University of Bath

PHD

Genetic and environmental factors influencing susceptibility and outcome in ankylosing spondylitis

Brophy, Sinead

Award date:
2001

Awarding institution:
University of Bath

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

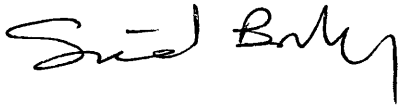
Download date: 22. May. 2019

Genetic and environmental factors influencing susceptibility and outcome in ankylosing spondylitis.

Submitted by Sinèad Brophy
For the degree of PhD of the University of Bath 2001

COPYRIGHT
Attention is drawn to the fact that copyright of this thesis rests with its author. This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the written consent of the author.

This thesis may be made available for consultation within the University Library and may be photocopied or lent to other libraries for the purposes of consultation



UMI Number: U601383

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

UMI U601383
Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.
All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

UNIVERSITY OF BATH
LIBRARY
55
28 NOV 2001
Ph.D.

Contents:

Acknowledgements

I would like to thank Kirsten MacKay, Matthew Brown and Michele Doran for making this study enjoyable and exciting.
Thank you to my supervisors, Dr Andrei Calin and Professor David Blake for all their help and advice.
Gordon Taylor advised me on the statistical analysis and appropriate methods for much of this work and Dr P Lewis on the secondary diseases study. I would like to say I am very grateful for all their help and guidance.
I would like to thank the Arthritis Research Campaign who funded me in this research.

Finally, I would like to say a big thank you to my dad who read all the first drafts of the PhD and the papers.

Collaborations with other institutes

This project includes work which was conducted collaboratively with, Matthew Brown and Kirsten MacKay from the Wellcome Trust Centre for Human Genetics, Oxford, Michele Doran at the Mayo Clinic, Rochester, and Helana Santos a visiting research fellow from the Instituto Português, Portugal.

Dr Kirsten MacKay, Dr Christopher Mack and Dr Andrei Calin devised a scoring system in 1996 for evaluating the spine and hips in AS. However, this system was not validated. The validation of the scoring system for AS (BASRI) was undertaken as part of this current study. The work was carried out in collaboration with Kirsten MacKay (Wellcome Trust, Oxford). The author, Sinèad Brophy, was first reader for the validation process and also carried out the analysis and interpretation. The second reader was Kirsten MacKay who also provided advice and amendments. The work employing the validated BASRI to describe the radiological progression of AS was performed and analysed by Sinèad Brophy.

The work on : eye, skin and bowel disease in spondylarthritis, interaction between age at onset and severity, impact of sex on inheritance of AS, interaction between sex, susceptibility factors and outcome in AS, concomitance of disease among affected relatives, and birth order in AS was performed by Sinèad Brophy.

Genetic typing of patients was performed at the Wellcome Trust Centre, Oxford in collaboration with Dr Matthew Brown. However, analysis on the identification of severity loci in AS was performed by Sinèad Brophy (with advice from Matthew Brown). The work on the role of susceptibility genes in severity was performed by Sinèad Brophy with blood results provided by the Wellcome Trust Centre for Human Genetics.

Dr Helena Santos (Visiting research fellow) collaborated on the AS and exercise study and Dr Michele Doran (Mayo Clinic, Rochester, MN) collaborated on the predictors of outcome in AS. In both cases this involved clinical interpretation (ie review of medical notes) by the medical doctors. The design, statistical analysis, and interpretation was conducted by Sinèad Brophy. The write up of the research for publication of these two studies was performed on a equal bases, with initial drafts by Sinèad Brophy and amendments and alterations suggested by the medical doctors.

Ethical approval

Ethical approval for this study was granted by the Bath local research ethics committee. The reference number for this approval is BA130/98-99.

1. Abstract

Ankylosing Spondylitis (AS) is the second most common form of inflammatory arthropathy after rheumatoid arthritis, affecting 1:200 men and 1: 500 women. Age at onset and severity are highly variable. The impact of sex and genetic transmission are poorly defined. Herein, a series of clinical, epidemiological and genetic studies explore these issues. The Bath National AS database (n= 5500) was used. 1) A new scoring system for radiographs was validated and implemented in order to examine the nature of progression in AS. 2) The influence of a) genetic and b) environmental variables on progression was examined. This involved an autosomal genome screen, a two year longitudinal study and control matched parent-child / sibling-sibling studies.

The data reveal : 1) Although symptoms begin in the mid twenties, the disease process may start as early as the first decade. The more severe the disease in the spine the more likely disease is to affect other areas, such as hips, skin, eyes and GI tract. 2 a). Severity is determined by genetics (chromosomes 13,18, 20, 21) and these genes are discrete from those determining susceptibility to AS (chromosome 6, 16). Furthermore, women carry more susceptibility factors and their children are more likely to develop AS and inflammatory bowel disease. The results from this study suggest that susceptibility genes may determine distribution of affected areas (eg bowel, skin, hip). However, severity genes determine the degree of inflammation in these areas. 2 b) Exercise, education and smoking may modify the activity of the disease (pain, function etc) but results show that radiological change appears to be an inherent phenomenon. These results pave the way for further research with the goal of modifying severity of AS. Future work should target the genes involved and identify their protein products, leading to improved prognosis for the first time.

2. Explanation of terms used

Susceptibility to disease : Predisposition to disease

Susceptibility genes: Genes which are associated with development of disease.

Severity of disease : The impact that the disease has on the patient. This can be measured or defined in terms of outcome measures (ie amount of radiological change, function of patient, disease activity of patient, need for surgery etc) at a specific point in time.

Severity genes : Genes which may be associated with severity of disease. However, there is no current evidence that severity genes exist.

Interaction between severity and susceptibility genes : How these genes overlap or affect one another : It is not known 1. If severity genes exist 2. If susceptibility genes work by making the disease more severe and therefore are severity genes 3. If severity and susceptibility genes are independent and separate entities.

Course of the disease/ progression of AS/ pathogenesis of disease/ evolution of disease : How the disease develops on a population level.

Outcome : The measurement of severity. An end result at a specific time point. Mortality, function, disease activity, radiological change, and prevalence of secondary disorders may all be used to give outcome of AS.

Secondary Diseases: Iritis, psoriasis and inflammatory bowel disease are all concomitant disorders associated with AS.

3. Outline of proposal

Ankylosing spondylitis is a chronic progressive inflammatory disorder affecting the axial skeleton and the peripheral joints. It is a condition which generally begins in the teens / early twenties and is unusual in that it is almost totally (95% in Caucasians) associated with one specific genetic marker (HLA-B27). However, AS is a polygenic disease. Therefore, there could be a great deal of interaction both between the different genetic components as well as between the genes and the environmental factors and this is likely to impact on the level of disease susceptibility and severity. There is marked individual variation in outcome and disease prognosis. Some individuals have minimal symptoms with only pelvic involvement, while others have widespread disease resulting in a curved spine and severe disability. There is no cure for the disorder. Moreover, it is uncertain if the long-term course of the disease can be altered. It is known that sex plays an important role in pathogenesis of disease with men having more severe axial disease. However, little is known on how genetic, sex and environmental factors interact to influence the disease course. Understanding the effects of these interactions will not only aid in the treatment of AS but also provide a model, which can be extrapolated to other polygenic inflammatory disorders.

3.1 Structure of investigation

This proposal is divided into two sections :

a) The nature of progression in AS : characteristics and disease evolution.
The validation of an existing radiographic scoring system in order to define the disease course in AS.

b) Genetic and environmental factors impacting on progression of disease.
The examination of how interactions between susceptibility and/or severity genes and environmental factors influence rate of progression in AS.

3.2 Hypothesis:

AS is an expression of **both** susceptibility genes **and** severity genes with environmental factors playing little part in disease outcome.

3.3 Aims and Purpose of the investigation

This study aims to examine the inter-relationship between genetics, sex and the environment in determining disease pathogenesis. Specifically to:

1. validate the Bath Ankylosing Spondylitis Radiology Index (BASRI).
2. describe the course of AS using BASRI in terms of radiological development and progression,
3. examine the relative contribution of genetics in terms of susceptibility and severity of disease.
4. to examine the relative contribution of environmental factors in terms of susceptibility and severity of disease.

4. Background

4.1 Ankylosing spondylitis : a historical perspective

The term ankylosing spondylitis (AS) comes from the Greek words ankylos (bent or crooked) and spondylos (vertebra)[1] . AS has been with man throughout the ages. It has been found in Egyptian mummies [Amenhotep II (Pharaoh in 1439-1413 BC) , Rameses the Great (1298-1232 BC) and his son and successor Merneptah] [2] [3] and ancient tribes world wide. It has been identified as the condition which Saint Banus (355-395 AD) suffered from (named Father Palm Tree in local language due to his stooped posture), forcing him to eat and sleep standing for 18 years [4] .This condition was recognized as distinct from Rheumatoid Arthritis by Hippocrates as early as the second century [5] . However it was not until 1898 that an accurate description of AS was published. Pierre Marie [5] outlined AS both in terms of post-mortem examinations of fusion and on patients posture and walking. Seventy five years later a major advance in understanding of AS occurred when it was shown that 95% of Caucasian patients all had one gene in common, HLA B27. Yet, AS is not a condition only found in man, a disease very much like ankylosing spondylitis has also been described in prehistoric crocodiles, monkeys and in horses.

4.2 What is the course of AS

AS is the second most common form of inflammatory arthritis (after rheumatoid arthritis) affecting 1 in 200 men (0.5%) and 1 in 500 women (0.2%). In general it occurs in patients who are genetically susceptible and exposed to an environmental trigger. It is thought to begin in the sacroiliac joints and progress up the spine. However, cases of AS without sacroiliac joint involvement have been reported [6] [7] [8]. Even so, the diagnosis of AS requires sacroliitis of grade 2 bilaterally or 3-4 unilaterally plus clinical criteria such as limited movement of lumbar spine, history of pain, limited chest expansion. However, 1.5%-10% of patients may be asymptomatic [9] . In addition, it could be argued that in the early stages plain radiographs are insufficient to detect changes in the sacroiliac joints [and other unrelated conditions such as tuberculosis, SAPHO syndrome, systemic lupus erythematosus, gout [10] or paraplegia [5] may also cause sacroliitis] . Mild disease

may never reach the threshold level of damage needed to achieve diagnosis. Women have milder disease than men in terms of radiographic change [1] and this may mean that it is the women who are less likely to be diagnosed. Thus, reports that the disease is less common in women could simply be a fault of the diagnostic criteria. All current research uses patients diagnosed under the New York Criteria and this might mean that many of the findings on AS are based on moderate and severe cases with a higher ratio of men than women. This will not only impact on our estimates of the incidence of AS but also presents a biases that research findings may only be based on the advanced diseased patients. Mild and early AS may not be studied under today's criteria. More importantly the criteria for classification have been developed for Caucasian patients and may not have been applicable to non-Caucasians.

The course of the disease differs among individuals but it also differs from country to country. It occurs at a younger age in developing countries. Juvenile AS [JAS] affects a greater proportion of patients in Mexico [11] , India [12] , and North Africa [13] than among European or American patents. JAS in general is associated with first symptoms in the knee or lower limbs.[14] Therefore, this means a greater proportion of patients in developing counties present with lower peripheral joint involvement. Ankylosing spondylitis is indigenous in Mexico and was present before European people arrived.[15] The knee, midtarsus and ankle are more frequently involved among these Mexican patients, but this condition is rarely associated with psoriasis. Yet, psoriatic arthritis is one of the most common forms of spondyarthritis (SpA) in Southeast Asia [16] . Thus, the type of clinical symptoms associated with AS do differ depending on country, whether this is ethic background or environmental differences is not clear. However, a severe outcome is more common in developing countries. For example, in Pakistan there was worse disease activity and function compared to an equivalent British Cohort [17] and in North Africa a higher proportion of patients have juvenile onset and suffer from peripheral arthritis, hip involvement, enthesopathies and are less likely to have axial changes than western patients [18]. A study in Morocco showed patients have more severe disease accompanied by hip involvement [19]. On the other hand in sub-Saharan Africa ankylosing spondylitis is extremely rare. In these patients (n=29) there was an older age at onset (age 32 years), no family history or psoriasis or iritis or

inflammatory disease [20]. Similarly, the prevalence of AS in Japan is very low at a rate of 2 per 100 000 [21] while prevalence in China is 2 per 1000 [22] . Among the Chinese patients there was more peripheral involvement but less iritis than in western countries and extra-articular manifestations are rare [23] . Patients in Saudi Arabia show a mean age of onset of 23.4 years, a positive family history in only 13 % of patients and iritis in 7% . Thus, the disease appears to be rare and shows a mild course with few complications.

Clearly the clinical pattern of disease can be influence by a number of factors including race, sex, age at onset, and associated conditions. In addition, differences in referral and follow-up practices and availability of rheumatology expertise and relevant resources may also explain some of the variation.

In western patients there is lower than normal bone density in early or mild AS. This early osteoporosis/osteopenia may result from disease specific inflammatory and cytokine-induced changes [24][25][26][27][28] . As the disease progresses greater than normal bone density (compared to controls) is observed as fusion takes place [28][30] (although there may be continuing loss of bone from specific areas of the skeleton). The fusion will spread from the lumber spine up to the neck. The hips and peripheral joints may also become involved. However, it is possible that the fusion is in fact a protective mechanism of the body protecting against fractures due to the osteoporosis [31]. Although AS is described as a chronic condition it may be argued that it is really an acute disease with many changes in the beginning but little active inflammation later. There are no reliable inflammatory markers. Erythrocyte sedimentation rate (ESR), plasma viscosity and C-reactive protein (CRP) are not abnormal in many patients with active disease [32] [33][34]. Other measures, such as such as IL-6, are found to be increased in the majority of patients with AS, but there is no correlation between IL-6 and disease activity [35] . The main symptoms of the condition are pain, fatigue, morning stiffness, tenderness, discomfort which can be measured by the Bath Disease Activity Index [BASDAI] [36] [Appendix 1] (this consists of 6 questions assessing on a 10 point scale the level of activity of disease). In defining outcome various approaches have been taken, culminating in the development of an international group (ASAS) formed to evaluate the best end-points for use in AS [37] [38] . The domains selected for measurement were: physical function, pain, spinal mobility, peripheral entheses, spinal radiograph and

patient global assessment. For function either the Bath functional index (BASFI) [a 0-10 scale (with 10 being the worst function and 0 being normal) assessing 10 aspects of daily function)] [39] [Appendix 1] or Dougados functional index (FI Dougados)[40] may be used. While for pain measurement two 100mm visual analogue scales (VAS) , one for night pain and the other for general pain are employed. The patient global assessment would be a VAS such as BAS-G [41] [Appendix 1] which consists of two question assessing patients global well-being over a 1 week or 6 month period.

