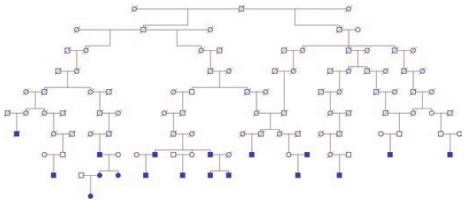


Figure 1 Pedigree of patients with ankylosing spondylitis (AS) extending over 10 generations. Disease status is known only for the later generations. This pedigree was created with the use of the deCODE genetics genealogy database. To protect the anonymity of the families, some of the unaffected relatives in the pedigree are not shown. The circles denote female family members and the squares denote male family members.

Table 1 Relative risk* estimates of ankylosing spondylitis (AS) in relatives in four generations of affected individuals with verified AS

Degree of relationship	RR (95% CI)	p Value†	Number of affected relatives
First-degree relatives	75.49 (60.19 to 93.87)	<0.001	112
Second-degree relatives	20.21 (15.15 to 30.20)	<0.001	50
Third-degree relatives	3.52 (2.20 to 5.67)	<0.001	28
Fourth-degree relatives	1.04 (0.58 to 1.66)	<0.476	18

*Risk estimates for AS are the RR (95% CI) for the estimated risk of having the disease itself (eg, the risk of AS in a relative of a patient with AS) as compared with the risk in 1000 sets of Icelandic control subjects.
†p Values are one sided and indicate the significance of the risk of disease in the combined relative groupings of all generational levels as compared with matched control subjects.

Table 2 Relative risk* for having ankylosing spondylitis (AS) for aunts and uncles, nephews and nieces, or cousins, of affected individuals with verified AS

Relationship	RR (95% CI)	p Value†	Number of affected relatives
Aunts and uncles	18.29 (12.93 to 27.63)	<0.001	20
Nephews and nieces	18.12 (12.41 to 27.63)	<0.001	20
Cousins	3.66 (2.27 to 5.72)	<0.001	26

*Risk estimates for AS are the RR (95% CI) for the estimated risk of having the disease itself (eg, the risk of AS in a relative of a patient with AS) as compared with the risk in 1000 sets of Icelandic control subjects.
†p Values are one sided and indicate the significance of the risk of disease in the combined relative groupings of all generational levels as compared with matched control subjects.

Geirsson AJ, Kristjansson K, Gudbjornsson B. *Ann Rheum Dis* (2010). doi:10.1136/ard.2009.125914

RR in spouses

The RR of spouses of patients was 6.64 (95% CI 0.88 to 12.80), but this value did not reach significance (p value=0.097). The RR for spouses of the controls was 0.952.

DISCUSSION

In the present study, we have used an extensive genealogical database, extending back to the late ninth century, for evaluating the relationships of all known patients with AS in Iceland. The study demonstrates a strong familiality of AS over three generations, but the risk of developing AS seems to disappear in the fourth generation of individuals with AS. The present results are the first to illustrate significant familiality of AS over several generations in a population-based cohort. Our study confirms results of previous studies of familiality of AS that have shown an increased prevalence of AS among siblings and FDRs of patients with AS.[5–7 12 13] These studies have illustrated a high RR of AS in siblings and FDRs of individuals with AS, with a λ_S (sibling recurrence RR=λ_S) of up to 82 and λ_{R2} (SDRs recurrence RR) of 10, compared to 75 and 20, respectively, in our study.

To our knowledge, only two studies have reported the prevalence of AS in SDRs and TDRs of individuals with AS.[5 13] In 1961, de Blecourt and coworkers published in the *Annals of Rheumatic Diseases* the prevalence of AS among 2862 relatives of 100 patients with AS and reported an 86% inclusion rate among relatives. They found that the RRs were around 10 for SDRs and 7.0 for TDRs, while we found a RR of 20 and 3.5 in SDRs and TDRs, respectively. In our study, we identified all known AS cases in Iceland and therefore were neither dependent on clinical evaluation of all the relatives of our patients nor on the prevalence value of AS in Iceland, which may explain the differences in our results. We report the RR for FDRs, SDRs and TDRs and also report that the risk of having AS mostly disappears in the fourth generations of our 259 index cases (RR 1.04; p=0.476), which is lower compared to previous report from Iceland on this issue.[13]

The observation of a RR of 75 and 20 for FDRs and SDRs, respectively, to develop AS is far higher than has been observed using the same methods for rheumatoid arthritis in Iceland, or 4.38 for FDRs and 1.95 for SDRs,[19] respectively and also well higher than we have reported for psoriatic arthritis in Iceland or 39.2 for FDRs and 12.2 for SDRs.[20] Thus, the present results demonstrate that genetic factors in AS probably play a stronger role in the development of AS than in rheumatoid arthritis or psoriatic arthritis, but that the genetic effects in all of these diseases extend over several generations. However, our study was not designed to elucidate any collective environmental or immunological factors, for example, HLA-B27, that may play a role in the pathogenetic processes of AS.

The prevalence of AS is much higher among men than women (4 to 1). Therefore, we looked at whether the RR of siblings was dependent on whether their mother or their father had AS. No significant difference in their RR was found in this respect.

All previous studies on familiality of AS have either presented only the prevalence value of AS among relatives to patients with AS or relied on estimates of the population prevalence of AS when calculating the λ values. In contrast, we based our study on complete ascertainment of all known cases of AS in Iceland as we did in our recent population-based study on the prevalence of AS in Iceland.[4] This we believe may alleviate some of the ascertainment bias that is inherent in studies that collect index cases and affected relatives for estimation of the familiar-related RR from specialised outpatient clinics only. These studies are likely to over-represent patients with more severe disease and families with more cases, which will influence the degree of heritability.[14] Meanwhile, our study used an unselected nationwide group of all known patients with verified AS in Iceland.

In conclusion, individuals with AS in Iceland are significantly more related to each other than randomly sampled Icelandic subjects for three generations. The present study used large, unselected family cohorts combined with an extended genealogy database to avoid the bias of traditional post hoc ascertainment of family members. These findings suggest that genetic factors play a stronger role in AS in comparison to rheumatoid arthritis and psoriatic arthritis.

Acknowledgements The authors would like to thank the staff of the Statistics Department at deCODE Genetics for their assistance with statistical analyses, especially Valdimar Búi Hauksson.

Funding The study was funded by the research foundations of the University Hospital in Iceland, the Society for Rheumatology in Iceland and the Wyeth Rheumatology Foundation in Iceland.

Patient consent Obtained. All participants gave their written informed consent.

Ethics approval This study was conducted with the approval of the Icelandic Bioethics Committee and the Icelandic Data Protection Commission.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Gran JT**, Husby G. The epidemiology of ankylosing spondylitis. *Semin Arthritis Rheum* 1993;**22**:319–34.
2. **De Angelis R**, Salaffi F, Grassi W. Prevalence of spondyloarthropathies in an Italian population sample: a regional community-based study. *Scand J Rheumatol* 2007;**36**:14–21.
3. **Gofton JP**, Robinson HS, Trueman GE. Ankylosing spondylitis in a Canadian Indian population. *Ann Rheum Dis* 1966;**25**:525–7.
4. **Geirsson AJ**, Eyjolfsdottir H, Bjornsdottir G, *et al*. Prevalence and clinical characteristics of ankylosing spondylitis in Iceland – a nationwide study. *Clin Exp Rheumatol* 2010. In press.
5. **de Blecourt J**, Polman A, de Blecourt-Meindersma. Hereditary factors in rheumatoid arthritis and ankylosing spondylitis. *Ann Rheum Dis* 1961;**20**:215–20.
6. **LeClercq SA**, Chaput L, Russell AS. Ankylosing spondylitis: a family study. *J Rheumatol* 1983;**10**:629–32.
7. **Bedendo A**, Glorioso S, Venturi Pasini C, *et al.* A family study of ankylosing spondylitis. *Rheumatol Int* 1984;**5**:29–32.
8. **van der Linden SM**, Valkenburg HA, de Jongh BM, *et al.* The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. *Arthritis Rheum* 1984;**27**:241–9.
9. **Järvinen P**. Occurrence of ankylosing spondylitis in a nationwide series of twins. *Arthritis Rheum* 1995;**38**:381–3.
10. **Brown MA**, Kennedy LG, MacGregor AJ, *et al.* Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997;**40**:1823–8.
11. **Pedersen OB**, Svendsen AJ, Ejstrup L, *et al.* Ankylosing spondylitis in Danish and Norwegian twins: occurrence and the relative importance of genetic vs. environmental effectors in disease causation. *Scand J Rheumatol* 2008;**37**:120–6.
12. **Brown MA**, Laval SH, Brophy S, *et al.* Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis* 2000;**59**:883–6.
13. **Thjodleifsson B**, Geirsson AJ, Björnsson S, *et al.* A common genetic background for inflammatory bowel disease and ankylosing spondylitis: a genealogic study in Iceland. *Arthritis Rheum* 2007;**56**:2633–9.
14. **Guo SW**. Inflation of sibling recurrence-risk ratio, due to ascertainment bias and/or overreporting. *Am J Hum Genet* 1998;**63**:252–8.
15. **Brewerton DA**, Hart FD, Nicholls A, *et al.* Ankylosing spondylitis and HL-A 27. *Lancet* 1973;**1**:904–7.
16. **Gulcher J**, Kong A, Stefansson K. The genealogic approach to human genetics of disease. *Cancer J* 2001;**7**:61–8.
17. **Gulcher JR**, Kristjánsson K, Gudbjartsson H, *et al.* Protection of privacy by third-party encryption in genetic research in Iceland. *Eur J Hum Genet* 2000;**8**:739–42.
18. **Reynisdottir I**, Gudbjartsson DF, Johannsson JH, *et al.* A genetic contribution to inflammatory bowel disease in Iceland: a genealogic approach. *Clin Gastroenterol Hepatol* 2004;**2**:806–12.
19. **Grant SF**, Thorleifsson G, Frigge ML, *et al.* The inheritance of rheumatoid arthritis in Iceland. *Arthritis Rheum* 2001;**44**:2247–54.
20. **Karason K**, Love TJ, Gudbjornsson B. A strong heritability of psoriatic arthritis over four generations. The Reykjavik psoriatic arthritis study. *Rheumatology* 2009;**8**:1424–8.