These are all aspects of the disease which may only be measured by patients subjective reports. There are very few objective and independent measures of the activity or severity of AS. For example, when disease activity is reported to decline as the disease progresses [42] it is possible that some of this decline may simply be a result of patients adaptation to living with the disease. Thus, psychological and social factors might affect these self assessment measures of AS.

A more objective approach would be to examine the disease in animals. The transgenic rat model develops a spontaneous inflammatory disease involving colitis and arthritis. For this condition to occur, T cells, gut bacteria (normal bacterial flora) and a high expression of B27 are needed.[43] . The earliest clinical manifestation is diarrhea which 100% of rats develop. The rat develops bowel disease, which is associated with increased production of cytokines, nitric oxide and myeloperoxidase. Most rats then develop peripheral arthritis, sacroiliitis and spondylitis with skin and nail changes appearing later. However, rats held in a germfree environment showed no evidence of gut or joint inflammation. Once the germfree rats are exposed to normal gut flora the entire disease process returns [44]. Transgenic mice with the HLA-B27 gene are more susceptible to bacterial infection than mice without the gene.[45] However most importantly, for the rat or mouse to develop any symptoms of AS a large number of copies of B27 are needed (ie gene copy number and level of expression of B27 is important for susceptibility). In addition, the strain of animal (ie genetic background) exerts a strong influence.

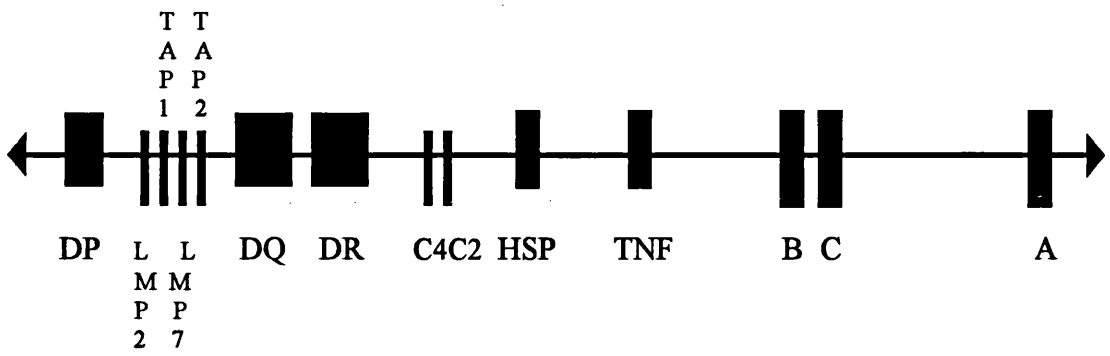
4.3 What causes AS ? : a) Genetics

A genetic susceptibility to disease is the first major contributor to developing AS. The link between HLA B27 and AS has been recognised for 25 years [46] [47]. This B27 association is consistent with a dominant or additive mode of inheritance. [48] There also exists weaker associations between this antigen and uveitis [49] [50] [51] and inflammatory bowel disease [52] [53]. The prevalence of AS world-wide varies within the different races, with frequency roughly following the distribution of HLA-B27. For example, B27 is absent among the Australian aborigines, and they do not suffer from AS. [54] The highest prevalence of both ankylosing spondylitis and B27 is found among the native American Indians (Haida, Pima) [54] [55] . It is also highly prevalent among the indigenous populations of the circum-polar regions [56]. This high frequency of the antigen may be explained by the finding that it is advantageous in generating viral specific immunity. For example, HIV infection progresses more slowly in HLA-B27 individuals [56]. Thus, it has been suggested that the reason the native American Indians have such a high presence of B27 is because these are the descendants of the people who survived small pox or other European viruses when the Spanish first arrived. Moreover, the overrepresentation of B27 concordance among dizygotic twin pairs suggests a survival advantage for this gene. [57] Approximately 10% of all B27 positive subjects develop AS.[58] [59] [60].

Why do only some people with B27 develop AS ?

It has been shown that the risk for developing the disease is 16 times greater in the HLA B27(+) relatives of AS patients than HLA-B27 (+) individuals of the population at large [58] [60]. In addition, as discussed above, in animal studies of B27(+) transgenic rats, the background strain of the rat will influence disease penetrance of the arthritis [61]. This indicates that the B27 antigen is important in developing AS but it is not sufficient by itself for the development of disease. For example, among the Fula ethnic group in The Gambia the gene B27 is nearly as common as among Caucasians. However, AS is extremely rare. This population may lack additional non-B27 susceptibility factors [62] . Conversely in a Lebanese population of 19 AS patients only 5 (26%) were B27 positive [63] and among a Zimbabwean population only 1 in 8 (13%) AS patients were B27 positive. [64] [65] . Therefore, non-B27 disease predisposing factors play a strong role in susceptibility

Figure 1: Representation of major histocompatability complex on chromosome 6



from Wordsworth and Brown [1] page180

to disease in these populations. This suggests that, in Western populations where the B27 gene is prevalent there is a strong and characteristic association between this gene and AS. However, non- B27 susceptibility factors also exist and can cause disease. In populations of low natural prevalence of B27 then these additional factors form an important component in predisposition to AS.

Other genes linked to AS

The HLA genes are within the major histocompatibility complex on Chromosome 6 [see figure 1], they encode molecules involved in recognition within the immune system. The class I molecules (such as those encoded by HLA B27) are involved in response to virus-infected cells and tumours. Class II molecules are expressed on specialised antigen-presenting cells (B lymphocytes, macrophages etc) and they act to stimulate antibody production and are involved in cellular responses. In addition, other loci within the major histocompatibility complex have immunological functions (cytokines, molecular chaperones etc) which potentially may be involved in influencing AS.

HLA B60 (a split of B40) increases susceptibility to AS in B27 positive [66] [67] and negative subjects [68]. It is believed to act in a synergistic manner with B27, increasing risk in the B27 / B60 positive individuals by a factor of three [69] . However, this is not confirmed by all studies [69] [70] [71] . Other HLA-B alleles which may have a role in susceptibility include HLA-B49 [69] , HLA-B16 [72] , HLA-B39 [73] [74] and HLA-B22 [75]. In addition, the genes of the class II molecules are speculated to have a role in susceptibility but again, studies have yielded conflicting results. HLA DR1 has been reported to increase risk of AS [71] [76] [77] albeit with only a minor effect [57] . DR4 may be associated with peripheral arthritis when AS is present [78] and DR2 has been reported to have similarities of peptide presentation with B27 [79] and has been associated with susceptibility to AS [80]. However, unlike the clear repeatability of the B27 association, the DR susceptibility effects are not consistently observed. For example, DR 8 may be important for the development of uveitis (iritis) in Japanese patients with AS [81], while Mexican and Norwegian studies do not support this finding. Nevertheless, the gene did influence other aspects of phenotypic expression, such as age at onset [82][83]. Another HLA class II gene (LMP2) may be the risk factor for

uveitis [84][85] and it is known that there is linkage disequilibrium between the DR 8 and LMP2 genes [86]. However, among a white Caucasian population the role of LMP2 in is not supported [87]. It is thought that non-HLA B27 alleles play a more significant role in the clinical expression of disease in Mexican patients [88] Thus, similar genes may have different roles depending on the ethnic background of the individual and perhaps environmental factors.

There is evidence of protective effects from certain subtypes of genes. For example, tumour necrosis factor encoded within the Class III region of the MHC may have alleles (TNF308 and TNF238.2) which have a protective role [89]. In addition, B35 is found in higher numbers than expected among unaffected relatives of AS patients [90]. Two subtypes of the B27 genes, B2706 and B2709 appear to be protective [91][92][93]. However, in the presence of the pathogenic B2704 subtype, the protective effects of B2706 are overridden. [94]

The presence of susceptibility (and perhaps protective genes) outside the MHC is supported by family and twin studies. The contribution of the MHC genes collectively has been calculated to be 50% or less of the total genetic contribution to the disease [57][67][95]. The T-cell receptor genes are on chromosome 7 and 14, and the T-cell receptor B locus may be involved in susceptibility [96]. In addition, it has been suggested since 1973 [97] that the susceptibility genes of the concomitant disorders (inflammatory bowel, psoriasis and iritis) associated with spondylitis may also act as susceptibility factors for AS. This hypothesis is supported by the finding that B27 negative patients suffer more psoriasis and inflammatory bowel disease [98] Thus, AS is clearly a polygenic disease, with many of the genes interacting. The phenotypic expressions depend presumably on the presence or absence of other genes.

Strikingly, a low prevalence of SpA can change abruptly as shown in sub-Saharan Africa with the advent of HIV & AIDS. In addition, studies of identical twins reveal disease concordance in only 50%-70% of pairs suggesting that not all of the susceptibility influences come from the genes, interaction with the environmental must also be taken into account.

b) Environment

Identical twin studies have shown even in patients with susceptibility to AS, the disease does not always develop. AS needs to be triggered by an environmental stimulus. This trigger is likely to be a bowel or urinary tract infection since many reactive arthropathies such as Reiter's Syndrome which are within the spondyarthritis family, are triggered by a bowel or genitourinary infection. [1] In addition, over 50% of patients with documented AS who underwent an ileocolonoscopy had evidence of inflammation of the bowel. [99]. In fact, gut inflammation is frequent in patients with spondylarthropathy, a quarter of whom have early features of Crohn's disease [52] . In addition, recent studies have indicated that the gut has a role in the severity of disease [101]. Among patients and their first-degree relatives there exists altered small intestine permeability which could possibly be genetically determined and may precede the development of bowel and joint symptoms. [102] Perhaps the strongest evidence for a environmental trigger comes from the HLA transgeneic rat. These animals develop bowel inflammation followed by arthritis, but if the rat is maintained in a sterile environment neither the inflammatory gastrointestinal disease, nor the arthritis develop. However, there is much debate as to which bacteria are involved. For example, the role of *Kebsiella* remains unknown [103]. The isolation of *Kebsiella* from patients with inactive disease was associated with a flare 2-3 months later, and it is claimed that bacteria may exacerbate disease [104]. AS patients have also been found to have elevated levels of antibodies against bacteria such as *Klebsiella* [105] The connection between the genetic and bacterial triggering agents is thought to lie in the way B27 is expressed. B27 is unique in binding poorly with β2-microglobulin (normally associated with class I molecules as they are assembled in the endoplasmic reticulum to be transported to the cell surface). Thus B27 positive individuals may have two forms of the B27 molecule: the normal B27~β2-microglobulin associated molecule, and the globulin free molecule. The latter have a low affinity for T cells. Thus, during the process of T cell selection, the T cells which recognise this molecule will not be identified as self/auto-reacting cells. These self-cells will not be deleted and destroyed. Several bacteria have sequences similar to those of

B27[107]. A bacterial infection will expand the T cells with the specificity for recognising self-B27 cells, initiating inflammation [56].

Yet, for every study identifying the role of specific bacteria, there are as many studies which have not found a link between gut flora and AS. [108][109] For example, it has been shown that when 82 AS patients were compared to 36 healthy controls there was no difference in faecal flora in terms of isolation rate of *Klebsiella* or other arthritogenic organisms [110]. However, it could be argued that the trigger is present at the beginning of the disease process but there is no reason why it should remain after this time. Thus, the absence of *Klebsiella* or other arthritogenic organisms among long term AS patient does not rule out its role as a trigger. In addition, the high frequency of juvenile-onset disease in developing countries may be a result of high incidence of infection in childhood. [111]. Support for this theory comes from findings that hip disease (which is more prevalent among juvenile onset patients in Morocco than among patients in France) is associated with environmental factors such as 'no running water in the home' in Moroccan AS patients. [112] Therefore, these data suggest that regardless of clinical status, gut infection may be the initial trigger for the immune response which leads to spondylitis. However, the putative triggers are likely to be common pathogens and not easily eliminated from the environment of susceptible individuals. It is also possible that there is not one trigger initiating AS, but instead a cascade of different bacteria, and each assault is progressively dealt with less well by the susceptible individuals. Thus, any gut pathogen with sequences similar to B27 or other susceptibility genes (and there are several bacteria of this type) may act as a trigger for the inflammation leading to AS.

4.4 Why is the disease in some patients severe and others mild ?

Many interlinking and overlapping variables affect severity of AS. For example some of the factors found to influence outcome include: dietary habits and a peripheral pattern of arthritis, which impact on the disease activity of AS (pain, fatigue, discomfort, morning stiffness etc) [106][113] . Smoking [114] is associated with worse clinical, functional and radiological outcome. Patients in the low education/occupational group have greater disease severity and poorer prognosis than those with higher occupational status [115][116]. Disability is more frequent

after heavy physical work or labour involving exposure to cold conditions, while sedentary work and vocational rehabilitation programs protect against long-term disability. [117] Cessation of work due to AS, before retirement age is associated with female sex, low education levels, uveitis and coexistence of non-rheumatic diseases. [116] Progression of disease is slowed by daily exercise [118], with women and younger patients showing most improvement [119].