Paper III

GASTROENTEROLOGY 2003;125:1598–1605

Subclinical Intestinal Inflammation and Sacroiliac Changes in Relatives of Patients With Ankylosing Spondylitis

INGVAR BJARNASON,* KRISTJAN O. HELGASON,† ÁRNI J. GEIRSSON,† GUDMUNDUR SIGTHORSSON,* INGA REYNISDOTTIR,§ DANIEL GUDBJARTSSON,§ ANNA S. EINARSDOTTIR,§ ROY SHERWOOD,* KRISTLEIFUR KRISTJANSSON,§ ÓLAFUR KJARTANSSON,† and BJARNI THJODLEIFSSON†

*Department of Medicine, Guy's, King's, St. Thomas' Medical School, London, England; †Department of Medicine, University Hospital of Iceland, Reykjavik; and §deCODE Genetics, Reykjavik, Iceland

Background & Aims: **It has been suggested that subclinical intestinal inflammation plays a pathogenic role in the spondylarthropathy of ankylosing spondylitis (AS). We assessed the possible presence and inheritance pattern of subclinical intestinal inflammation in first-degree relatives of patients with AS. The relationship between this inflammation and the subjects' HLA-B27 genotype as well as computerized tomographic sacroiliac abnormalities was also assessed.** ***Methods:*** **A total of 124 of 213 (58%) available first-degree relatives of 47 patients with AS in Iceland underwent investigation for intestinal inflammation (fecal calprotectin concentration), HLA-B27 genotyping, and computerized tomography of the sacroiliac joints.** ***Results:*** **A total of 41% of the first-degree relatives had subclinical intestinal inflammation, whereas 15 of 17 spouses were normal. Variance components analyses suggest that the inheritance pattern of this inflammation is affected by a major additive gene. Some sacroiliac changes, suggestive of early AS, differed significantly between subjects with and without subclinical intestinal inflammation (mean diameter of subchondral cysts [2.9 vs. 1.2 mm; $P = 0.026$] and blurring of joint margins [9 of 44 (20%) vs. 1 of 41 (2%); $P = 0.02$]). Intestinal inflammation and sacroiliac changes did not relate to the subjects' HLA-B27 status.** ***Conclusions:*** **Many first-degree relatives of patients with AS appear to have an inherited abnormality that leads to subclinical intestinal inflammation. The association between the presence of this inflammation and the sacroiliac changes suggests that it may play a pathogenic role in the spondylarthropathy of AS.**

In the past century, an interesting link has been found between some intestinal diseases and arthritis.[1] It is most obviously seen in the inflammatory bowel diseases (IBD) ulcerative colitis and Crohn's disease (CD). These are associated with 3 patterns of arthritis,[2] one of which is a spondylarthropathy. This spondylarthropathy (requiring both clinical and radiologic features) is found in 1%–6% of patients with classic IBD, whereas radiologic sacroiliitis is more common and evident in 18% of patients with classic IBD[3] and as many as 53% are affected when modern techniques are used.[4] Clinically, this spondylarthropathy is almost identical to that of idiopathic ankylosing spondylitis (AS). However, there is a difference in the prevalence of HLA-B27 in the 2 spondyloarthropathies; the prevalence in IBD, while still high, is significantly lower than in AS.[5]

Idiopathic AS usually occurs without overt signs of intestinal inflammation, but ileocolonoscopy studies[6–9] show a high prevalence of asymptomatic intestinal inflammation. This usually involves the ileum, although some reports also show a microscopic colitis.[10,11] There seems to be a close relationship between this subclinical intestinal inflammation and the inflammation seen in patients with CD. Hence, the prevalence (AS, 20%–80%; CD, 35%–80%) and type of inflammation (presence of giant cells, granulomas, and fissures: AS, 7%–19%; CD, 8%–17%) seen on ileal biopsy are almost identical in AS and CD.[11] Furthermore, about 10% of patients initially diagnosed with AS associated with this subclinical ileitis develop classic IBD when restudied some years later.[12,13] These findings have led to 2 important suggestions about the nature and importance of the intestinal inflammation in AS.[11] First, it is suggested that the intestinal inflammation in AS may represent subclinical CD, that is, it might share a pathogenic event (genetic or environmental) that does not usually by itself progress to the full phenotype of CD. Second, it is suggested that the intestinal inflammation may play a pathogenic role in the arthropathy of AS. One proposed

Abbreviations used in this paper: AS, ankylosing spondylitis; CT, computerized tomography.

0016-5085/03/$30.00
doi:10.1053/j.gastro.2003.08.035

mechanism associates the inflammation with increased intestinal permeability to luminal antigens and/or bacteria with an HLA-B27–dependent immune response to elements of fibrocartilage resulting in the arthropathy.[11]

There are other similarities between the 2 diseases that strengthen these suggestions. Patients with AS and CD have a high prevalence of increased intestinal permeability.[14,15] Moreover, the prevalence of increased intestinal permeability in first-degree relatives of patients with AS (10%–60%) and CD (10%–54%) is similar.[11,14,15] In the case of relatives of patients with CD, it is suggested that the intestinal permeability changes are consequent to subclinical intestinal inflammation.[16] The pattern of this subclinical inflammation conforms to an autosomal additive trait, suggesting an inherited underlying genetic susceptibility (a risk factor) that requires an environmental factor for the full phenotype expression of CD.[16] If the intestinal inflammation associated with AS truly represents subclinical CD, it would seem possible that the 2 diseases share this genetic risk factor.

The purpose of this study was to assess the prevalence and possible inheritance pattern of subclinical intestinal inflammation in first-degree relatives of patients with AS. In a subgroup of relatives, we assessed if the presence of intestinal inflammation related to the subjects' HLA-B27 genotype or skeletal changes suggestive of early AS.

Patients and Methods

This study was performed in Iceland, which has a particularly well-characterized homogeneous population of 280,000. Icelanders are mostly of Scandinavian origin and, until recently, have lived in relative isolation for 1100 years; therefore, they are ideally suited for genetic studies.

This was a 2-part study. First, we studied the prevalence and possible mode of inheritance of subclinical intestinal inflammation in first-degree relatives of patients with AS. Second, we studied the possible consequences of this subclinical intestinal inflammation by assessing the prevalence of skeletal abnormalities that are suggestive of early AS by computerized tomography (CT). These findings were also assessed in relation to the subjects' HLA-B27 genotype status.

Altogether, 53 of 54 patients with AS who were approached for this study by mail and telephone participated. This represented all of the patients with AS under the care of the rheumatologists at the Reykjavik University Hospital and just >25% of all patients in Iceland with AS. The diagnosis of AS had been established at least 1 year before this study, and all patients met the diagnostic criteria for definite AS as defined by the modified New York criteria.[17] There was clustering of cases in 9 families with 2 first-degree relatives with AS; in one family, there were 4 first-degree relatives with AS.

Intestinal studies in patients with AS are complicated by the fact that these patients are treated with a number of drugs that cause or modify intestinal inflammation.[18] All of the patients with AS in this study were receiving or had received conventional nonsteroidal anti-inflammatory drugs (NSAIDs) for prolonged periods, which can lead to intestinal inflammation (NSAID enteropathy).[18] At the time of this study, 7 were not receiving any treatment, 41 were taking NSAIDs, and 21 were taking sulfasalazine (which may reduce the intestinal inflammation associated with AS[19] as well as that due to NSAIDs[20]). Furthermore, 4 were taking prednisolone and 3 were taking methotrexate, both of which may reduce intestinal inflammation in patients with IBD.

Table 1. Demographic Details of Patients With AS Studied, Their First-Degree Relatives, and Their Spouses

	Patients with AS	First-degree relatives	Spouses of patients with AS
No.	47	124[a]	17
Sex (M/F)	37/10	51/73	3/14
Age, yr (mean ± SD)	40 ± 12	45 ± 16	38 ± 11

[a]A total of 213 first-degree relatives, which excludes those younger than 16 and older than 80 years of age, were potentially available for study.

The first-degree relatives of the 53 patients who participated were approached for the first part of the study. Six patients had no relatives available for study because the relatives lived abroad or in remote places in Iceland. The remaining 47 patients had 213 first-degree relatives potentially available for study (excluding those who were younger than 16 years and older than 80 years), and 124 (58%) participated. Eighty-nine relatives (42%) did not participate because they had predetermined exclusion criteria, were living abroad, or declined participation because of frailty or various other reasons. The predetermined exclusion criteria for the patients and relatives were age younger than 16 years and older than 80 years; severe neurologic, psychiatric, endocrine (including diabetes mellitus), cardiovascular, pulmonary, hepatic, or renal diseases; malignancy; and pregnancy. Relatives with established gastrointestinal disease were excluded, as were those misusing alcohol (n = 14) and those taking NSAIDs (n = 8).[14,21] Low-dose aspirin (≤300 mg/day) was not an exclusion criteria (n = 5) because, unlike conventional NSAIDs, aspirin does not cause small bowel inflammation.[11,14,21]

Seventeen spouses also underwent studies for intestinal inflammation; if they were affected similar to the patients or first-degree relatives, this would suggest that environmental rather than genetic factors might be responsible for the inflammatory changes. The couples had been living together for 4–33 years (mean, 8 years). The spouses were subject to the same exclusion criteria as the first-degree relatives and were not taking aspirin or NSAIDs.

Table 1 shows the demographic details of the patients, first-degree relatives, and spouses studied. Of the 124 first-degree relatives studied, 100 were randomly invited to participate in the CT study. Fifteen declined (mostly those younger

than 25 years and concerned about the radiation); therefore, 85 (32 men [38%] and 53 women [62%]) underwent CT of the sacroiliac joints to assess the possible consequence of the subclinical intestinal inflammation. All but 4 of these underwent HLA-B27 genotyping.

Participants attended an investigational unit at the Icelandic National University Hospital. A medical and drug history was taken, all were questioned on back symptoms, and physical examination was performed with emphasis on spinal mobility (Schober test), chest expansion, and signs of enthesitis. Blood samples were taken for HLA-B27 genotyping, and subjects were provided with a container to collect a stool sample.

All subjects provided written informed consent. The studies were approved by the National University Hospital ethical committee, the Radiation Protection Institute, and the Data Protection Authority of Iceland. All subjects were aware of the relatively high dose of radiation (2.65 millisieverts) received during the CT scan.

Intestinal Inflammation

Intestinal inflammation was assessed by measurement of calprotectin in feces. Calprotectin is a neutrophil-selective protein that is also present in small quantities in other polymorphonuclear white cells. Its presence in feces relates quantitatively to the neutrophil flux to the gastrointestinal tract, that is, it is proportional to the degree of acute inflammation.[21–23]

Subjects provided a stool sample within 3 days of visiting the investigational unit. The samples were usually received within 8 hours of passing the stool, and 20-g portions were frozen and stored at −20°C. Calprotectin is resistant to bacterial degradation and is stable in feces at room temperature for at least 1 week.[22] After thawing, 5-g aliquots were processed for quantitative measurements by a sensitive and specific enzyme-linked immunosorbent assay as previously described.[21] The within-assay coefficient of variation was 1.2%, and the between-assay variation was 15%.

The normal range of fecal calprotectin excretion and concentration was established in 163 healthy Icelandic volunteers during these studies, mostly from health care professionals and their immediate families (88 men and 75 women; mean age, 46 ± 8 years; range, 19–72 years). None of the controls had a first-degree relative with IBD or a chronic arthritic condition (excluding osteoarthritis).

HLA-B27 Genotyping

Genomic DNA was isolated from whole blood according to standard protocols, and HLA-B27 status was determined by polymerase chain reaction as previously described.[24]

Assessment of Inheritance

Variance component analysis was performed[25] to identify possible factors controlling the calprotectin concentrations in first-degree relatives of patients with AS. This modeling allows for a single major gene affecting the trait as well as the trait being affected by environmental influences or an additive polygenic component. The polygenic and environmental influences are not distinguishable in the type of analysis, and their effect will hereafter be referred to as polygenic. The variance of trait measurement is partitioned into an additive variance (s_a), a dominance variance (s_d), a polygenic variance (s_p), and a residual variance (s). The variance of a measurement is the sum of these components, and the covariance between 2 individuals is $k\,s_a + z\,s_d + p\,s_p$, where k is the kinship coefficient of the 2 (the expected proportion of alleles they share identically by descent), p is the number of alleles shared identically by descent at the major gene, and z is 1 if 2 alleles are shared identically by descent at the major gene and 0 otherwise. These models/hypotheses will be considered.

H_0 $s_a = s_d = s_p = 0$ No genetic factor

H_p $s_a = s_d = 0, s_p \geq 0$

No major gene but a polygenic factor

H_a $s_d = 0, s_a \geq 0, s_p \geq 0$

A major additive gene and a polygenic factor

H_d $s_a = 0, s_d \geq 0, s_p \geq 0$

A major dominance gene and a polygenic factor

H_{ad} $s_a \geq 0, s_d \geq 0, s_p \geq 0$

A major additive and dominance gene and a polygenic factor

Three classes of tests were performed. To establish that there is a familial effect, H_0 was tested against all the other hypotheses. To establish that there is a major gene affecting the trait, H_p was tested against H_a, H_d, and H_{ad}. Finally, to compare how the major locus dominance and additive components fit the data, H_a and H_d were tested against H_{ad}. Testing was performed using a likelihood ratio test, assuming multivariate normality. Note that the estimate of polygenic variance component under H_p is an estimate of the heritability of log-calprotectin levels.

CT Imaging

The sacroiliac joints were examined with CT (CT Lightspeed; General Electric, Milwaukee, WI), and 3.75-mm contiguous scans were taken over the sacroiliac joint with the subjects in a supine position and the gantry angled craniocaudally parallel to the sacrum and the sacroiliac joints. The estimated effective radiation dose equivalent received during this procedure was 2.65 millisieverts. All images were read independently by 2 radiologists with no knowledge of the clinical findings. Numeric data were generated as the mean from the 2 observers. Overall, there was excellent agreement between the 2 observers, with only a single interobserver variation that was resolved by discussion.

A widely accepted scoring system for the evaluation of sacroiliitis with CT is not available. For the purpose of this study, a system was devised based on a conventional radiography scoring system that describes 6 stages of arthritis from grade 0 (normal) to grade 5 (bony ankylosis in the sacroiliac joints).[26] The CT score in the relatives fell within grade 0 (normal) and 1 (suspicious for early AS changes), and none had grade 2 (definite AS changes) or higher. Grade 1 included the presence, number, and size of subchondral cysts/erosions and blurring of joint margins. The predetermined grade 1 criteria also included subchondral sclerosis (sacral/iliac), osteitis condensans ilii, joint space narrowing (<2 mm wide), and air streak in the sacroiliac joint, but these were so infrequently encountered that reliable statistical analysis between groups was not possible.

Statistics

A Shapiro–Wilks W prime test showed that the fecal calprotectin data from the patients with AS and their relatives were not all normally distributed. Values for these are therefore presented as median and ranges, with the upper limit of normal for measurement of fecal calprotectin concentrations defined as below the 95th percentile of the median. We used the Welch modified 2-sample t test to test the difference in means of the relatives of patients and of controls, assuming the variance in the relatives and the controls was unknown and not necessarily the same in the 2 groups.

Radiologic data are presented as confidence intervals calculated by the t test and, where appropriate, the Mann–Whitney test and Fisher exact test. Kendall's τ was used for correlations.

Results

Intestinal Inflammation

The median fecal calprotectin value from controls was 3 mg/L (range, 0.1–15.5 mg/L; upper 95% confidence limits, 10.8 mg/L). Figure 1 shows the fecal calprotectin concentrations from the study groups. Significantly increased fecal calprotectin concentrations ($P < 0.0001$) were observed in patients with AS (median, 21.5 mg/L; range, 2–600 mg/L) and their first-degree relatives (median, 8.6 mg/L; range, 0.7–305 mg/L). Fecal calprotectin concentrations in the spouses of patients with AS did not differ significantly from controls (median, 4 mg/L; range, 0.1–21.5 mg/L), with 15 of 17 having values within the reference range. By comparison, 78% and 41% of the patients with AS and their first-degree relatives, respectively, had fecal calprotectin concentrations above the reference range.

HLA-B27 Genotype

Of the 47 patients with AS who underwent the fecal calprotectin studies, 42 (89%) were HLA-B27 positive. Of the 93 first-degree relatives, 53 (57%) and 40 (43%) were HLA-B27 positive and negative, respectively. The median fecal calprotectin concentrations did not differ significantly ($P > 0.6$) between the HLA-B27–positive (median, 11.9 mg/L) and HLA-B27–negative (median, 8.1 mg/L) relatives.

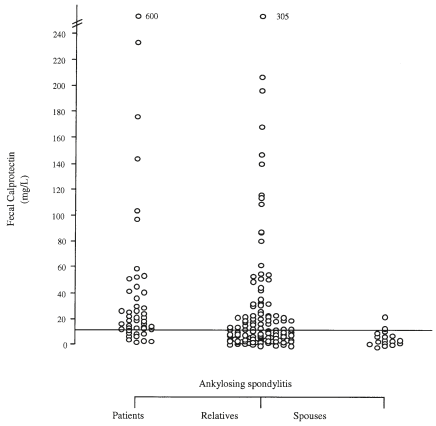


Figure 1. Fecal calprotectin concentrations in patients with AS, first-degree relatives, and spouses. The *horizontal line* indicates the upper normal limit of fecal calprotectin concentrations. A total of 78% of patients with AS and 41% of their first-degree relatives have increased calprotectin concentrations, representing intestinal inflammation. Two spouses had evidence of low-grade intestinal inflammation.

Assessment of Inheritance

We used the log-transformed calprotectin measurements in the analyses, because these transformed values are less skewed than the original values. Table 2 shows the results of variance component analyses for the first-degree relatives of patients with AS. The observed heritability is 57% and is significantly different from 0 ($P = 2 \times 10^{-15}$ when testing H_p against H_0). There is strong evidence for the presence of a major additive gene influencing calprotectin levels, and its additive variance component is estimated as 73% ($P = 0.002$ when testing H_a against H_p). Interestingly, in the presence of the additive variance component, the polygenic component is estimated as 0, showing the strength of the effect of the estimated gene effect. There is no evidence for a dominance component; adding a dominance component does not fit the data better than having only a polygenic component ($P = 0.4$ when testing H_d against H_p). Also, adding a dominance component to a model with a polygenic and an additive component does not fit the data better ($P = 1$ when testing H_{ad} against H_a).

Table 2. Results of Variance Components Analysis

Model	*df*	Additive component (%)	Dominance component (%)	Polygenic component (%)	*P* vs. H_0	*P* vs. H_p	*P* vs. H_{ad}
H_p	1			57	2×10^{-15}		
H_a	2	73		0	2×10^{-16}	0.002	1
H_d	2		42	55	8×10^{-15}	0.4	0.003
H_{ad}	3	73	0	0	6×10^{-16}	0.007	

NOTE. Estimates of variance components are reported as percentages of the total variance. *P* values are for testing the given model against H_0 and against H_p and for testing H_{ad} against the given model. The *df* of each model is given and in the first set of tests is compared with the 0 *df* of H_0.

Figure 2 shows the pedigrees (families in which 50% or more eligible relatives were studied) where relatives are identified as having increased or normal calprotectin concentrations.

CT Imaging

CT of the sacroiliac joints was grade 0 in 50 (59%) of the first-degree relatives of the patients with AS, whereas 35 (41%) had grade 1 changes; the most consistent changes were the presence of subchondral cysts (n = 33) and blurring of joint margins (n = 10).

Table 3 shows the overall results that are presented separately according to the subjects' HLA-B27 genotype and the presence of subclinical intestinal inflammation. There was no significant difference ($P > 0.6$) in the prevalence or type of sacroiliac CT changes between the HLA-B27–positive and HLA-B27–negative subjects.