However, it is difficult to separate the effects of one variable from another. For example, the influence of smoking may actually be a reflection of social status, occupation (manual labour) or lack of exercise.

In addition, other predictors of severity such as; hip disease [120], peripheral joint involvement and gut inflammation [106] may point to a genetic rather than environmental component. One study using clinical entry variables to predict long term outcome identified severe disease as being associated with the presence of: hip arthritis, ESR>30mm/h, sausage-like finger or toe, oligoarthritis, onset < 16 years, limitation of the lumbar spine and poor efficiency of nonsteroidal anti-inflammatory drugs. [121] However, clearly here, there is a risk of circular reasoning with those already doing badly destined to do less well.

Trauma, or immobilisation due to injury has been implicated in precipitating AS. However, it may be that immobilisation might bring the already existing disease to the patent's attention. [122] Associated with this is the realisation that the onset of AS symptoms may not necessarily indicate the beginning of AS. Conceivably, the time when the trigger is pulled may precede symptoms by years or even a decade.

Thus, pain, function and progression of AS may be influenced by inter-related factors such as education, occupation and exercise. However one of the most important factors in determine severity of AS is sex.

Hormones as an environmental effect on severity

There exists an obvious and dramatic difference in susceptibility and severity of AS between the sexes. Men are 2.5 times more likely to suffer from AS [123]. Most studies suggest that most of the diseases associated with B27 (eg. Reiter's disease, AS, PsAS) occur much more commonly in men. [124] Women have milder disease in terms of axial involvement although extra-spinal manifestations such as hip involvement and iritis occur in equal frequency to men. [125][126] Pregnancy does

not influence the overall course of AS. However, peripheral arthritis and anterior uveitis occurred less frequently during pregnancy compared to the 6 months post delivery. [127]

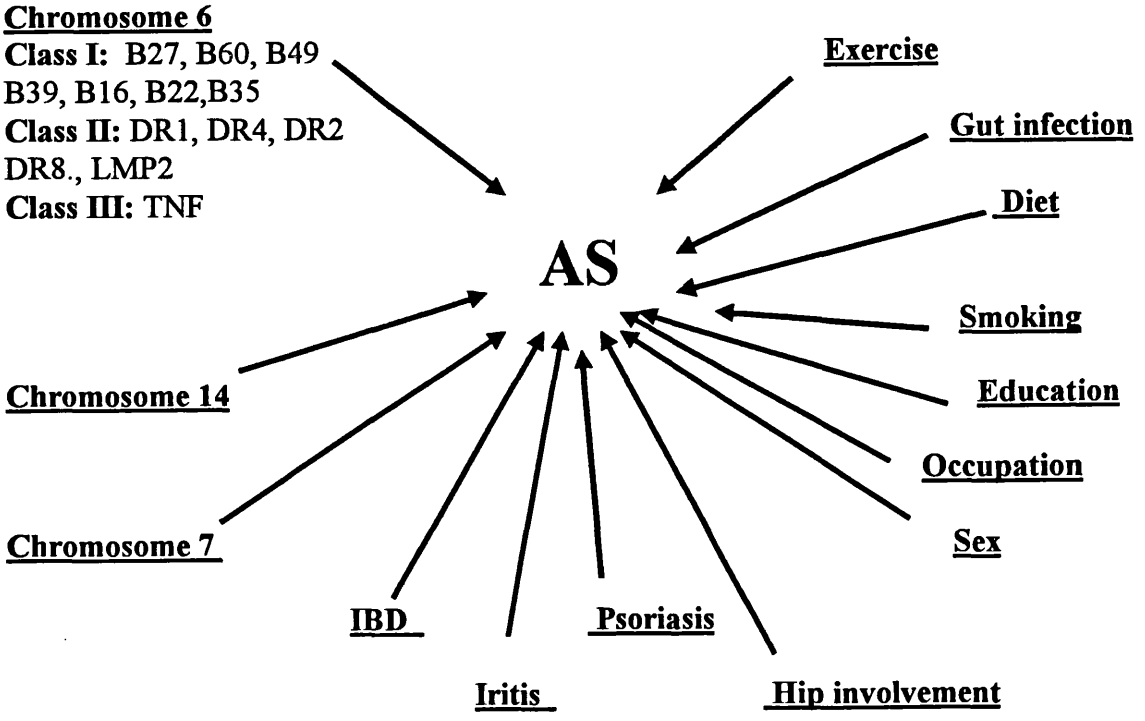
In general, symptoms of AS are uncommon before puberty, but rapidly increase in incidence during the teen and young adult years. Conceivably, testosterone levels play a role in AS, male patients have higher testosterone levels than average [128][129][130]. Moreover, the MHC of the mouse has been shown to encode genes which are responsible for regulating testosterone [131]. HLA susceptibility may be modulated by differences in circulating sex hormone levels. However, when corrected for confounding variables (serum extraction to eliminate interference with water-soluble substance such as phenylbutazone) the elevated testosterone levels are no longer seen in AS compared to controls. [132][133] [134] In addition, it is not clear what the effect of pain from a chronic disease will have on hormone levels. Hormone levels may have an influence in AS but measuring their levels and showing their effect on an inflammatory disease such as AS is difficult.

4.5 Aspects of the disease which remain unknown.

It is not know when the disease process of AS actually begins. In some patients at least there is asymptomatic radiological change [9] . Thus , the onset of symptoms may not actually be the time of onset of the disease for many patients. When the disease onset occurs may be at the time of exposure to the trigger. Alternatively there may be a latent period between the timing of the trigger and first disease specific change. The cascade of environmental triggers may begin as early as in utero. If this were the case then a mother with AS may be exposing her genetically susceptible child to the triggering agents as an embryo. There may be different forms of AS. Women may have a different type of disease (ie less severe axial involvement but more peripheral change) which is passed on to her children. Patients with hip disease or a family history may have a different expression of disease than sporadic or non-hip patients. There may be some predictors (environmental or genetic) to suggest which form of AS the person will develop. Most importantly it is not known what effect the susceptibility genes such as B27 and B60 have on outcome. People who are diagnosed easily may be the most severe and appear the

most susceptible. Perhaps susceptibility genes also determine severity. These are the aspects of the disease which will be specifically targeted within this study.

Figure 2: Factors affecting Ankylosing Spondylitis



5. Rational of proposal

The hypothesis that AS is an expression of both susceptibility and severity genes with environmental factors playing little part in disease outcome is explored in this project.

1. The natural progression of disease is examined by validating and implementing a radiographic scoring system.
2. The extent to which this natural disease course is determined by genetics or environmental factors is assessed.

5.1 The nature of progression in AS

A number of measures (ie. metrology, function, global well-being and disease activity indices) are used simultaneously by researchers and clinicians to monitor outcome in spondylitis. However, the comparison of individuals with AS is often difficult as many of the measures are subjective (ie pain, fatigue and movement). To determine the natural course of disease it was felt that a fixed and objective measure was needed. Radiographs can represent an unbiased, 'gold standard' for the measurement of disease status. In addition, they are generally available on most patients, as they are required within the Rome and New York Criteria for the diagnosis of AS. However, to date the existing measures using radiographs are time consuming to use, do not include the entire spine or address hip involvement and have been shown to be insensitive to change [135][136]. Little work has been done to assess disease progression in radiological terms. Mackay et al developed a set of criteria for a new radiology score in 1996 [137] but this has not been validated. Thus, this study will validate and implement this measure of

(a) assessing spinal and hip disease thereby creating the opportunity to describe the progression of AS in an objective way.

(b) A general description of the disease course and development of spondylitis will be presented (eg average change in disease per year [linear or sigmoid progression]) using the new radiology measure.

A description of the disease course among subsets of AS patients (ie male/ female and hip/ non-hip) will be offered using the new radiology measure.

This section of the work will then allow an investigation into what influence genetic and environmental factors have on the rate of this disease development and how they affect the natural history of AS.

5.2 (a) The influence of genetic factors:

The hypothesis (see page 4) is that the expression of AS may result from a combination of severity and susceptibility genes. The study will explore the relationship and overlap between these two types of genes.

(i) Secondary disorders: As reported earlier, B27 negative spondylitis patients are more likely to have concomitant disorders (psoriasis or IBD). This suggests that the secondary diseases associated with AS (inflammatory bowel disease, psoriasis, iritis) may share susceptibility genes with AS. These genetic factors may be additive or have synergistic effects on one another. The genes themselves may have effects in modulating disease expression of the AS. Thus, the hypothesis, that there are shared over-lapping genes between AS and the associated inflammatory conditions (iritis, psoriasis, inflammatory bowel disease), will be investigated by the study.

(ii) Susceptibility genes: The susceptibility genes which have been identified (ie B27, B60 and DR alleles) may not be 'pure' susceptibility markers but may also have a severity effect. The study will investigate the impact of established susceptibility genes on severity.

(iii) Age of onset: Similarly, patients with a young age at onset who have an increased number of genetic susceptibility markers may have a different disease expression than late onset patients (who may carry fewer susceptibility genes but more severity genes). These cohorts will be compared within the project.

(iv) Sex: It is known that disease prevalence differs between men and women. There are 2.5 men affected for every woman with disease. This difference may be due to genetic factors in the sex chromosomes. If this were the case then there should be a difference in the sex ratio of children who inherit the disease depending on the sex of the affected parent. (eg an X chromosome susceptibility gene will always be passed on by an affected father to the daughter but not to their sons). The study will investigate the sex ratio of affected children of parents with disease.

(v) Inheritance of phenotypic expression: Assuming that the combination of severity to susceptibility genes creates the clinical expression of disease and women develop the disease less often (compared to men). Then, women should require more susceptibility factors than do men to develop disease. Thus, the children of women with AS should inherit a higher proportion of susceptibility genes (and less severity genes) than offspring of men. This higher susceptibility may influence outcome. By comparing the expression of disease in children inheriting the disease maternally as opposed to paternally, the affect of these susceptibility factors can be investigated.

(vi) Concomitance of disease among affected relatives: Little is known about factors influencing outcome. A comparison of the expression of disease in parent-child and sibling-sibling pair should aid in understanding the extent to which disease expression is inherited. Eighty percent of AS patients have sporadic disease (ie no previous member in their family with spondylitis) and 20% have a family history of the condition (ie have other relatives with AS). It is feasible that these may represent two slightly different forms of the disease. Those with a family history may have more susceptibility factors but milder disease (hence its ability to be transmitted). A comparison of familial and sporadic disease will further examine the interaction between susceptibility and severity factors.

(vii) Identification of loci associated with severity:

Greater similarity of disease severity is observed in monozygotic compared with dizygotic twins, although no individual severity measure achieved statistical significance [57]. Collaborative work with The Wellcome Trust, Oxford suggests there may be a recessive genetic model for influencing disease activity and function. Further work into forming this model will involve identifying the loci which are associated with variation in severity measures.

Thus this section of the study investigates the over-lap of severity and susceptibility genes and the contribution that genetic factors play in outcome of AS.

5.2 (b) The influence of environmental factors

Little is known about how environmental factors influence severity. Herein factors such as entry variables (ie education, area of first symptom, smoker etc) and exercise are explored and their impact on outcome is examined.

(i) Exercise: How much exercise patients do on a regular bases will be evaluated. Demographic and clinical variables that might influence adherence to exercise will be examined.

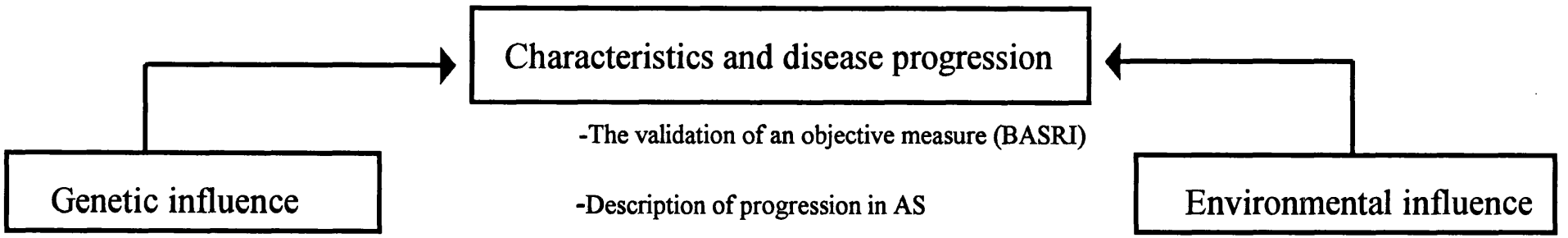
(ii) Physiotherapy/education: A 2 year longitudinal follow-up of the patients attending the Royal National Hospital for Rheumatic Diseases will be performed. The long-term improvement gained by exercise therapy will be evaluated.

(iii) Entry variables: The natural history of AS in the individual is poorly defined and there is a great deal of individual variation in terms of prognosis. It is possible that demographic factors with which the patient presents can be used to predict long term outcome [121]. Taking the disease index scores (ie BASRI [radiology], BASDAI [disease activity], BASFI [function]) of patients now and correlating with presentation as defined by the patients demographic characteristics (such as; smoker, occupation, education level etc.) may give an indication of factors associated with a severe prognosis.

(iv) Birth Order: HLA-B27 transgenic mice with a lower birth order have an increased prevalence of ankylosing enthesopathy. If the same were true in humans, an environmental explanation (such as a lower incidence of childhood diseases among the first born children) would be implied. The study will examine if the AS patient is more likely to be a first born child.

The disease course will be investigated using a newly validated outcome measure. The relative overlap and role of the severity vs susceptibility genes in progression of AS will be examined along with the effect of environmental variables such as exercise and demographic details in the prognosis of disease [Figure 3]

Figure 3: Genetic and environmental factors influencing susceptibility and outcome in ankylosing spondylitis:



Overlap between severity and /or susceptibility genes.

- Secondary diseases
- Severity effect of 'susceptibility' genes
- Age at onset
- Sex and inheritance
- Sex and severity
- Disease concordance among family members
- Loci associated with severity

- Exercise
- Physiotherapy
- Entry variables (smoker, Sex , occupation, education)
- Birth order

6. Methods and Results

6.1 The nature of progression in AS: characteristics and disease evolution.

In order to determine the history and characteristics of disease development in ankylosing spondylitis an objective scoring system was needed. Thus, the Bath Ankylosing Spondylitis Radiology Index (BASRI) was validated to provide this measure for this study. However, patients with hip disease represent a small and distinct subset of individuals and hip disease is more difficult to quantify and validate than spinal disease. This is because of the difficulty distinguishing between AS hip changes and those of osteoarthritis. Therefore, the two regions of disease (ie the index for spinal disease and the index documenting hip disease) were validated and described separately. The BASRI -spine [BASRI-s] describes spinal status, BASRI-hip [BASRI-h] describes hip involvement and BASRI-total [BASRI-t] describes the combination of these two indices. Using these indices the general disease evolution of AS will be determined and the differences between sub-sets of patients such as male /female, and those with hip disease/non-hip will be examined.

6.1 (a) The validation of BASRI

(i) The spine :

Objective: To validate the scoring system for spinal involvement devised by MacKay et al. [138] This system should be reproducible, simple and quick to use, incorporate the entire spine (anteroposterior & lateral) and pelvis and be sensitive to change. The discriminating features of each radiological severity group were defined by 3 experienced readers [138] [Appendix 2].

Methods: Radiographs of 188 consecutive patients with AS and 89 without AS were scored randomly and blindly by 3 readers to validate the BASRI. Two hundred and sixty-three SI joint, 160 lumbar spine and 145 cervical spine radiographs from the AS cohort were scored,

assessing intra and interobserver variation using a kappa statistic (as data tended to cluster towards worse disease).

Sensitivity to change over time was determined by scoring serial radiographs of 58 patents, assessing 177 time intervals of 1,2,3 and 4 yrs. Radiographs were obtained on 2 occasions for each individual. The two time intervals were separated by 12, 24, 36 or 48 months. They were available on 20, 31, 28 and 23 individuals respectively. All radiographs were blinded as to the date of radiograph and a Wilcoxon signed rank test for nonparametric data was used to determine the earliest point at which sensitivity to change became apparent.

The specificity and positive predictive value against other rheumatic conditions was assessed using 305 radiographs from the AS cohort and 78 radiographs from 89 non-AS patients. Radiographs of non-AS patients were interspersed with the AS patient's films, such that readers were unaware of the diagnosis. A cut-off of grade 2 (definite) disease was used and all radiographs were then classified into one of two groups, those with and those without AS changes. The SI joints were not viewed at this point.

The non-AS cohort consisted of consecutive outpatients who were attending the Royal National Hospital for Rheumatic Disease and who had cervical and lumbar spine radiographs obtained for evaluation of symptoms. The mean age was 57.9 +/- 16.8 yrs and the sex ratio was 1:3 (males: female). This cohort included 41 patients with rheumatoid arthritis, 21 with mechanical back pain, 10 with fibromyalgia, 10 with osteoporosis and 7 with psoriatic arthritis.

Results: Intraobserver variation. Two hundred blinded SI joint radiographs were assessed twice by a single observer, with 86% complete agreement, giving a kappa score of 0.69. Results for the lumber and cervical spine showed 75% complete agreement, with kappa 0.65 and 81% complete agreement with kappa 0.73. The main errors were reading the SI joint as grade 3 rather than grade 4 on 12/200 (6%) of cases [Figure 4] and distinguishing suspicious (grade 1) from mild (grade 2) disease in the cervical spine in 5 cases [Figure 5].

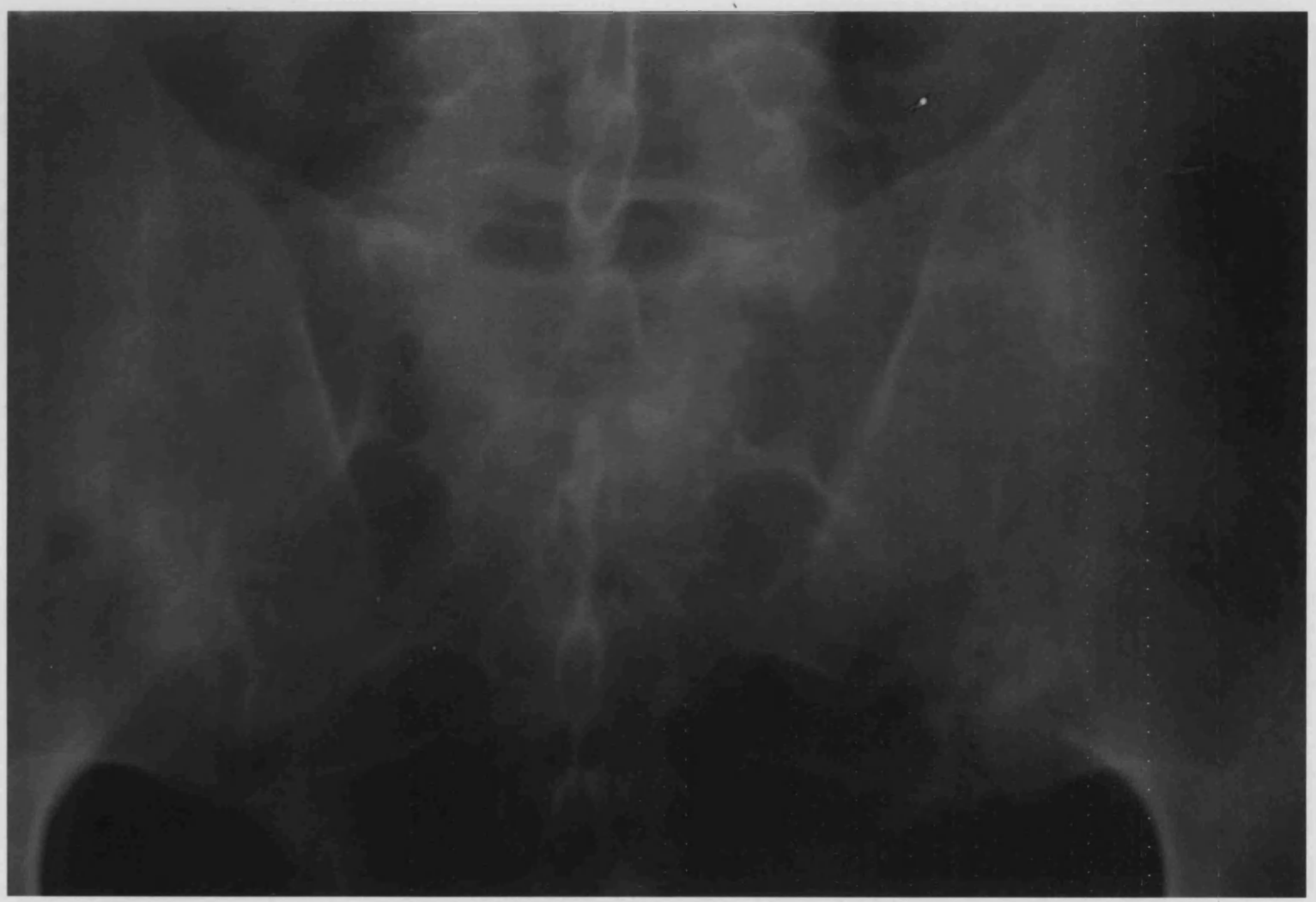


Figure 4
Plain radiograph of SI joint demonstrating errors in distinguishing Grade 3 (moderate) from Grade 4 (severe)

Variation of >1 grade occurred only twice when scoring the lumbar spine and never when scoring the SI joints or the cervical spine.

Interobserver variation: Reproducibility between two readers was assessed using 261 SI joint radiographs scored by each assessor on separate occasions. There was 78% complete agreement between observers, with kappa score of 0.55. The 160 sets of lumbar spine radiographs used reached 73% complete agreement (κ= 0.64) and the 145 cervical spine radiographs reached 79% complete agreement (κ=0.69) Both the lumbar and cervical spine scores outperformed the established NY criteria for SI joint assessment. Difficulty in separating grades 3 and 4 existed in SI joint assessment (8 occasions)[Figure 4], while distinguishing mild from suspicious disease was the main problem for the lumber and cervical spine scoring (5 occasions). Discrepancies of >1 grade occurred while scoring the SI joints in 3 of 263 films, the lumber spine in 5 of 160 films, and the cervical spine in 10 of 145 radiographs.

Disease specificity: Specificity for the lumbar spine was 0.89, and that for the cervical spine was 0.83. The positive predictive value for the lumbar spine was 0.97 and that for the cervical spine was 0.95.

Sensitivity to change: Using Wilcoxon's signed rank test for nonparametric data, the BASRI-s demonstrated a significant change in radiological score ($p<0.001$) at 24 months for the SI joints, the lumbar spine, and the cervical spine. Where the time interval between radiographs was 12 months, 30% of cases showed changes of at least 1 grade within this period, but this is not statistically significant ($p<0.07$).

Interpretation of the study: The BASRI was reproducible, with intra- and interobserver variations equivalent to those of the NY criteria. The main problem for grading the lumbar and cervical spine was distinguishing suspicious disease from mild disease on 5 occasions because of difficulty in determining whether squaring of the vertebra was present. A

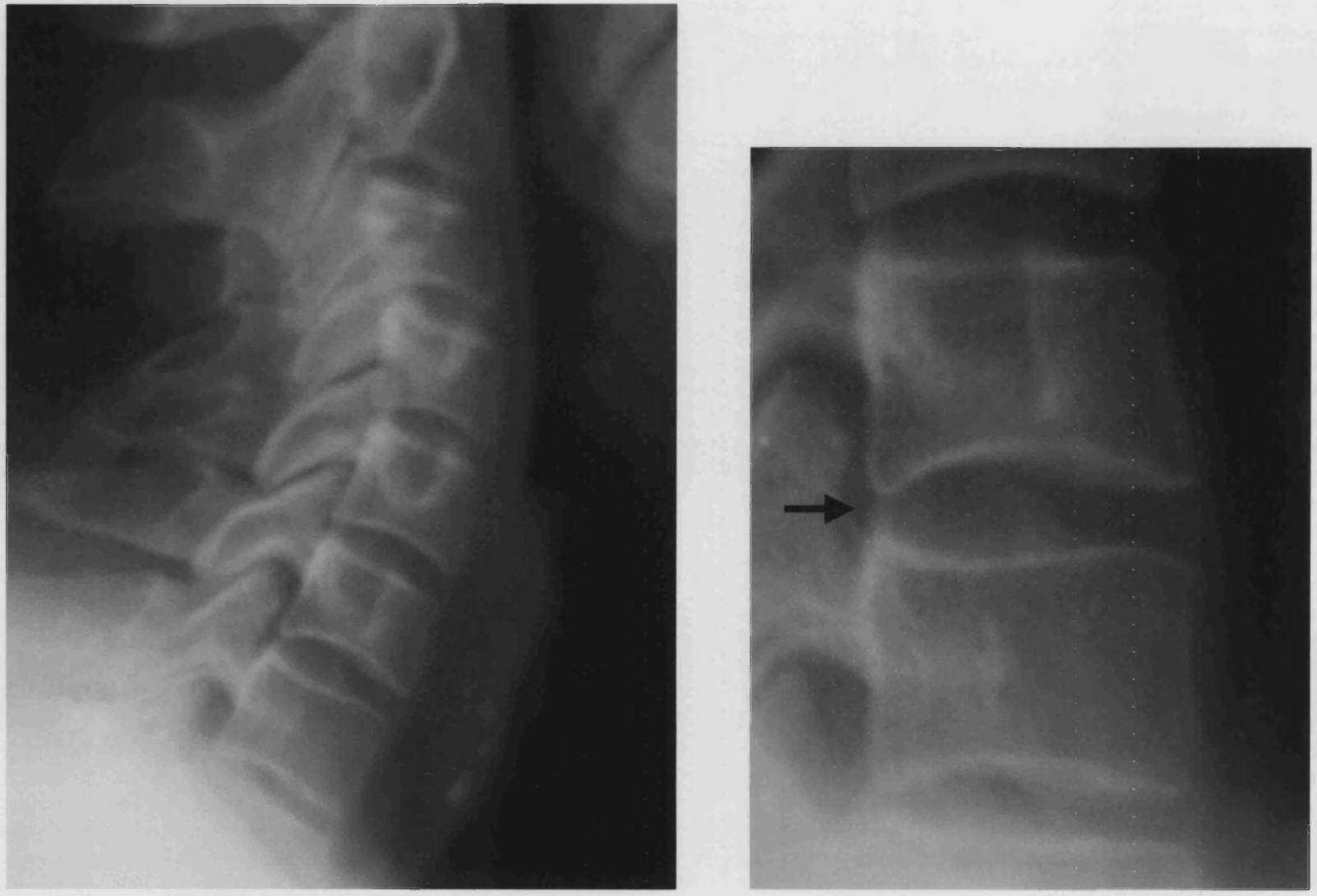


Figure 5
Plain radiograph of cervical spine demonstrating errors in distinguishing Grade 1 (suspicious) from Grade 3 (fusion)

potential method of overcoming this problem for the spine would be to measure each vertebra individually (in cases where distinguishing the grade is a problem) to determine the presence or absence of squaring as done in the method described by Ralston et al [136]. The BASRI was found to be sensitive to change over a 2 year period, which suggests that radiographs at intervals of <2 yrs for either routine or study purposes are not warranted. To maintain simplicity, the BASRI does not pick up minor radiological change. However, by not differentiating between grades such as bamboo spine and >3 vertebrae fused, the score does suffer from a ceiling effect. Yet, the lack of radiological progression to severe spinal disease in the majority of cases suggests that the potential ceiling effect is of little consequence. The BASRI fulfils the OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials) filter goal: truth, discrimination and feasibility [139].