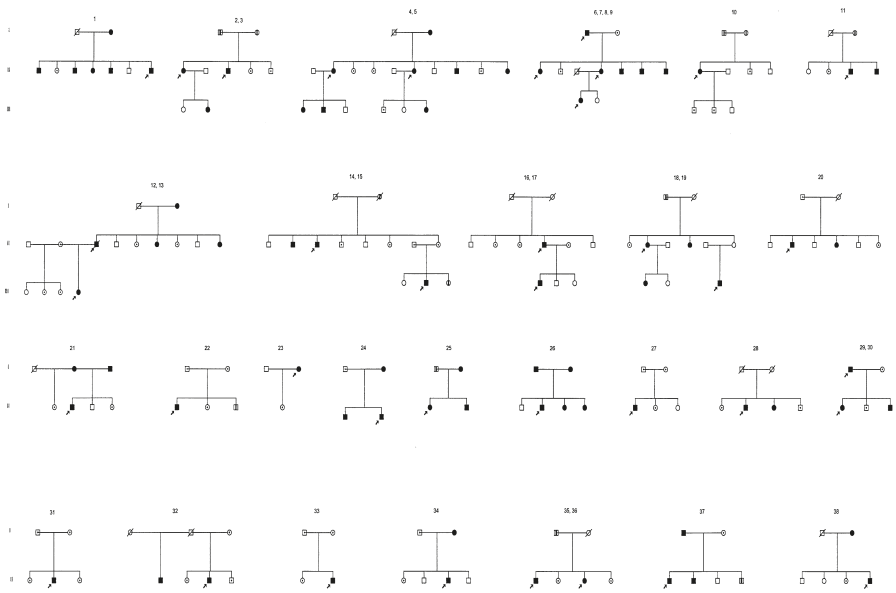


Figure 2. The pedigrees of 38 patients with AS. A *box* represents a male, and a *circle* represents a female. *Blank boxes* and *circles* indicate that the subject was not studied. *Shaded boxes* and *circles* indicate subjects with increased fecal calprotectin concentrations. Those with a *dot* indicate subjects with normal concentrations. *Arrows* identify index cases of AS. Deceased subjects have an *oblique line* through the box or circle, and a *vertical line* indicates that the subject was outside the age limits for the study. Analyses of the inheritance pattern (text) of the subclinical intestinal inflammation among the first-degree relatives of the patients with AS show a pattern consistent with an additive trait.

Table 3. Sacroiliac CT Changes, HLA-B27 Genotype, and Fecal Calprotectin

	HLA-B27 positive (n = 45)	HLA-B27 negative (n = 36)	Increased fecal calprotectin concentration (n = 44)	Normal fecal calprotectin concentration (n = 41)
Subjects with subchondral cysts (%)	19/45 (42)	13/36 (36)	21/44 (48)	12/41 (29)
No. of subchondral cysts (average no. of cysts/relatives)	45 (1.0)	39 (1.1)	59 (1.3)	29 (0.7)
Mean total cystic diameter (*mm*) of subchondral cysts (95% confidence interval)	2.0 (1.1–2.5)	2.1 (1.3–3.0)	2.9 (1.7–4.2)	1.2[a] (0.4–2.0)
Blurring of joint margins (%)	5/45 (11)	5/36 (14)	9/44 (20)	1/41 (2)[b]

NOTE. Of the 10 subjects with blurring of joint margins, 7 were men and 3 were women. Five each were HLA-B27 positive and HLA-B27 negative.
[a]Differs significantly ($P = 0.026$) from relatives with increased fecal calprotectin concentrations.
[b]Differs significantly ($P = 0.02$) from relatives with increased fecal calprotectin concentrations.

The total number and average number of subchondral cysts per subject did not differ significantly ($P = 0.076$ and $P = 0.058$, respectively) between first-degree relatives with and without subclinical intestinal inflammation. However, those with subclinical intestinal inflammation had significantly larger cysts ($P = 0.026$). Nine of the 10 subjects ($P = 0.02$) with blurring of the joint margins had evidence of subclinical intestinal inflammation. All subjects with blurring of joint margins had subchondral cysts apart from one with subclinical intestinal inflammation and the one subject without the inflammation. All of these CT changes were independent of age and sex.

Fecal calprotectin concentrations in the first-degree relatives who had CT changes (median, 12.2 mg/L) did not differ significantly ($P = 0.87$) from those without (median, 8.0 mg/L) abnormality. A further analysis was performed to assess if fecal calprotectin concentrations in subjects with CT changes differed according to whether they were HLA-B27 positive (18.6 mg/L; range, 0.80–86.2 mg/L) or HLA-B27 negative (9.3 mg/L; range, 1.8–50.1 mg/L), but this did not differ significantly ($P = 0.11$).

Symptoms and signs of spinal disease (back pain, spinal mobility, enthesitis, morning stiffness) did not differ significantly ($P > 0.4$) between the 2 groups (HLA-B27 positive vs. HLA-B27 negative; normal vs. increased fecal calprotectin concentrations).

Discussion

Our results show a high prevalence of subclinical intestinal inflammation among first-degree relatives of patients with AS. Of the models tested, the pattern of inheritance is most consistent with an additive model, suggesting that there is a genetic susceptibility to the development of the subclinical intestinal inflammation. Furthermore, the association of the subclinical intestinal inflammation with some of the CT abnormalities suggests that the intestinal inflammation may play a pathogenic role in the sacroiliac bone changes.

The intestinal manifestations of CD and AS share certain features, which suggests that they share a common etiology and pathogenesis.[7–9,11] In the first-degree relatives of patients with CD, a persuasive case can be made for the idea that the underlying cause of the subclinical intestinal inflammation (found in 50%) is genetically determined,[16] perhaps involving defective neutrophil function, and that the increased intestinal permeability[14] is consequent to this inflammation.[16] In the current study, we show that patients with AS have significantly increased fecal concentrations of calprotectin comparable to patients with CD.[16,23] However, the interpretation of this finding is hampered by the fact that all of the patients with AS were receiving or had received NSAIDs, which can cause an enteropathy.[14] However, a similar proportion of first-degree relatives of patients with AS (who had not received NSAIDs) had subclinical intestinal inflammation, as in relatives of patients with CD and of comparable severity.[16] Furthermore, the pattern of inheritance among both groups of relatives is consistent with the presence of a major additive gene when assessed with established and accepted techniques that are commonly applied to segregation analysis of quantitative traits.[27] Fifteen of 17 spouses of patients with AS had normal calprotectin concentrations, which further suggests but does not exclude that genetic rather than external factors are of pivotal importance in the development of this inflammation. Although the precise localization and nature of the subclinical intestinal inflammation in the first-degree relatives of patients with AS is uncertain, it did not relate to their HLA-B27 genotype. Collectively, these findings suggest that first-degree relatives of patients with CD and AS share a common genetic risk factor for the development of subclinical intestinal inflammation. The full phenotype expression of CD might only be brought about by the

cumulative effects of additional genetic and/or possibly environmental interactions under these circumstances.[28]

The clinical course and radiographic appearances of the spondylarthropathy associated with IBD and idiopathic AS are often indistinguishable.[2,29] The high prevalence of intestinal inflammation in patients with AS and certain other spondyloarthropathies has suggested that it plays a pathogen role in the arthropathy.[9,11,19] This intestinal-spondylarthropathy interaction hypothesis is strengthened experimentally by the finding that transgenic rats, which strongly express the human HLA-B27 and β_2-microglobulin genes, develop colonic inflammation and arthritis.[11,30] The activity of the colitis and arthritis in these rats is dependent on the presence of certain intestinal bacteria. This intestinal bacterial-host interaction is of interest because a similar mechanism is implicated in the pathogenesis of CD[28] and modification of the enteric bacterial flora is currently being exploited for therapeutic purposes in CD and various other diseases.[31]

In the present study, the intestinal-spondyloarthropathy interaction was studied by assessing the presence of sacroiliac bone changes in the first-degree relatives and correlating the findings with the results of the calprotectin test. The CT studies are complicated by at least 2 related factors. First, there are no large population studies from Iceland or elsewhere that define "normality" or "nonpathologic" age-related changes. Second, although the CT diagnostic criteria for clinically evident AS (bony ankylosis of the sacroiliac joints) are straightforward and widely recognized, there is no consensus as to the most sensitive CT criteria for the early evolving bony lesions of AS. In an attempt to overcome these problems, we extrapolated an accepted radiologic scoring system for the early changes suggestive of AS over to the CT method. The CT scans would be expected to be somewhat more sensitive than radiology and thus explain our higher prevalence of skeletal abnormalities in the first-degree relatives of patients with AS than previous (radiographic) studies.[32,33] The strength of the scoring system is that it does not include common age-related changes such as that associated with osteoarthritis and osteoporosis. The main drawback is that subchondral cysts/erosions and blurring of joint margins are not pathognomic for early AS. Nevertheless, there was excellent agreement between the radiologists in their interpretation of the CT findings, neither of who had any knowledge of the clinical or laboratory results. For the whole group of relatives, 41% had grade 1 changes without any significant association/correlation with HLA-B27 status, sex, or age. This lack of association is in keeping with there being a significant genetic susceptibility to the severity of AS that is largely conferred by complex genes other than HLA-B27.[34,35] However, there was a statistically nonsignificant tendency for an increased prevalence and number of subchondral cysts in relatives with as opposed to without subclinical intestinal inflammation. Furthermore, the mean cyst diameter was significantly greater (the larger the cysts, the more likely they are believed to represent a pathologic process) than in those with normal calprotectin concentrations, and there was a significant clustering of cases with abnormal joint margins in the high calprotectin group, most of whom also had cysts. These findings, although not specific for early AS, add some further weight to an intestinal-spondylarthropathy interaction. The reason why not all of the relatives with subclinical intestinal inflammation develop evidence of sacroiliac bone changes or why those that develop it do not progress to the full AS phenotype may again be that such progression requires additional interacting risk factors (genetic or environmental).

The complex inheritance pattern of AS is in common with many chronic diseases. AS falls best within the "complex disease trait" category of inheritance in which various environmental and additive genetic combinations (risk factors) need to be present, with the HLA-B27 genotype particularly important, before a "threshold" is reached that leads to the full phenotype of AS. The results from this study lead us to 2 main conclusions. First, the subclinical intestinal inflammation that we have identified in the first-degree relatives of patients with AS may represent the consequence of inherited genetic defects. This inflammation (frequency and inheritance pattern) closely resembles that found in first-degree relatives of patients with CD[16] and provides further evidence that the 2 diseases share common pathogenic factors. Second, this subclinical intestinal inflammation may play a pathogenic role in the sacroiliac changes on CT that have certain features that might represent the early stages of AS.