Table 1: Validation of BASRI-spine

		Radiographs (number)	Kappa
Intraobserver variation	Sacroiliac joints	263	0.69
	Lumbar spine	160	0.65
	Cervical spine	145	0.73
Interobserver variation	Sacroiliac joints	263	0.55
	Lumbar spine	160	0.64
	Cervical spine	145	0.69
			Specificity
Specificity	Lumbar spine	188 (AS) vs 89 (non-AS)	0.89
	Cervical spine	188 (AS) vs 89 (non-AS)	0.83
Sensitivity		Lumber & Cervical spine & sacroiliac joints	
12 months		20	$p<0.07$
24 months		31	$p<0.001$
36 months		28	$p<0.001$
48 months		23	$p<0.001$

(ii) The hip :

Objective: To validate the scoring system for hip involvement devised by MacKay et al. Patients developing hip disease represent a small, distinctive subset of patients, developing more severe disease, at a younger age (15-19 yrs). Hip disease in AS has not been satisfactorily defined. The Larsen score (originally designed for grading radiological change in rheumatoid arthritis) has occasionally been applied to the hip radiographs of AS patients. However, this score is less reproducible if used for assessing conditions where there is increased bony deposition, as occurs in AS. [140]

The Bath Ankylosing Spondylitis Radiology Hip Index, (the BASRI-hip) was designed to address these problems. No additional radiographs are required to score the BASRI-hip if an antero-posterior (AP) pelvic view has already been taken to review the sacroiliac (SI) joints.

Methods: Following definition of the scoring system [141] [Appendix 3], radiographs of 134 consecutive patients with AS and 100 without AS were chosen to assess the reliability, predict sensitivity to change and determine disease specificity. The mean age of the AS population was 45.9 [+/- 10.6 yrs and the sex ratio was 4.8 : 1 (males : females)]. The non-AS cohort were consecutive outpatients attending the Royal National Hospital for Rheumatic Diseases (RNHRD), a tertiary referral centre. Pelvic x-rays, in this group, had been taken for the evaluation of symptoms. Their mean age was 54.5 +/- 17.5 yrs and the sex ratio was 1:2 (males : females). The cohort included 48 with Rheumatoid Arthritis, 21 with osteoarthritis or mechanical back pain, 7 with fibromyalgia, 3 with osteoporosis, 12 with psoriatic arthritis, 4 with polymyalgia rheumatica, 3 with inflammatory bowel disease and 2 with trochanteric burstis.

Inter-observer variation: Two independent observers (Sinead Brophy, Michele Doran) scored the 234 radiographs in a blinded fashion, on separate occasions, to assess inter-

observer variation. All sacroiliac joints had been obscured and radiographs from the non-AS cohort interspersed such that the readers were unaware of the diagnosis.

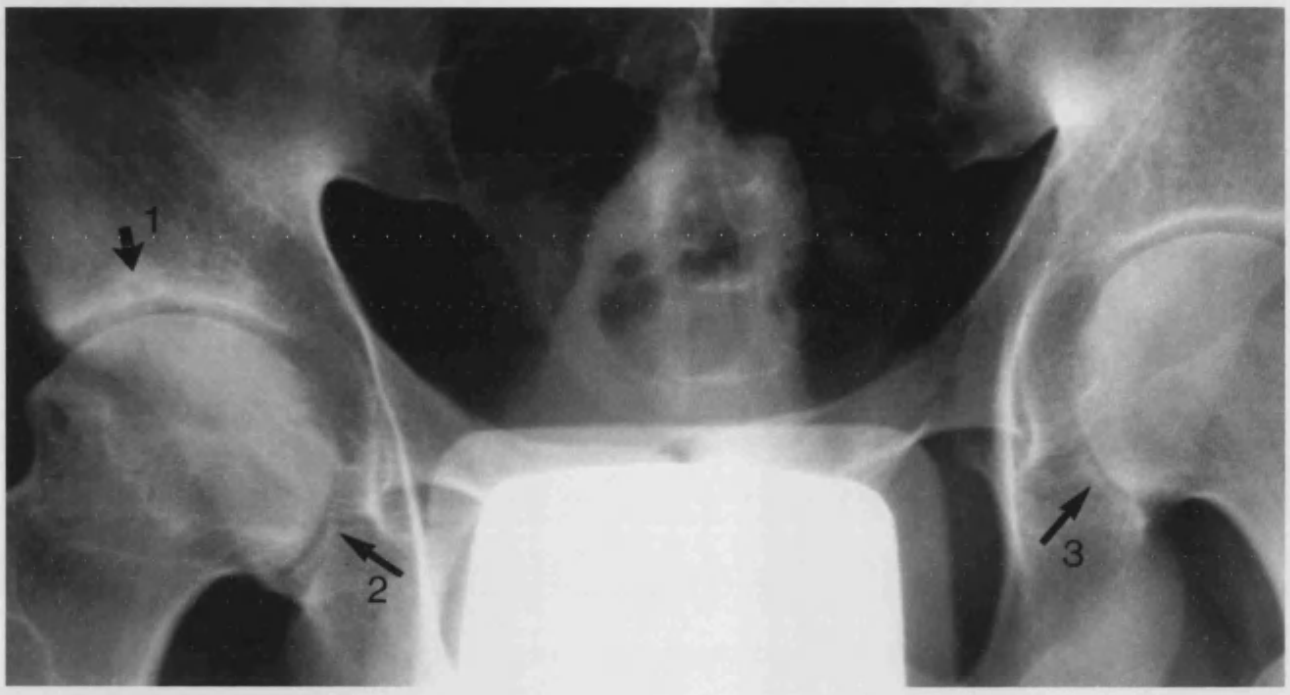
Intra-observer variation: One hundred AS radiographs were chosen by a non-reader and scored by a single observer (Sinead Brophy) on two consecutive sessions, assessing intra-observer variation. A Kappa statistic was used to determine the significance of inter- and intra-observer variability.

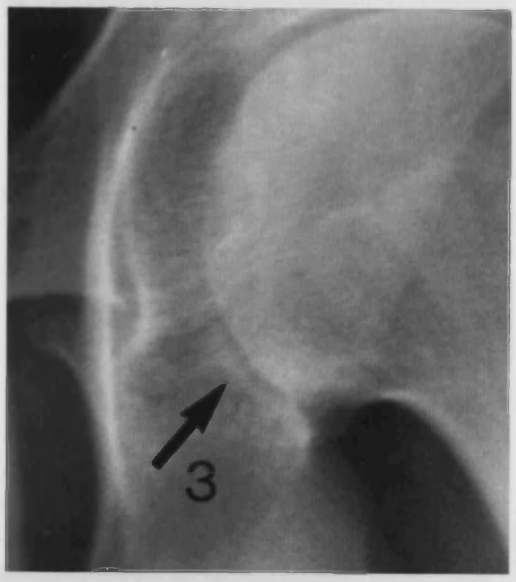
Disease Specificity: The specificity of BASRI-h against other rheumatic conditions was assessed using 234 radiographs. A cut off of grade 2 (definite) disease was used and all radiographs were then classified into one of two groups: those with and those without AS changes.

Sensitivity to change: Sensitivity to change over time was determined by scoring 438 serial radiographs of 122 patients, assessed at time intervals of 1,2,3 or 4 years. Patients were included if pelvic radiographs were available on two occasions 12,24, 36 or 48 months apart. Pairs of x-rays were available in 60, 65, 55 and 55 cases, respectively.

The mean time from diagnosis of these 122 patients was 18 years (range 0-44 years). All radiographs were blinded as to the name and date of the radiograph and a Wilcoxon signed rank test for non-parametric data was used to determine the earliest point at which sensitivity to change became apparent.

Results: One hundred pelvic (AP) radiographs were assessed twice by a single observer with 94% complete agreement for the right hip and 96% complete agreement for the left hip. The Kappa scores (see table 3) showed good agreement at 0.86 and 0.91 respectively for the right and left hips. The main errors were distinguishing between suspicious (grade 1) and minimal (grade 2) disease in 4% of cases. As the definition is 'narrowing of less than 1 cm' the main difficulty was deciding if narrowing was present when both hips were affected (ie normal vs narrowed) [Figure 6]





1- erosion

2 & 3 - possible narrowing

Figure 6
Plain radiograph of hip joint demonstrating errors in distinguishing if narrowing is present

Inter-observer variation. Reproducibility between two readers was assessed using 134 pelvic (AP) radiographs scored by each observer on separate occasions, revealing a 83% complete agreement between readers for the right hip and 84% complete agreement for the left hip. Kappa scores showed good agreement at 0.75 and 0.74 respectively. Distinguishing minimal from suspicious disease was again the main problem in 6% of cases.

Sensitivity to change. Using a Wilcoxion signed rank test for non-parametric data, BASRI demonstrated a significant change in radiological score ($p<0.001$) at 1 year for the hip.

Disease specificity: A BASRI-hip score of grade 2 was used as the cut off to determine AS radiological change. Disease specificity is defined as 'the proportion of true positives that are correctly identified as such and is 1 minus the false negative rate' [142] . Hence, the disease specificity of the BASRI-hip in this study was 1-0.24 = 0.76 (76%). If non-AS patients with a total hip replacement (THR) were excluded (because they automatically score grade 4, whatever the reason for their THR), disease specificity was 0.83. Hence, 24 of the 200 hips studied in the non-AS cohort scored more than 2.

Scoring speed. The mean time taken to score a pair of hips, on a single AP pelvic radiograph, is 10 seconds

Interpretation of this study: The only radiological scoring system currently in use for the AS hip has been the Larsen score, which was designed primarily to describe the hip in Rheumatoid Arthritis. Larsen states in his paper that the score is not as reliable when used to grade conditions, such as psoriatic arthritis or AS, where new bone formation is a prominent feature [140]. The BASRI-hip, in conjunction with the BASRI-spine, was designed to fill a perceived gap in the range of outcome measures for AS.

The BASRI-hip was developed in an identical way to the BASRI-spine score and was equally reproducible. During the validation of BASRI-hip, the sacroiliac joints were covered prior to scoring because sacroilitis (as part of the NY criteria) was a necessary entry

requirement for any AS patient in the study. Although BASRI-hip was shown to be disease specific it did not perform as well as the BASRI-spine mainly because a hip replacement always scores grade 4 whatever its cause. Of the 100 patients included in the non-AS cohort, 24 were graded as if they had AS (i.e. they had scores > 2) but 7/24 scored grade 4 because of a hip replacement. If these 7 were excluded, only 17/100 scored >2 giving a specificity of 83%. Of the remaining 17 with scores greater than two, 13 had severe RA. It may be that the BASRI-hip is describing certain aspects of inflammatory arthritis eg circumferential joint space narrowing which is central to both RA and AS.

Little information is available regarding radiographic sensitivity to disease progression and at what frequency repeat radiographs should be performed. The BASRI-hip was found to be sensitive to change over a one-year period. By definition, the patients used to study sensitivity to change each had more severe hip disease than other patients with only a single pelvic x-ray. This could mean that sensitivity to change becomes apparent at one year only in those with severe hip disease. However, in an effort to reduce radiation dosage, it is the hospital policy to perform a single pelvic radiograph to assess both the sacroiliac joints and hips together. Hence, the additional pelvic x-ray may have been taken to assess the sacroiliac joints rather than the hips and this was the case in at least half of the patients included. The radiographs in the study were blinded for date. This confirms that the BASRI-hip can determine 'forward progression' (i.e. it can identify the earlier of 2 radiographs performed on the same individual).

Table 2: Validation of BASRI-hip

		Radiographs (number)	Kappa
Intraobserver variation	Left hip	100	0.91
	Right hip	100	0.86
Interobserver variation	Left hip	234	0.74
	Right hip	234	0.75
			Specificity
Specificity		234 (AS) vs 100 (non-AS)	0.76
Sensitivity			
12 months		60	$p<0.001$
24 months		65	$p<0.001$
36 months		55	$p<0.001$
48 months		55	$p<0.001$

6.1 (b) Natural History.

(i) Radiological progression in AS:

Objective: The natural history of AS is poorly understood. However, using BASRI to examine the disease history and radiographic progression of AS will allow this study to examine the influence of genetic and environmental factors on the rate of this disease development and their affect on the course of AS.

Aims: 1) to examine the progression of the disease cross-sectionally and evaluate factors associated with hip, lumber or cervical involvement. 2) to examine the longitudinal progression of disease among individuals in whom a diagnosis was made and radiographs taken within two years of developing first symptoms. 3) to construct a centile chart both for reference and to further aid in describing the disease history of AS. 4) to examine the rate of radiological change and estimate by extrapolation the age when first radiological change may occur. Assuming rate of progression before symptom onset is equal to rate of progression after symptom onset, the extrapolation can indicate age of first radiological change. [Appendix 4]

These four approaches were designed to reflect the natural history of AS, including first radiological change, rate of progression, variation seen among individuals in relation to the general AS population and factors determining this variation.

Methods: All existing radiographs of 571 AS patients attending the Royal National Hospital for Rheumatic Diseases (n= 2,284 radiographs) were scored using BASRI by two trained independent readers (SB, AAl-S). [Discrepancies between scores were rescored by a third trained reader (KM) and a consensus was reached]. Patients were selected if they had been diagnosed according to the New York Criteria, had full sets of X rays and complete notes (including age of symptom onset, disease duration, family history, etc). Where more than

one set of radiographs were available on a patient a random number table was used to select the set for inclusion in the cross sectional database.

1. Cross sectional progression of AS (n=571 patients): A plot was made of percentage of patients with regional involvement (lumbar, cervical and hip) at each year of disease duration. A cut off of grade 2 or more (ie mild disease) on the BASRI scale was used to define involvement of the joint. Univariate analysis was used to examine factors (ie secondary diseases, delay in diagnosis, age, family history, disease activity) associated with cervical, hip and lumbar involvement. Multivariate analysis was performed using these factors.