References

1. Bywaters EGL. Historical aspects of the etiology of rheumatoid arthritis. Br J Rheumatol 1988;27(suppl II):110–115.
2. Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. Gut 1998;42:387–391.
3. Wright V, Watkinson G. Articular complications of ulcerative colitis. Am J Proctol 1966;17:107–115.
4. de Vlam K, Mielants H, Cuvelier C, De Keyser F, Veys EM, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease: prevalence and HLA association. J Rheumatol 2000;27:2860–2865.
5. Russell AS, Percy JS, Schlaut JS, Sartor VE, Goodhart JM, Sher-

baniuk RW, Kidd EG. Transplantation antigens in patients with Crohn's disease: the linkage of associated ankylosing spondylitis with HLA-W27. Am J Dig Dis 1995;20:359–361.
6. Leirisalo-Repo H, Repo H. Gut and spondyloarthropathies. Rheumatol Dis Clin North Am 1992;18:23–35.
7. Mielants H, Veys EM, Cuvelier C, De Vos M, Botelberghe L. HLA B27 related arthritis and bowel inflammation: Part 2. Ileocolonoscopy and bowel histology in patients with HLA B27 related arthritis. J Rheumatol 1985;12:294–298.
8. Mielants H, Veys EM, De Vos MM, Cuvelier C. Gut inflammation in the pathogenesis of idiopathic forms of reactive arthritis and in the peripheral joint involvement of ankylosing spondylitis. In: Mielants H, Veys EM, ed. Spondylarthropathies. Involvement of the gut. Amsterdam: Excerpta Medica, 1987:11–21.
9. Mielants H, Veys EM, Cuvalier C, De Vos M. Ileocolonoscopic findings in seronegative spondyloarthropathies. Br J Rheumatol 1988;27(suppl II):95–105.
10. Leirisalo-Repo H, Turunen U, Stenman S, Helenius P, Seppala K. High frequency of silent inflammatory bowel disease in spondylarthropathy. Arthritis Rheum 1994;37:23–31.
11. Smale S, Natt RS, Orchard TR, Russell AS, Bjarnason I. Inflammatory disease and spondylarthropathy. Arthritis Rheum 2001; 44:2728–2736.
12. De Vos M, Cuvelier C, Mielants H, Veys E, Barbier F, Elewaut A. Ileocolonoscopy in seronegative spondylarthropathy. Gastroenterology 1989;96:339–344.
13. Mielants H, Veys EM, Cuvelier C, De Vos M, Goemaere S, De Clercq L, Schatteman L, Gyselbrecht L, Elewaut D. The evolution of spondyloarthropathies in relation to gut histology: II. Histological aspects. J Rheumatol 1995;22:2273–2278.
14. Bjarnason I, Macpherson AJM, Hollander D. Intestinal permeability: an overview. Gastroenterology 1995;108:1566–1581.
15. Martinez-Gonzales O, Cantero-Hinojosa J, Paule-Sastre P, Gomez-Magan JC, Salvatierra-Rios D. Intestinal permeability in patients with ankylosing spondylitis and their healthy relatives. Br J Rheumatol 1994;33:644–647.
16. Thjodleifsson B, Sigthorsson G, Cariglia N, Reynisdottir I, Gudbjartsson DF, Kristjansson K, Meddings JB, Gudnason V, Wandall JH, Andersen LP, Sherwood R, Kjeld M, Oddsson E, Gudjonsson H, Bjarnason I. Subclinical intestinal inflammation: an inherited abnormality in Crohn's disease relatives? Gastroenterology 2003;124:1728–1737.
17. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for the modification of the New York criteria. Arthritis Rheum 1984;27:361–368.
18. Bjarnason I, Hayllar J, Macpherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine. Gastroenterology 1993;104:1832–1847.
19. De Vos M, Mielants H, Cuvelier C. Ileitis in the spondylarthropathies. In: Allan RN, Rhodes JM, Hanauer SB, Keighley MRB, Alexander-Williams J, Fazio VW, eds. Inflammatory bowel disease. New York: Churchill Livingstone, 1997:451–460.
20. Hayllar J, Price AB, Smith T, Macpherson A, Gumpel MJ, Bjarnason I. Nonsteroidal antiinflammatory drug-induced small intestinal inflammation and blood loss: effect of sulphasalazine and other disease modifying drugs. Arthritis Rheum 1994;37:1146–1150.
21. Tibble J, Sigthorsson G, Foster R, Scott D, Roseth A, Bjarnason I. Faecal calprotectin: a simple method for the diagnosis of NSAID-induced enteropathy. Gut 1999;45:362–366.
22. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating calprotectin in feces: a methodologic study. Scand J Gastroenterol 1992;27:793–798.
23. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M, Bjarnason I. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;47:506–513.
24. Calvert GT, Shore EM. Human leukocyte antigen B27 allele is not correlated with fibrodysplasia ossificans progressiva. Clin Orthop 1998;346:66–70.
25. Abney M, McPeek MS, Ober C. Estimation of variance components of quantitative traits in inbred populations. Am J Hum Genet 2000;66:629–650.
26. Dale K. Radiographic grading of sacroiliitis in Bechterew's syndrome and allied disorders. Scand J Rheumatol 1980;9(suppl 32):92–97.
27. Cardon LR, Garner C, Bennett ST, Mackay IJ, Edwards RM, Cornish J, Hegde M, Murray MAF, Reid IR, Cundy T. Evidence for a major gene for bone mineral density in idiopathic osteoporotic families. J Bone Miner Res 2000;15:1132–1137.
28. Shanahan F. Crohn's disease. Lancet 2002;359:62–69.
29. Orchard TR, Thiygaraja S, Welsh KI, Wordsworth BP, Gaston JHS, Jewell DP. Clinical phenotype is related to HLA genotype in the peripheral arthropathies in inflammatory bowel disease. Gastroenterology 2000;118:274–278.
30. Rath HC, Ikeda JS, Linde HJ, Schomerich J, Wilson KH, Sartor RB. Varying cecal bacterial loads influences colitis and gastritis in HLA-B27 transgenic rats. Gastroenterology 1999;116:310–319.
31. Farrell RJ, LaMont JT. Microbial factors in inflammatory bowel disease. Gastroenterol Clin North Am 2002;31:41–62.
32. Contu L, Capelli P, Sale S. HLA B27 and ankylosing spondylitis: a population and family study in Sardinia. J Rheumatol 1977; 3(suppl):18–23.
33. Konieczna B, Stawarz M, Mackiewicz S. Occurrence of HLA antigens in patients with ankylosing spondylitis and in their families. Arch Immunol Ther Exp (Warsz) 1983;31:153–160.
34. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, Taylor A, Calin A, Wordsworth P. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–1828.
35. Hamersma J, Cardon LR, Bradbury L, Brophy S, van der Horst-Bruinsma I, Calin A, Brown MA. Is disease severity in ankylosing spondylitis genetically determined? Arthritis Rheum 2001;44: 1396–1400.

Received April 19, 2003. Accepted August 21, 2003.

Address requests for reprints to: Ingvar Bjarnason, M.D., M.Sc., F.R.C.Path., F.R.C.P.(Glasg), D.Sc., Guy's, King's, St. Thomas' Medical School, Bessemer Road, London SE5 9PJ, England. e-mail: ingvar.bjarnason@kcl.ac.uk; fax: (44) 20-7346-6474.

Supported in part by a Biomedical Research Collaboration grant from the Wellcome Trust (ref. 048461) and the Arthritis Research Campaign, United Kingdom (ref. B0627).

Paper IV

ARTHRITIS & RHEUMATISM
Vol. 56, No. 8, August 2007, pp 2633–2639
DOI 10.1002/art.22812

A Common Genetic Background for Inflammatory Bowel Disease and Ankylosing Spondylitis

A Genealogic Study in Iceland

Bjarni Thjodleifsson,[1] Árni J. Geirsson,[1] Sigurdur Björnsson,[1] and Ingvar Bjarnason[2]

Objective. **Patients with ankylosing spondylitis (AS) and ~50% of their first-degree relatives may have a genetic abnormality that results in subclinical intestinal inflammation. This study was undertaken to examine the familial occurrence and cosegregation of AS and inflammatory bowel disease (IBD) in order to determine whether there is a shared genetic risk factor in families.**

Methods. **The Icelandic genealogy database and population-wide data on all living Icelanders diagnosed as having AS (n = 205) and/or IBD (n = 1,352) were used to estimate the risk ratios of AS for relatives of patients with AS, the risk ratios of IBD for relatives of patients with IBD, and the cross-risk ratios of AS for relatives of patients with IBD or of IBD for relatives of patients with AS. The mean kinship coefficients for each disease were calculated. The control population for disease risk calculations comprised 10,000–100,000 sets of matched Icelandic subjects.**

Results. **First-, second-, and third-degree relatives of patients with AS had risk ratios of 94, 25, and 3.5, respectively, indicating an increased risk of developing AS (each $P < 0.0005$), while first-, second-, and third-degree relatives of patients with IBD had risk ratios for IBD of 4.4, 2.2, and 1.4, respectively (each $P < 0.0001$). The cross-risk ratios of IBD were 3.0 and 2.1 in first- and second-degree relatives of patients with AS, respectively, and were the same for AS in first- and second-degree relatives of patients with IBD. With the exception of Crohn's disease, the risk of having AS, ulcerative colitis, or IBD in spouses of patients with these diseases did not differ significantly from that in controls. Calculation of the kinship coefficients confirmed these patterns of familial risk.**

Conclusion. **Patients with AS or IBD in Iceland are significantly more related to each other than are randomly sampled control subjects, in terms of an increased risk of either or both conditions developing in third-degree relatives. These findings suggest that one or more undiscovered genetic variants may underlie the risk of both diseases.**

An interesting link has been found between some intestinal diseases and arthritis (1). This is most obviously illustrated in chronic inflammatory bowel disease (IBD), Crohn's disease, and ulcerative colitis. IBD is associated with 3 patterns of arthritis (2), one of which is a spondylarthropathy. This spondylarthropathy is not uncommon, with 3–6% of patients with classic IBD being affected (3), while radiologic sacroiliitis is evident in up to 18% of patients. The spondylarthropathy associated with IBD differs from that of idiopathic ankylosing spondylitis (AS) in that the prevalence of HLA–B27, although still high (20–40%), is significantly lower than in AS (which characteristically has a prevalence of HLA–B27 of ~90% [4]).

Both IBD and AS show familial clustering and may coexist in a patient. Evidence from studies of twins and other first-degree relatives suggests that the genetic basis is somewhat stronger for IBD than for AS (5–8). Apart from the potential role of the NOD2 gene in Crohn's disease and the recently reported role of the IL23R gene in IBD (9), the susceptibility loci for both IBD and AS remain largely unknown. The at-risk vari-

Supported by the Landspitali-University Hospital Research Fund.

[1]Bjarni Thjodleifsson, MD, PhD, Árni J. Geirsson, MD, Sigurdur Björnsson, MD: Landspitali University Hospital, Reykjavik, Iceland; [2]Ingvar Bjarnason, MD, DSc: Guy's, King's College, and St. Thomas' Hospitals Medical School, London, UK.

Address correspondence and reprint requests to Bjarni Thjodleifsson, MD, PhD, Landspitali University Hospital, Hringbraut, Reykjavik IS-101, Iceland. E-mail: bjarnit@landspitali.is.

Submitted for publication January 13, 2007; accepted in revised form May 4, 2007.

ants in NOD2 (R702W, G908R, and 3020insC) have been reported in 15–25% of patients with Crohn's disease in some European populations (10,11), but not in the Icelandic population (12). Disease expression in AS, in contrast, is largely, but not exclusively, genetically based, with non–HLA–B27 genes potentially playing a significant role (1–4,12–19). Alterations in the NOD2 gene do not appear to confer susceptibility to AS (20), but in a recent report, CARD15 gene polymorphisms were identified in a subgroup of patients with AS whose disease was associated with chronic intestinal inflammation. (21)

Idiopathic AS usually occurs without overt signs of intestinal inflammation. However, ileocolonoscopy studies (13–15) have shown a high prevalence of asymptomatic inflammation of the terminal ileum and microscopic colitis (22,23). The nature of this inflammation is uncertain, but there is a close similarity to the inflammation of IBD, particularly in Crohn's disease (24). The resemblance is enhanced by the observation that first-degree relatives of patients with AS (21–60%) (17) and first-degree relatives of patients with Crohn's disease (10–54%) (18,19) have increased permeability of the small intestine. In relatives of patients with Crohn's disease, the findings suggest that the changes to intestinal permeability are a consequence of subclinical intestinal inflammation (12). The inheritance pattern of this subclinical inflammation conforms to the profile of an additive trait. The same subclinical inflammation is evident in relatives of patients with AS, and it also conforms to the profile of an additive trait (25).

On the basis of these findings and other observations (13–15), it appears that the intestinal inflammation in AS may represent a form of subclinical IBD, which, in particular, resembles Crohn's disease. If so, this implies that these diseases share a pathogenic event that, in the case of AS, may progress to the full phenotype of Crohn's disease (16). An important first step in examining this hypothesis is to demonstrate convincingly the coinheritance of these conditions; if this effort is successful, it has the potential to facilitate the identification of a common step in the pathogenesis of each disease that may be amenable to targeted treatment.

Icelanders have several features that render them an attractive study population for assessing the heritability of IBD/AS. In this regard, Icelanders are relatively homogeneous with respect to the environment, cultural aspects, and genetic factors. Iceland has extensive genealogic records that cover the familial data of the whole nation, together with a bureaucratically small and accessible health care system. In the present study we leveraged these resources to 1) determine the degree of relatedness of IBD and AS in relatives of patients with IBD/AS, as compared with that in random population samples, and 2) assess the risk ratios of IBD and AS individually and combined among relatives of patients with IBD and patients with AS at varying degrees of genetic distance.

PATIENTS AND METHODS

IBD and AS registries. The IBD registry is a population-based sample of data from all patients diagnosed as having IBD in Iceland for the 50-year period 1950–2000, most of whom have been described in detail elsewhere (26–28). The patients were born in the years 1885–1990. This group consisted of a total of 1,352 patients, including 1,091 with ulcerative colitis and 261 with Crohn's disease; 8 patients had indeterminate colitis and were therefore excluded from this study. The included patients were participants of 3 different studies that had the same principal investigator (26–28).

The diagnoses of these patients were biopsy based and independently reviewed; the disease in all patients was confirmed to fulfill the accepted diagnostic criteria (29). Each patient was required to have had at least 1 year of followup, and many patients have undergone decades of reevaluation and had received confirmation of their final diagnosis. The distribution of inflammation in the patients with ulcerative colitis was as follows: 54% with involvement of the rectum only, 30% with involvement of the procto-sigmoid, 5% with left-sided colitis, and 11% with extensive colitis. The distribution of inflammation in the patients with Crohn's disease was as follows: 55% with involvement of the colon only, 19% with ileocolonic involvement, 25% with involvement of the ileum only, and 1% with involvement of the stomach.

The AS registry is also a population-based sample and includes data from all patients diagnosed as having AS in Iceland for the period 1950–1996. There were 205 patients with AS, with a male:female ratio of 1.7:1. The criteria for the diagnosis of AS were the American College of Rheumatology modified criteria (30). Associated peripheral arthritis was found in 98 patients (48%), uveitis in 53 patients (26%), Crohn's disease in 1 patient (0.5%), and ulcerative colitis in 9 patients (4%).

Genealogy database. DeCODE Genetics (Reykjavik, Iceland) has built a computerized genealogy database of more than 760,000 individuals (31). The database contains records of all living Icelanders, comprising more than 290,000 individuals, and a large proportion of data from subjects of Icelandic descent who have ever lived in the country are in the database. The genealogy of the entered individuals is recorded from multiple sources, including church records, censuses from previous centuries, and, more recently, published genealogies.

The genealogy database is essentially complete and spans from the 18th century to the current time, thus allowing distant relationships to be traced accurately (32). Use of this database allows investigation into the relationships among patients for both diseases, as well as assessment of the various levels of relatedness. It also allows the creation of matched

control groups for use in assessing the statistical significance of the results.

To ensure anonymity of the patients in the present study, the social security numbers of participants were sent to the Data Protection Commission of Iceland for encryption before arriving at the laboratory (33). Thus, all medical information was imported to deCODE Genetics with encrypted identifiers. Both the Icelandic Bioethics Committee and the Icelandic Data Protection Commission approved the present study protocol, and all study participants gave their written informed consent.

Assessment of inheritance. The risk ratio in relatives is a measure of the risk of disease in a relative of an affected person as compared with the risk in the population as a whole. This measure is directly related to the power to identify, or map, susceptibility genes (34). Obtaining valid estimates of the risk ratio is, however, not straightforward, because many sampling schemes may lead to biased or inaccurate estimates (35). The use of a population-based group of patients eliminates some of this potential sampling bias. In addition, a near-complete genealogy database facilitates identification of patients who are related to other patients.

In the present study we determined the risk ratio for IBD and the risk ratio for AS in a previously described Icelandic population (32), in separate analyses and in combined analyses of the diseases. Empiric P values and 95% confidence intervals were estimated in relation to the distribution of risk ratios calculated for 10,000 sets of matched control subjects from Iceland, with each control subject matched to each patient by sex, year of birth, and number of ancestors in the 5 preceding generations (32).

The cross-risk ratio was used to estimate the relatedness between AS and IBD, as well as the relatedness of the subcategories ulcerative colitis and Crohn's disease. The cross-risk ratio is an estimate of the risk of IBD or its subcategories ulcerative colitis and Crohn's disease in relatives of patients with AS, as compared with the risk in the population as a whole. This was computed in a manner similar to that for the risk ratio, using the patients with AS as the proband and determining the number of IBD cases among their relatives. The cross-risk ratio was symmetric, so we did not consider the risk of AS among relatives of patients with IBD separately (32).

The mean pairwise kinship coefficient (KC) was calculated for all of the patients with IBD and all of the patients with AS. The KC values were compared with the distribution of the mean KCs among 100,000 sets of control subjects who were matched to the patients in the same manner as described above for the risk analyses. The KC is the probability that 2 randomly selected alleles at an autosomal locus, 1 from each individual, are inherited from a common ancestor. The results of KC calculations were refined by excluding the genetic contributions from relatives at 1 or 2 meioses, up to and including 5 meioses. Genetic contributions from the first- and second-degree relatives may dominate the results, and therefore the resulting values were compared with the distribution of values for the 100,000 matched control groups. The KC is expressed as a genealogic index of familiality, calculated as the mean KC $\times$ 100,000 (36). All P values reported were nominal.

RESULTS

Pedigrees. Figure 1 shows a representative pedigree of 8 patients with IBD and 8 patients with AS (1 of these patients had both conditions) extending over 6 generations. Fourteen of the patients were related within and at a distance of 6 meioses and could be traced to a common pair of ancestors. The pedigrees demonstrated significant clustering of patients with AS and significant clustering of patients with IBD within families.

Risk ratios in relatives. The risk ratio estimates and cross-risk ratio estimates for disease in relatives of affected patients are shown in Table 1. The risk ratios for AS were 94, 25, and 3.5 (all $P < 0.0005$) among first-, second-, and third-degree relatives of affected patients, respectively. The risk ratios for IBD were 4.4, 2.2, and

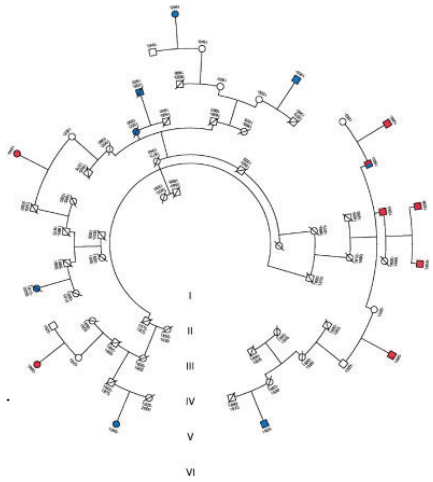


Figure 1. Pedigree of patients with inflammatory bowel disease (IBD) and patients with ankylosing spondylitis (AS) extending over 6 generations. The representative pedigree shows 8 patients with IBD (blue symbols) and 8 with AS (red symbols), 1 of whom had both conditions. Except for 2 patients with IBD, all of the patients could be traced to a common pair of ancestors. Disease status is known only for the later generations. This pedigree extends over 6 generations, as indicated by the Roman numerals, and was created with the use of the deCODE Genetics genealogy database. To protect the anonymity of the families, some of the unaffected relatives in the pedigree are not shown. The circles denote female family members, and the squares denote male family members.