2. Longitudinal progression of AS: Patients with radiographs taken within 2 years of symptom onset (n=20) and who had more than one set of films were selected. The BASRI score was plotted against disease duration.

3. Percentile reference curve for BASRI. : The curve was assembled using the method described by Altman and Chitty [143] . A quadratic regression model was fitted to the raw data with means weighted according to sample number in at each disease duration point. Cross sectional data (with each patient included only once) was used to develop the reference centiles (n=571).

4. Rate of progression and extrapolation to time of first radiological change: Patients with two sets of radiographs taken 10 years apart were selected. Those patients with first radiographs taken at 0-2 years, 10(+/-2) years and 20 (+/-2) years from time of symptom onset were used to examine rate of progression of disease over any 10 year period. The progression rate was calculated not in relation to total score but in relation to remaining score, to remove the ceiling effect. The formula to calculate relative progression (ie the score change per amount of score available to change [144]) was as follows:

(the score of the designated year) - (the score of the previous year)

---

the total score (16) - (the score of the previous year)

Using this estimate of rate of progression of radiological change, an extrapolation to time of first radiological change was performed. Assuming rate of progression before symptom onset is equal to rate of progression after symptom onset, then extrapolation can indicate age of first radiological change.

Statistical methods: SPSS was used for the univariate & multivariate analysis (multiple regression) and the percentile estimates using a quadratic regression model.

Results: **1. Progression of disease** The cross sectional data of all 571 patients was plotted against disease duration [Figure 7]. More patients have lumbar involvement than cervical disease at all points in the disease duration ($p<0.001$) and 86% of those with cervical disease had also lumbar spine changes. Patients with cervical changes alone (14%) had a shorter disease duration [18 (sd: 10 yrs) vs 23 (sd: 10 yrs) respectively ($p=0.006$), CI: 5.2 (1.5-8.9)] than those with both lumbar and cervical change. After 25 years of disease, approximately 75% of patients have cervical spine involvement and 85% have lumbar spine involvement. The factors associated with level of cervical involvement include [Table 3]: disease duration, degree of hip disease, degree of lumbar spine disease and a history of iritis. A model of these factors explains 59% [$p<0.0001$] of the variation seen in cervical spine involvement. The factors associated with level of lumbar spine disease were disease duration and level of cervical involvement. A model of these factors explain 36% [$p<0.0001$] of the variation seen in lumbar spine involvement. Only degree of cervical spine involvement and disease duration was associated with level of hip disease, describing 19%

[p<0.0001] of the variation seen in level of hip disease. Factors not associated with progression to lumbar, cervical or hip disease include disease activity [BASDAI], delay in diagnosis, family history, bowel disease and presence of psoriasis.

2. Longitudinal progression: Individual plots of patients confirmed the findings from cross sectional study. [Figure 8]. There is a great deal of variation in radiological score among patients at time of first symptoms of spondylitis. There is a very slow general rate of progression with some individuals having bursts of rapid change. However, these times of change can occur at any time in the course of the disease.

3. Percentile reference curve for BASRI: The percentiles of 571 patients were plotted against disease duration (defined as years from time of first symptom of AS). Quadratic regression models were fitted to the weighted means (weighted for number of observations at each time point) at each disease duration point [Figure 9]. The proportions of cases falling outside the 10th and 90th centile were calculated and confirmed the goodness of fit. [<10th centile range = 7.5%-10.2%, ; >90th centile range 6.2%-11.2%].

4. Rate of progression and extrapolation to age of first radiological change: On a BASRI scale of 2-16 the change in score was 2.9 (+/-3), 3.2 (+/1.3) and 1.6 (+/-1) for disease duration time points of 0-10 yrs, 10-20 yrs and 20-30 yrs respectively. [Table 4]. There was no significant difference between rate of radiological progression in these three time periods (p=0.163). If progression rate was calculated not in relation to total score but to remaining score to remove the ceiling effect, the progression rate was 30% of remain change (sd: 0.3), 40% of remaining change (sd:0.3) and 35% remaining change (sd: 0.4) at time points 0-10 yrs, 10-20 yrs and 20-30 yrs respectively. Thus, rate of radiological change was linear. [Figure 10]

If the change in AS is linear with an average change over any 10 year period of 2.55 (36%), then the rate of change is 1 point on the BASRI scale every 4 years. If average BASRI score at time of symptom onset is 5 when the patient is aged on average 28 (see table 4)

then it would have been an estimated 20 years (eg 5 x 4 years =20 yrs) before symptom onset that radiological change began or at age 8 (assuming rate of change before symptoms is equal to rate of change after symptom onset). [Figure 11]

Interpretation of the study: In terms of time of first radiological change, rate of progression, variation seen among individuals in relation to general AS population and factors determining this variation this study shows:

1. In the majority of patients, AS begins in the SI joints and progresses up the spine (the thoracic spine is not evaluated within the BASRI score). Thus, spinal involvement is largely an expression of disease duration.

2. The hips become involved in about 20-30% of individuals and may predict a more severe outcome for the cervical spine.

3. AS is a linearly progressing condition with about 35% change or an increase of 2.5 on the BASRI (2-16) scale every 10 years.

4. This rate of change can be extrapolated backwards to estimate time of first radiological changes to be at approximately age 8 years.

However, AS is defined as symptomatic sacroiliitis and therefore by definition all our patients had sacroiliitis. Conceivably some patients may begin with cervical or thoracic disease and then have descending involvement or may indeed have spinal disease without SI involvement. However, this is most likely a rare phenomenon [145] [142].

The patients followed longitudinally from time of onset of first symptoms are those who have received an early diagnosis and have been referred to a tertiary referral centre. Thus, these subjects probably represent a severe group with perhaps the most rapidly progressing disease. Yet, even among this population of patients the radiographic progress of the disease appears to be quite slow and gradual.

A reference centile chart for BASRI has been developed based on outpatients from the RNHRD and individuals attending an in-patients intensive physiotherapy and education course which is comprised of people from across the UK and N Ireland. This population should provide a representative sample which may be applied to other patients around the world. The centile chart may differ between populations of male vs female, hip vs non-hip disease patients and those with and without iritis. With greater numbers the reference centile chart may be made more specific to different subgroups of AS patients. Using the centile chart showed that there is a great deal of variation and spread among patients with AS [ie half of the patients are spread over 40% of the entire BASRI scale at 10 yrs disease duration (50% of patients lie between 4 and 9.5 on the BASRI scale)]

This study examined a hospital population and showed linear progression of disease. However, this study probably suffers from left censoring and as such it would be expected that the most severe cases are referred to this tertiary centre. This would normally imply that there would be differential exclusion of patients with mild disease early in the course of their illness allowing for their inclusion only later as the disease became more problematic.[147]. Thus, patients with inherently milder disease are seen later in their course than those with more severe disease. The progression in this case would appear to be rapid at the beginning and slower as disease duration progresses. However, this selection bias does not appear to be observed in the AS population therefore perhaps lending weight to the finding that AS is a linearly progressing condition. Although it is theoretically possible that the patients who do continue to attend the hospital visits are the ones who continue to

have disease and this disease may in fact 'burn out', there has been has as yet been no evidence among non-hospital patients that the disease goes into remission [148].

The extrapolation to age of first radiological change is based on the assumption that rate of change before symptoms (generally SI changes) are equal to change after symptoms (generally spinal change). Only by investigating the rate of SI change will we be able to truly estimate the timing of first radiological damage. This would require an MRI of young individuals who are in the highest risk groups such as those with a family history, iritis etc [149].

In summary, radiographic progression appears to be constant over the course of AS. With advancing disease duration there is advancing radiographic change, the involvement of the lumbar spine increases the chances that the cervical spine will become affected. Hip disease and iritis, separately point to worse cervical involvement. There is a great deal of variation and spread among patients with AS. However, the disease progresses at an estimated rate of 2.5 points on the BASRI scale or 35% every 10 years. This rate can be used to make a very crude extrapolation to timing of first radiological change which could be from as young as aged 8. However, the time interval between the pulling of the trigger and onset of disease remains unknown.

Table 3: Factors associated with progression to cervical spine, lumbar spine or hip involvement.

	Factors associated with Cervical spine		**Factors associated with Lumbar spine**		**Factors associated with Hip**	
	Correlation	Univariant analysis	Correlation	Univariant analysis	Correlation	Univariant analysis
Disease Duration	0.3	p<0.0001	0.4	p<0.0001	0.2	p=0.005
Age	0.3	p<0.001	0.4	p<0.0001	0.02	
BASDAI	-0.02		-0.05		0.05	
Delay	0.1		0.01		-0.1	
	Means	Univariant analysis	Means	Univariant analysis	Means	Univariant analysis
Family history	0 = 2.1, 1= 1.8		0 = 2.4, 1 = 2.2		0 = 0.7, 1 = 1.0	
Hip disease	0 = 1.7, 1 = 3.3	p<0.0001	0 = 2.1, 1 = 3.0	p<0.0001		
Cx disease			0 = 1.1, 1 = 2.2	p<0.0001	0 = 0.1, 1 = 0.5	p<0.0001
IBD	0 = 2.1, 1 = 1.5		0 = 2.4, 1 = 2.0		0 = 0.8, 1 = 0.8	
Iritis	0 = 1.8, 1 = 2.5	p=0.001	0 = 2.2, 1 = 2.6	p=0.02	0 = 0.7, 1 = 0.9	
Lx	0 = 0.5, 1 = 2.5	p<0.0001			0 = 0.3, 1 = 0.9	p<0.0001
Psoriasis	0 = 2.0, 1 = 2.3		0 = 2.2, 1 = 2.6		0 = 0.8, 1 = 0.8	
Sex	0 = 1.7, 1 = 2.2	p=0.0001	0 = 2.0, 1 = 2.4	p=0.003	0 = 0.8, 1 = 0.8	

Table 4:

Disease duration	**Age**	**BASRI (Year 1)**	**BASRI (Year 10)**	**Change**	**Percentage change**
0-10 yrs (n=15)	28.3 (+/- 10)	5.0 (+/- 2)	8.0(+/-4)	2.9 (+/-3)	29%
11-20 yrs (n=20)	35.8 (+/-11)	8.2 (+/-4)	11.3 (+/-3)	3.1(+/-2.5)	40%
21-30 yrs (n=19)	43(+/-5)	9.6(+/-3)	11.4(+/-3)	1.6(+/-1)	36%
p value				p=0.1	p=0.4

Figure 7: Percentage of patients with regional involvment (hip, cervical spine, lumbar spine) over disease duration

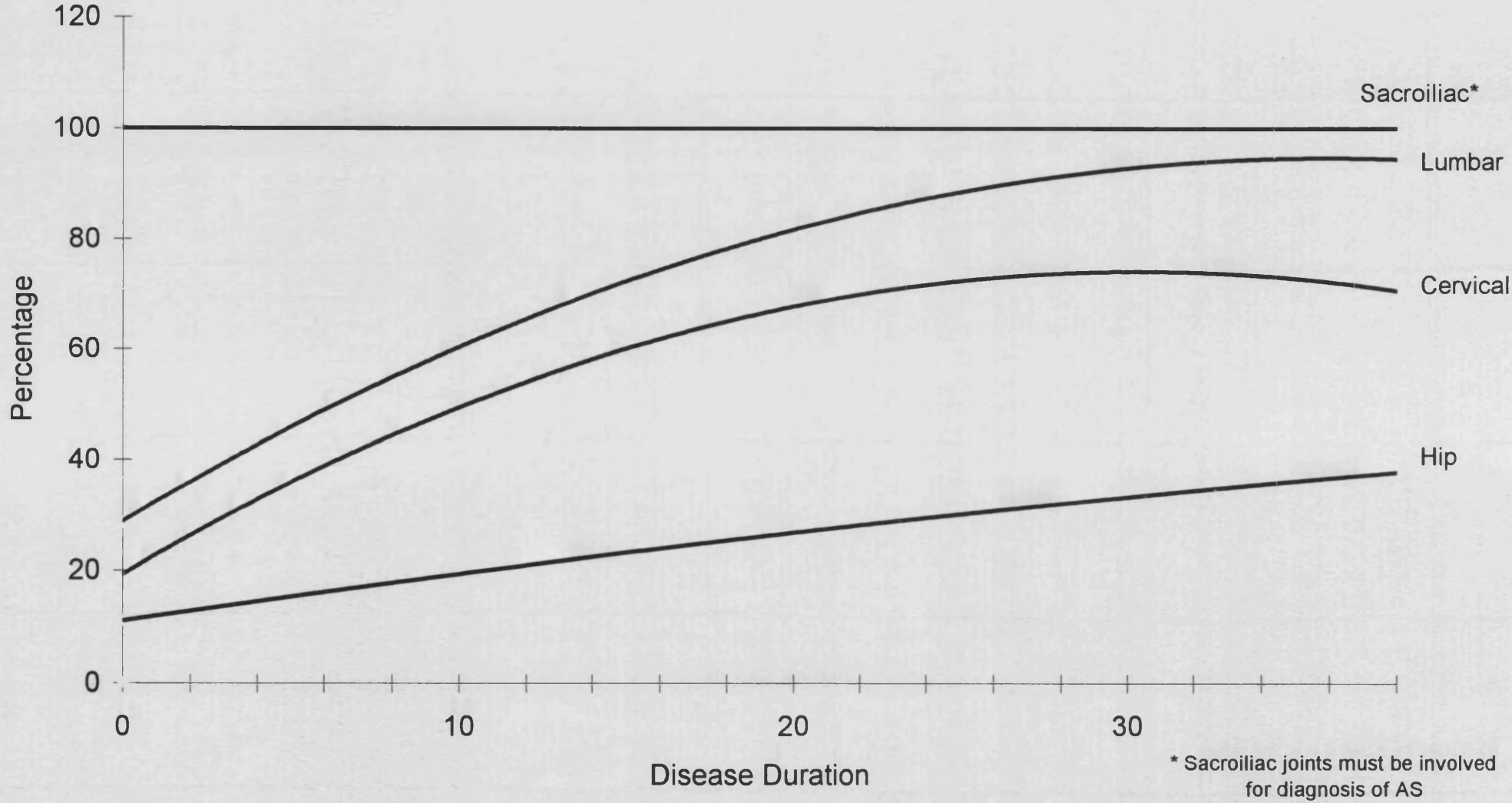


Figure 8: Longitudinal radiographs taken from time of symptom onset [n=20 indiviuals]

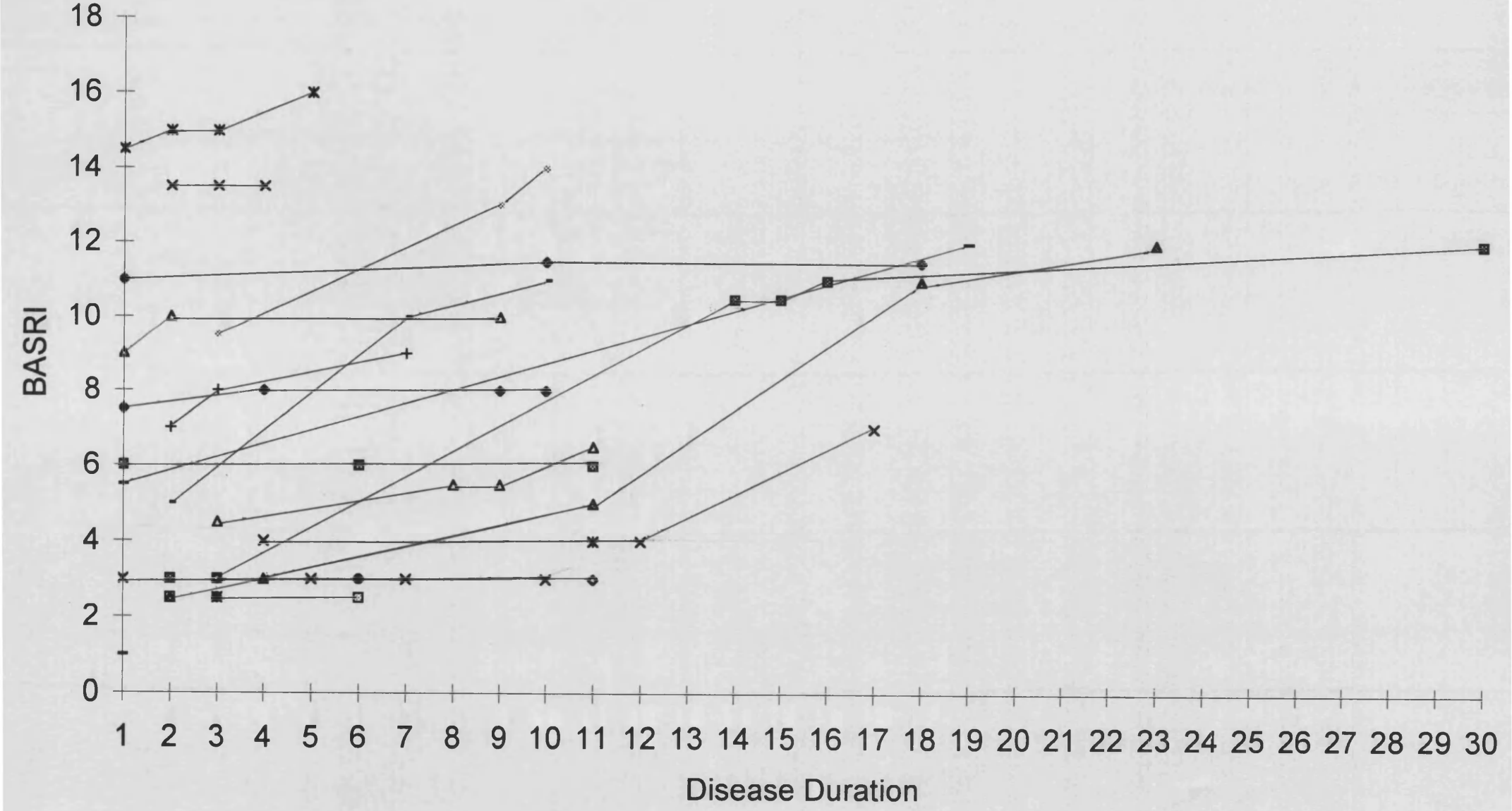


Figure 9:

Centile chart for BASRI (Quadratic weighted means) n=571

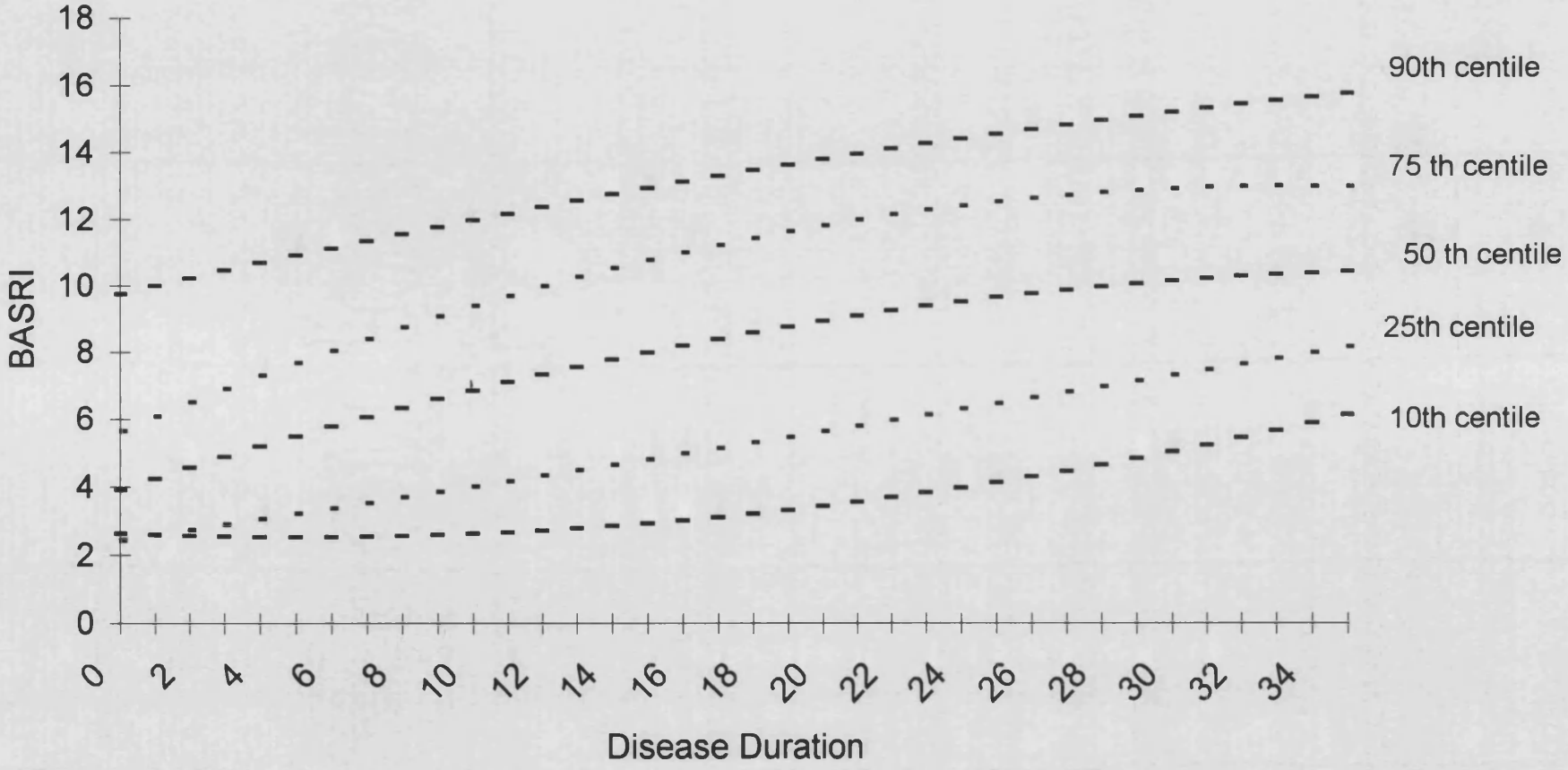


Figure 10: Rate of radiological change over 10 yr intervals (n=53 individuals)

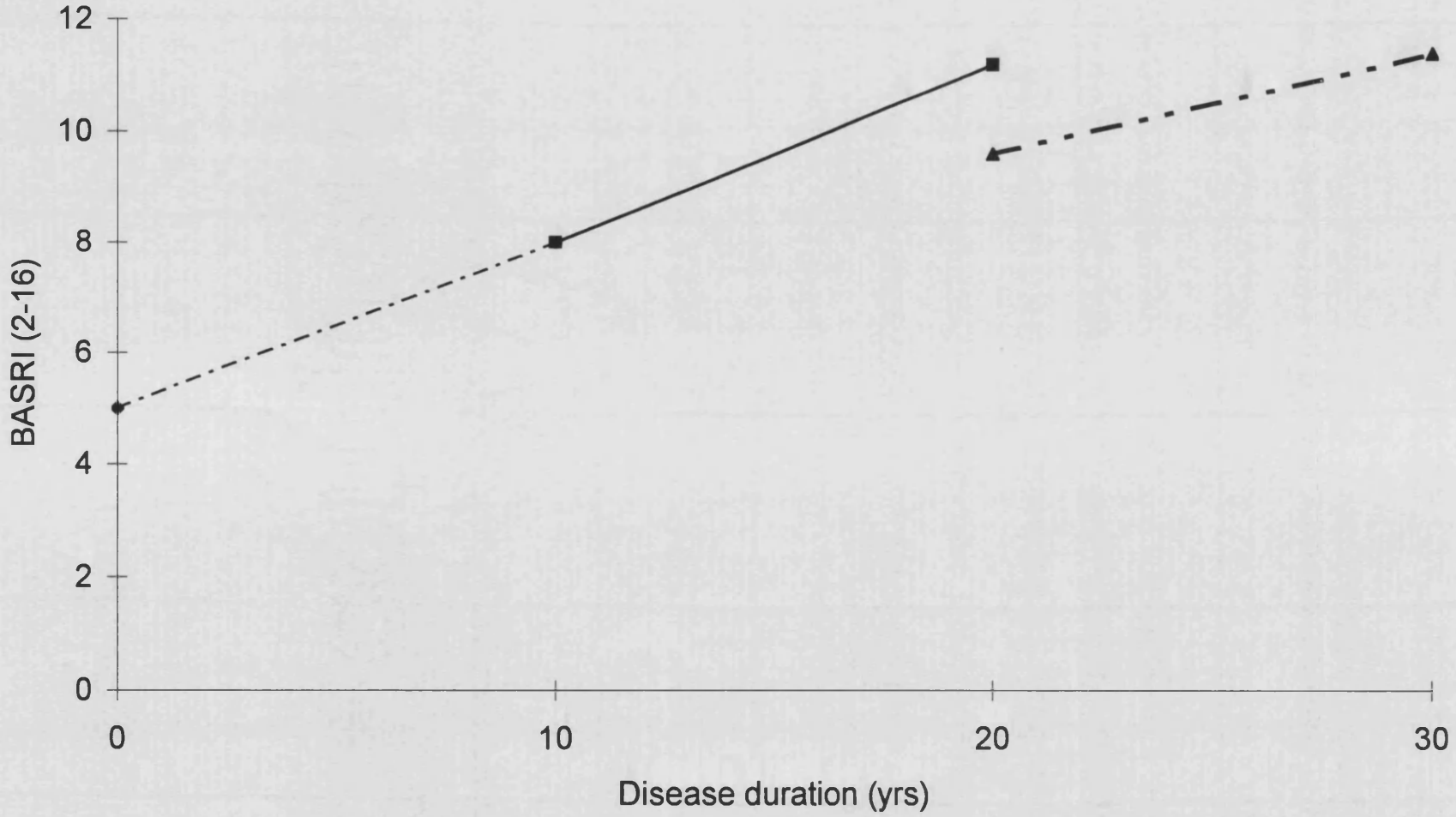
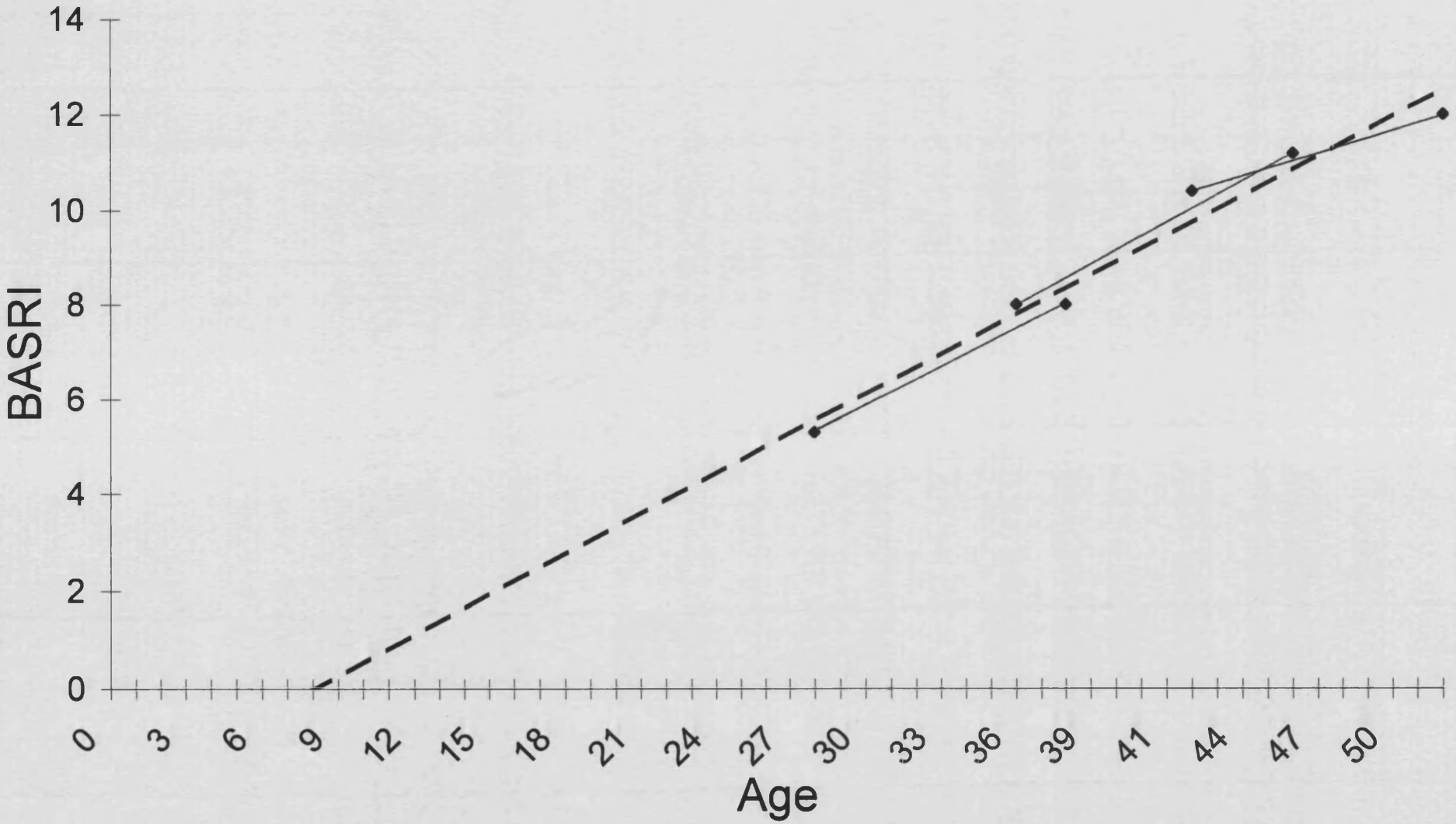