Table 1. Risk ratio estimates and cross-risk ratio estimates of disease in relatives of affected patients*

Disease	No. of patients	Risk ratio or cross-risk ratio estimate (95% CI)					*P*, combined†		
		First-degree relatives	Second-degree relatives	Third-degree relatives	Fourth-degree relatives	Fifth-degree relatives	First- to fifth-degree relatives	First- and second-degree relatives	Third- to fifth-degree relatives
AS	205	94 (74–114)‡	25 (16–36)‡	3.5 (1.6–8.2)‡	0.9 (0.3–1.9)	1.4 (0.8–2.1)	<0.0001	<0.0001	0.0039
Crohn's disease	261	3.7 (1.1–8.5)‡	1.9 (0.7–5.6)	0.3 (0.1–1.0)‡	0.8 (0.5–1.4)	1.1 (0.74–1.4)	0.066	0.0054	0.94
Ulcerative colitis	1,091	2.9 (1.6–5.0)‡	2.0 (1.3–3.1)‡	1.1 (0.8–1.5)	1.2 (0.9–1.4)	0.9 (0.8–1.1)	<0.0001	<0.0001	0.36
IBD	1,352	3.0 (1.8–4.6)‡	2.1 (1.4–2.9)‡	1.0 (0.7–1.3)	1.1 (0.9–1.3)	0.9 (0.8–1.1)	<0.0001	<0.0001	0.68
Crohn's disease	261	5.9 (1.8–11.7)‡	2.6 (0.9–6.9)	3.6 (2.2–5.9)‡	0.7 (0.3–1.5)	1.5 (1.0–2.0)‡	<0.0001	0.0003	0.0001
Ulcerative colitis	1,091	2.1 (1.1–3.5)‡	1.4 (0.9–2.1)	1.2 (0.9–1.6)	1.4 (1.2–1.7)‡	1.0 (0.9–1.2)	<0.0001	0.0028	0.0039
IBD	1,352	2.9 (1.8–4.8)‡	1.6 (1.1–2.3)‡	1.7 (1.3–2.0)‡	1.3 (1.1–1.5)‡	1.1 (1.0–1.3)‡	<0.0001	<0.0001	0.0001
Ulcerative colitis	1,091	5.4 (4.3–6.9)‡	2.7 (2.2–3.3)‡	1.3 (1.1–1.6)‡	1.3 (1.1–1.5)‡	1.13 (1.0–1.2)‡	<0.0001	<0.0001	<0.0001
IBD	1,352	4.8 (3.9–5.8)‡	2.4 (2.0–2.9)‡	1.3 (1.1–1.5)‡	1.3 (1.1–1.4)‡	1.1 (1.0–1.2)‡	<0.0001	<0.0001	<0.0001
IBD	1,352	4.4 (3.6–5.3)‡	2.2 (1.8–2.6)‡	1.4 (1.1–1.5)‡	1.3 (1.1–1.4)‡	1.1 (1.0–1.2)‡	<0.0001	<0.0001	<0.0001

* Risk estimates for each primary heading of disease (ankylosing spondylitis [AS], Crohn's disease, ulcerative colitis, and inflammatory bowel disease [IBD]) are the risk ratio (95% confidence interval [95% CI]) for the estimated risk of having the disease itself (e.g., the risk of AS in a relative of a patient with AS) as compared with the risk in 10,000 sets of Icelandic control subjects, whereas the risk estimates for each secondary subheading of disease are the cross-risk ratio (95% CI) for the estimated risk of having that disease in relation to the primary heading disease (e.g., the cross-risk of Crohn's disease in a relative of a patient with AS) as compared with that in the control population, in first- through fifth-degree relatives of affected patients.
† *P* values are 1-sided and indicate the significance of the risk of disease in the combined relative groupings of all generational levels, close relatives (first- and second-degree only), and distant relatives (third- through fifth-degree only) as compared with matched control subjects.
‡ The 95% CI does not include 1.0 (1-sided $P < 0.025$).

1.4 (all $P < 0.0001$) among first-, second-, and third-degree relatives of affected patients, respectively. The cross-risk ratios for IBD were 3.0 and 2.1 in first- and second-degree relatives of patients with AS, respectively, and were the same for AS in first- and second-degree relatives of patients with IBD. The estimates of risk and cross-risk in relatives of patients with ulcerative colitis and in relatives of patients with Crohn's disease were comparable.

Risk ratios in spouses. The risk ratio estimates for disease in spouses of affected patients are shown in Table 2. The risk ratio for having AS or Crohn's disease in spouses of affected patients was 0 (i.e., none of the spouses had either of these diseases). Moreover, the risk ratio for having ulcerative colitis or IBD in spouses was not significant. In contrast, the risk ratio for having Crohn's disease in spouses of patients with Crohn's disease was significantly increased ($P = 0.003$), whereas no increase in the risk ratio was observed for spouses of patients with ulcerative colitis.

Kinship coefficients. Table 3 shows the relatedness of IBD and AS separately and combined. On the basis of the KC, it was evident that patients with IBD were significantly more related to each other than were population controls. The KC was significant in relatives up to the fourth generation. Similar to the findings from the risk analyses, even stronger relationships among AS patients were evident up to the first 3 generations. Moreover, patients with IBD and patients with AS were more closely related to each other, even after exclusion of second-degree relatives (grandparents/grandchildren or first cousins), than were population controls.

DISCUSSION

Many previous epidemiologic studies have demonstrated the involvement of genetic components in the

Table 2. Risk ratio estimates of disease in spouses of affected patients*

Disease	No. of patients	No. of spouses	Risk ratio (95% CI)	*P*†
AS	205	196	0	1.0
Crohn's disease	261	196	0	1.0
Ulcerative colitis	1,091	196	1.1 (0.0–4.3)	0.54
IBD	1,352	196	0.9 (0.0–3.9)	0.61
Crohn's disease	261	232	21 (4.5–30)	0.003
Ulcerative colitis	1,091	232	0	1.0
IBD	1,352	232	3.5 (0.9–8.2)	0.033
Ulcerative colitis	1,091	1,101	1.3 (0.3–4.6)	0.35
IBD	1,352	1,101	1.1 (0.3–4.1)	0.43
IBD	1,352	1,366	1.4 (0.5–4.8)	0.22

* Risk estimates are the risk ratio (95% CI) for the estimated risk of having the disease as a spouse of an affected patient, as compared with that in the control population. See Table 1 for definitions.
† Significance of the risk ratio in spouses versus controls.

Table 3. Kinship coefficients (KCs) for the risk of developing IBD and AS, separately and combined*

Exclusion for KC calculation	IBD			AS			AS vs. IBD		
	KC	KC for controls (95% CI)	*P* vs. controls	KC	KC for controls (95% CI)	*P* vs. controls	KC	KC for controls (95% CI)	*P* vs. controls
No exclusions	14.5	11 (10–11)	<0.00001	75.3	8.2 (6.0–11)	<0.00001	12.9	11 (10–12)	0.00005
Exclusion									
First-degree relatives	13.4	11 (10–11)	<0.00001	44.2	8.0 (6.0–11)	<0.00001	11.7	11 (9.8–12)	0.011
Second-degree relatives	11.6	10 (9.5–11)	<0.00001	19.1	7.6 (5.8–9.7)	<0.00001	11.2	10 (9.5–11)	0.0079
Third-degree relatives	10.2	9.4 (8.9–9.8)	0.00017	10.2	7.1 (5.6–8.8)	0.00075	10.0	9.6 (9.0–10)	0.13
Fourth-degree relatives	9.1	8.5 (8.1–8.9)	0.0038	7.0	6.2 (5.0–7.6)	0.12	9.2	8.8 (8.2–9.3)	0.060
Fifth-degree relatives	7.7	7.4 (7.1–7.8)	0.079	6.5	5.3 (4.3–6.3)	0.013	8.1	7.7 (7.3–8.2)	0.059

* Values are the mean pairwise KC calculated for patients with IBD and patients with AS, as compared with the distribution of the mean KC in 100,000 groups of matched control subjects. The KC value is the probability that 2 randomly selected alleles at an autosomal locus, 1 from each individual, are inherited from a common ancestor. See Table 1 for other definitions.

risk of IBD as well as in the risk of AS. Our results confirm these observations, and also show that heritability in Iceland is much stronger in close relatives of patients with AS than in close relatives of patients with IBD. Furthermore, we provide the first direct evidence to support a common genetic component for IBD and AS, which may be highly significant in view of the fact that patients with AS frequently have a form of chronic intestinal inflammation resembling that of classic IBD (13–15).

The approach taken in the present study has several methodologic advantages over those taken in previous studies (7,35,37–39), since many prior studies were limited in size and assessed limited extended relationships. Our study was based on a nationwide, population-based sample containing all patients diagnosed as having IBD and/or AS over a relatively long, defined period, which reduces the potential for selection bias. The Icelandic genealogy database uniquely permits investigation of multiple levels of relationships among patients and allows comparisons with a population-based sample of controls. In this study, the risk ratio for disease in spouses of patients with ulcerative colitis and spouses of patients with AS who lived in the same environment did not differ from that of controls, indicating that environmental factors did not substantially affect the results in subjects in these disease categories. Similarly, the demonstration of familial aggregation of IBD and AS beyond the nuclear family (in which shared environmental components may predominate) supports the role of genetic influences on the risk of these 2 diseases.

In contrast, our results showed a higher rate of Crohn's disease in spouses of patients with Crohn's disease than in control subjects, suggesting that they may be more related to each other than are randomly selected pairs of Icelanders, or that the risk of developing Crohn's disease may be influenced by a common environmental factor that is only observed later in life. However, the robustness of this finding is somewhat undermined by the fact that the significance levels were critically dependent on a small sample size in which only 2 spouses had Crohn's disease of a total of 232 subjects, and this was reflected by the wide confidence interval. Although there is a possibility that selection bias could have affected these results, all patients with Crohn's disease in Iceland seek their medical care at the same medical centers, rendering selection bias less likely to occur.

Another key new finding from this study is the elevated cross-risk ratio between IBD and AS, which was demonstrated in both first- and second-degree relatives. This finding further suggests that there are genetic components shared by these complex diseases. Previous ileocolonoscopy studies in patients with AS have shown substantial similarities in the macro- and microscopic appearances of the terminal ileum as compared with that in patients with Crohn's disease (13–17), and other investigators have found a high prevalence of colitis among patients with AS. There is also the possibility of an association between Crohn's disease and AS that is indicated by the fact that the 2 coexist in the same individual more often than do ulcerative colitis and AS. Both twin studies and other genetic studies have shown that there is a stronger genetic component in the risk of developing AS (40) and Crohn's disease (6,41) than in the risk of developing ulcerative colitis. Similar relationships are evident when subclinical disease markers are assessed in first-degree relatives of patients with these disorders (12).

Thus, relatives of patients with AS and relatives of patients with Crohn's disease may have a higher

prevalence of increased intestinal permeability and subclinical intestinal inflammation (12,25) than is observed in relatives of patients with ulcerative colitis (42,43). When taken together, the results of the present study and those from previous studies suggest that there is a strong and common genetic component to the risk of AS and IBD. However, the nature of this genetic abnormality is speculative. There is, almost certainly, not an effect from the IBD1 gene, since the at-risk variants in the NOD2 gene are not found in Icelandic patients with Crohn's disease (12) and the prevalence of this genotype is not increased in AS (44).

Our results provide strong evidence that a molecular-genetic approach should be utilized in patients with these diseases, in order to identify the putative common genetic abnormality in AS and IBD. If the approach proves successful, this will open the possibility of identifying a common early pathophysiologic event in both AS and IBD that may be amenable to new and selective treatments.

ACKNOWLEDGMENT

We thank the staff of deCODE Genetics for access to the data and assistance with statistical analyses.

AUTHOR CONTRIBUTIONS

Dr. Thjodleifsson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Thjodleifsson, Geirsson, Björnsson, Bjarnason.

Acquisition of data. Thjodleifsson, Geirsson, Björnsson, Kárason (nonauthor; deCODE Genetics), Kristjánsson (nonauthor; deCODE Genetics), Reynisdóttir (nonauthor; deCODE Genetics), Stefánsson (nonauthor; deCODE Genetics).

Analysis and interpretation of data. Thjodleifsson, Stefánsson (nonauthor; deCODE Genetics).

Manuscript preparation. Thjodleifsson, Geirsson, Björnsson, Bjarnason, Stefánsson (nonauthor; deCODE Genetics).

Statistical analysis. Thorvaldsson (nonauthor; deCODE Genetics), Halapi (nonauthor; deCODE Genetics), Hauksson (nonauthor; deCODE Genetics).

REFERENCES

1. Bywaters EG. Historical aspects of the aetiology of rheumatoid arthritis. Br J Rheumatol 1988;27 Suppl 2:110–5.
2. Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. Gut 1998;42:387–91.
3. Wright V. Ankylosing spondylitis: aetiology. Proc R Soc Med 1966;59:451–3.
4. Maksymowych WP. Ankylosing spondylitis: at the interface of bone and cartilage. J Rheumatol 2000;27:2295–301.
5. Halfvarson J, Bodin L, Tysk C, Lindberg E, Jarnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology 2003;124:1767–73.
6. Orholm M, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins: results of a nationwide study. Scand J Gastroenterol 2000;35: 1075–81.
7. Peeters M, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, et al. Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 1996;111:597–603.
8. Lee JC, Lennard-Jones JE. Inflammatory bowel disease in 67 families each with three or more affected first-degree relatives. Gastroenterology 1996;111:587–96.
9. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006;314:1461–3.
10. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599–603.
11. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603–6.
12. Thjodleifsson B, Sigthorsson G, Cariglia N, Reynisdottir I, Gudbjartsson DF, Kristjansson K, et al. Subclinical intestinal inflammation: an inherited abnormality in Crohn's disease relatives? Gastroenterology 2003;124:1728–37.
13. Leirisalo-Repo M, Repo H. Gut and spondyloarthropathies. Rheum Dis Clin North Am 1992;18:23–35.
14. Mielants H, Veys EM, Cuvelier C, De Vos M. Ileocolonoscopy and spondyloarthritis. Br J Rheumatol 1988;27:163–4.
15. Mielants H, Veys EM, Cuvelier C, De Vos M. Subclinical involvement of the gut in undifferentiated spondylarthropathies. Clin Exp Rheumatol 1989;7:499–504.
16. Mielants H, Veys EM, De Vos M, Cuvelier C, Goemaere S, De Clercq L, et al. The evolution of spondyloarthropathies in relation to gut histology. I. Clinical aspects. J Rheumatol 1995;22:2266–72.
17. Vaile JH, Meddings JB, Yacyshyn BR, Russell AS, Maksymowych WP. Bowel permeability and CD45RO expression on circulating CD20+ B cells in patients with ankylosing spondylitis and their relatives. J Rheumatol 1999;26:128–35.
18. May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? Gastroenterology 1993;104:1627–32.
19. Teahon K, Smethurst P, Levi AJ, Menzies IS, Bjarnason I. Intestinal permeability in patients with Crohn's disease and their first degree relatives. Gut 1992;33:320–3.
20. Van der Paardt M, Crusius JB, de Koning MH, Murillo LS, van de Stadt RJ, Dijkmans BA, et al. CARD15 gene mutations are not associated with ankylosing spondylitis. Genes Immun 2003;4:77–8.
21. Laukens D, Peeters H, Marichal D, Vander Cruyssen B, Mielants H, Elewaut D, et al. CARD15 gene polymorphisms in patients with spondyloarthropathies identify a specific phenotype previously related to Crohn's disease. Ann Rheum Dis 2005;64:930–5.
22. De Vos M, Cuvelier C, Mielants H, Veys E, Barbier F, Elewaut A. Ileocolonoscopy in seronegative spondylarthropathy. Gastroenterology 1989;96(2 Pt 1):339–44.
23. Porzio V, Biasi G, Corrado A, De Santi M, Vindigni C, Viti S, et al. Intestinal histological and ultrastructural inflammatory changes in spondyloarthropathy and rheumatoid arthritis. Scand J Rheumatol 1997;26:92–8.
24. Smale S, Natt RS, Orchard TR, Russell AS, Bjarnason I. Inflammatory bowel disease and spondylarthropathy [review]. Arthritis Rheum 2001;44:2728–36.
25. Bjarnason I, Helgason KO, Geirsson AJ, Sigthorsson G, Reynisdottir I, Gudbjartsson D, et al. Subclinical intestinal inflammation and sacroiliac changes in relatives of patients with ankylosing spondylitis. Gastroenterology 2003;125:1598–605.

26. Bjornsson S. Inflammatory bowel disease in Iceland during a 30-year period, 1950-1979. Scand J Gastroenterol Suppl 1989;170: 47–9.
27. Bjornsson S, Johannsson JH, Oddsson E. Inflammatory bowel disease in Iceland, 1980-89: a retrospective nationwide epidemiologic study. Scand J Gastroenterol 1998;33:71–7.
28. Bjornsson S, Johannsson JH. Inflammatory bowel disease in Iceland, 1990-1994: a prospective, nationwide, epidemiological study. Eur J Gastroenterol Hepatol 2000;12:31–8.
29. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl 1989;170:2–6.
30. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
31. Gulcher J, Kong A, Stefansson K. The genealogic approach to human genetics of disease. Cancer J 2001;7:61–8.
32. Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, Arnason S, et al. Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS Med 2004;1:e65.
33. Gulcher JR, Kristjansson K, Gudbjartsson H, Stefansson K. Protection of privacy by third-party encryption in genetic research in Iceland. Eur J Hum Genet 2000;8:739–42.
34. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 1990;46:222–8.
35. Guo SW. Inflation of sibling recurrence-risk ratio, due to ascertainment bias and/or overreporting. Am J Hum Genet 1998;63: 252–8.
36. Cannon-Albright LA, Thomas A, Goldgar DE, Gholami K, Rowe K, Jacobsen M, et al. Familiality of cancer in Utah. Cancer Res 1994;54:2378–85.
37. Halme L, Turunen U, Helio T, Paavola P, Walle T, Miettinen A, et al. Familial and sporadic inflammatory bowel disease: comparison of clinical features and serological markers in a genetically homogeneous population. Scand J Gastroenterol 2002;37:692–8.
38. Binder V. Genetic epidemiology in inflammatory bowel disease. Dig Dis 1998;16:351–5.
39. Brophy S, Taylor G, Blake D, Calin A. The interrelationship between sex, susceptibility factors, and outcome in ankylosing spondylitis and its associated disorders including inflammatory bowel disease, psoriasis, and iritis. J Rheumatol 2003;30:2054–8.
40. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–8.
41. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins: a study of heritability and the influence of smoking. Gut 1988;29:990–6.
42. Loranskaia ID, Zorin SN, Gmoshinskii IV, Shirina LI, Iurkov M, Mitrofanova IP, et al. Permeability of intestinal barrier for macromolecules in patients with Crohn's disease and ulcerative colitis. Klin Med (Mosk) 1999;77:31–3. In Russian.
43. Resnick RH, Royal H, Marshall W, Barron R, Werth T. Intestinal permeability in gastrointestinal disorders: use of oral [99mTc] DTPA. Dig Dis Sci 1990;35:205–11.
44. Brown MA, Brophy S, Bradbury L, Hamersma J, Timms A, Laval S, et al. Identification of major loci controlling clinical manifestations of ankylosing spondylitis. Arthritis Rheum 2003;48:2234–9.