Figure 11: Extrapolation from time of onset of symptoms



(ii) Subsets of AS patients: variations in disease progression:

Objective: To describe the progression and disease development of ankylosing spondyltis among subsets of AS patients (Male vs Female / Hip vs non-hip) [Appendix 5].

Methods: The difference in progression in a) male vs female and b) hip vs non-hip was assessed. Sets of radiographs (n=423) of patients with known disease duration were selected and scored. Independent t tests or wilcoxon rank sum test and chi-squared were used to evaluate mean scores. The SPSS software program was used for analyses.

Results: a) The mean BASRI-t was higher for men than women (8.9 vs 7.2 respectively; $p<0.001$ Figure 12). Disease duration was comparable (21 vs 20 yrs ; $p<0.27$). More men than women had severe disease in the SI joints (odds ratio, 1.74 (1.1-2.7) $p<0.016$). More men than women had severe lumbar spine disease (2.6 (1.4-4.6); $p<0.001$) More men than women had severe cervical spine disease (2.3 (1.3-3.9) $p<0.002$). The numbers of men and women with severe hip disease were comparable at all stages of disease.

b) The BASRI-s was higher for those with hip disease ($p<0.001$ Figure 13). There was no difference in disease duration between the 2 groups (20 and 21 yrs respectively: $p<0.2$).

Interpretation of study: a) Men have more severe disease than women in the SI joints, lumbar spine, and the cervical spine, but not in the hips. b) Hip disease is a marker for more severe axial progression in AS (in both men and women).

Thus, subsets of patients with more progressive (or milder) disease exist in ankylosing spondylitis.

Figure 12

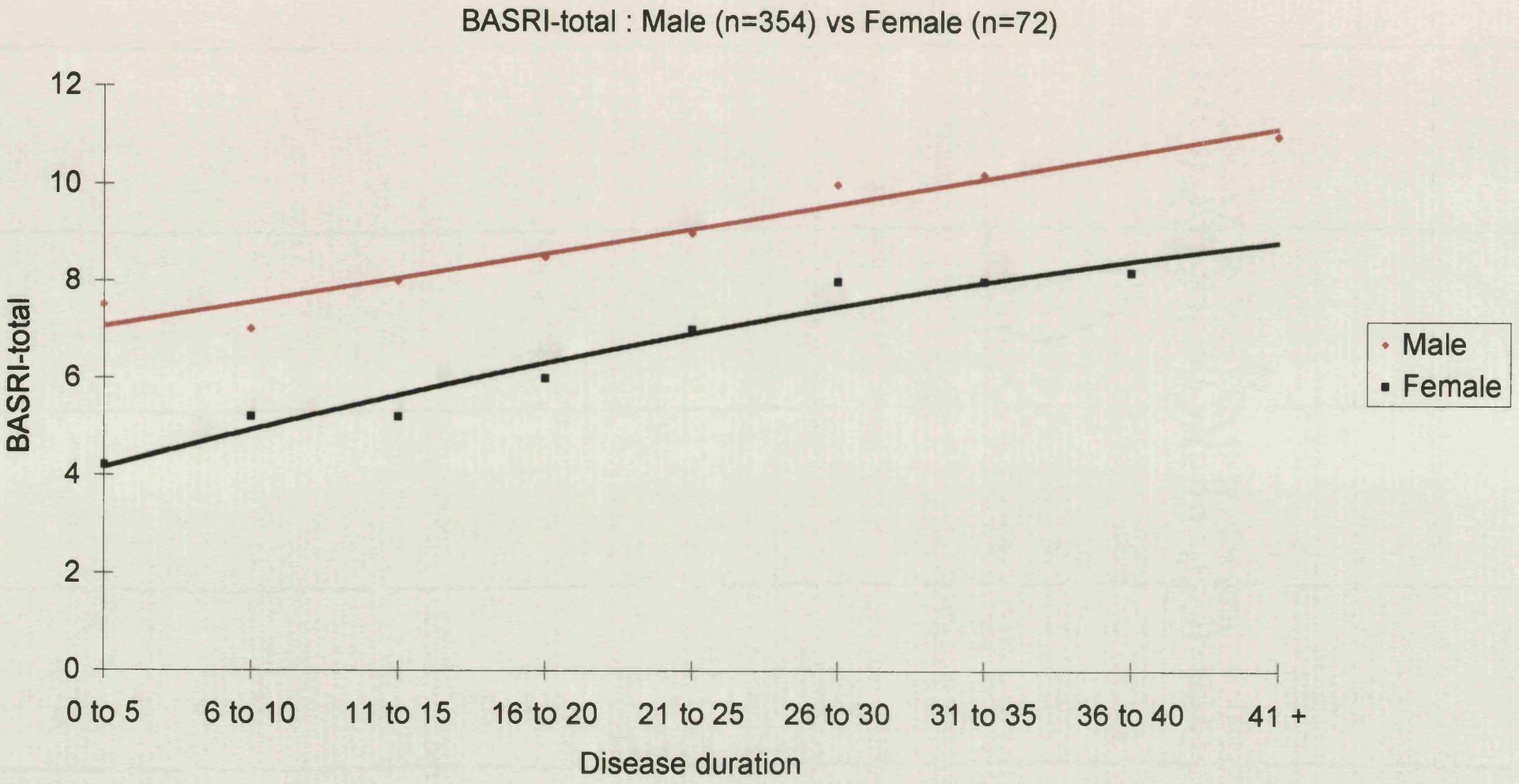
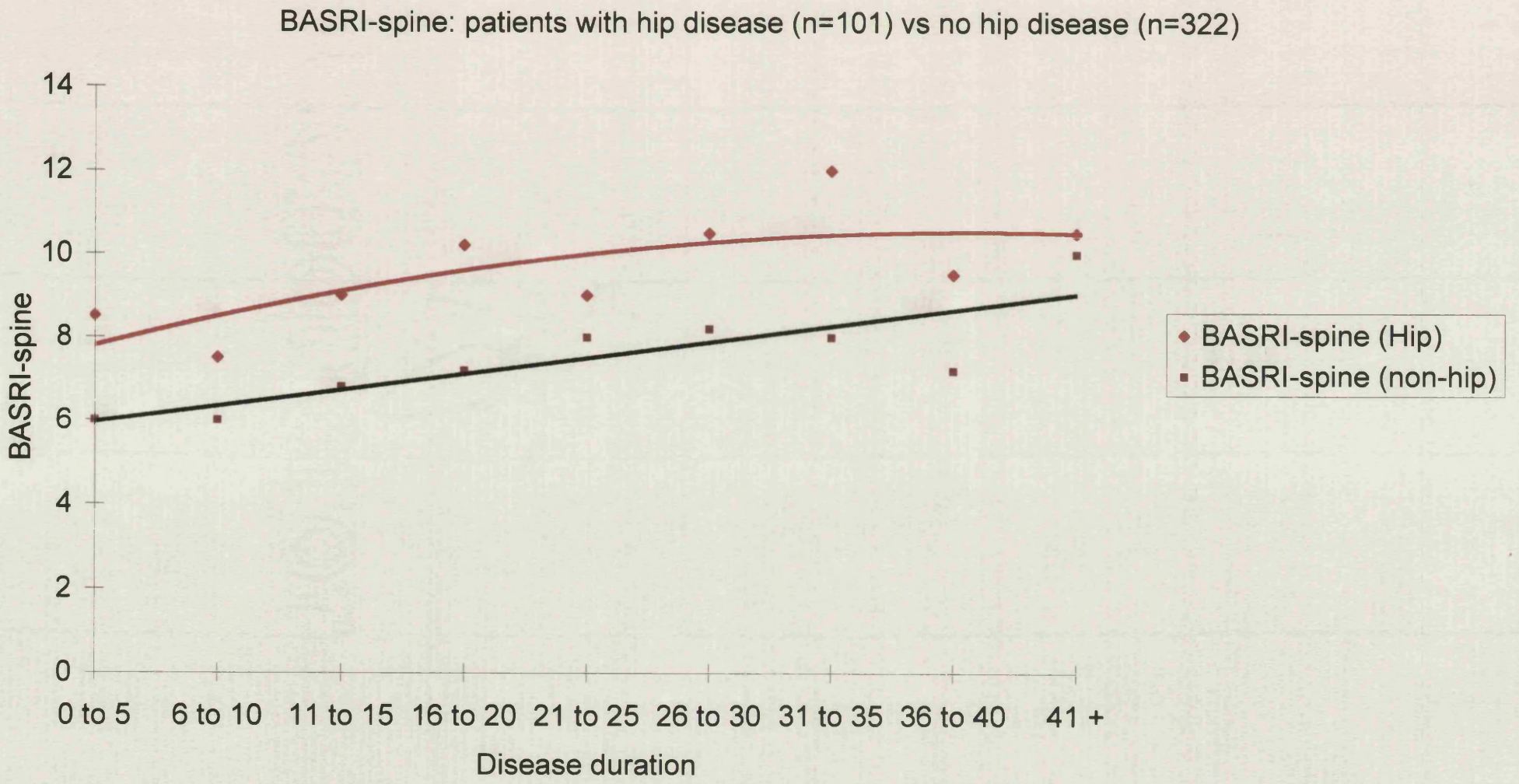

BASRI-total : Male (n=354) vs Female (n=72)
BASRI-total
12
10
8
6
4
2
0
0 to 5
6 to 10
11 to 15
16 to 20
21 to 25
26 to 30
31 to 35
36 to 40
41 +
Disease duration
Male
Female

Figure 13


BASRI-spine: patients with hip disease (n=101) vs no hip disease (n=322)
BASRI-spine
14
12
10
8
6
4
2
0
0 to 5
6 to 10
11 to 15
16 to 20
21 to 25
26 to 30
31 to 35
36 to 40
41 +
Disease duration
BASRI-spine (Hip)
BASRI-spine (non-hip)

6.2 The influence of genetic and environmental factors:

i) Genetics

AS is a polygenic disease, therefore it can be expected that there will be overlap and interaction between susceptibility and severity factors. Susceptibility is clearly genetically determined but as yet there is little knowledge as to what factors influence disease progression and outcome. Herein, we examine the overlap between susceptibility and severity factors by exploring:

a) Evidence for an overlap of susceptibility genes involved in both AS and the secondary disorders (iritis, psoriasis, and inflammatory bowel disease) b) Impact of known susceptibility genes on disease activity and function c) Impact of age at onset (ie susceptibility factors) on outcome d) Impact of parental sex on transmission of disease to the next generation e) Inheritance of phenotypic expression f) Concomitance of disease expression among family members and g) Identification of possible loci on the genome involved in severity.

This should establish

1. the influence of susceptibility factors on disease expression and
2. if severity genes exist and where in the geneome they might lie.

5.2 (a) Eye, skin and bowel disease in spondylarthritis : Genotypic, phenotypic and environmental factors.

Objective: Patients with ankylosing spondylitis (AS) can develop psoriasis (Ps) or inflammatory bowel disease (IBD), whereas those with psoriasis may develop joint involvement. The link between these diseases might be genetic, with shared susceptibility genes. This hypothesis is supported by the finding that B27 negative spondylitis patients are

more likely to have secondary forms of disease linked to psoriasis or IBD [98] . Thus, in the absence of the B27 susceptibility effect, genes such as those for the other inflammatory conditions may be important in predisposing the individual to ankylosing spondylitis. Alternatively, these conditions may be linked by environmental factors, the inflamed gut and skin allowing the conduit of pathogenic triggers which induce AS. The HLA-B27 transgenic rat develops clinical features including: inflammatory gastrointestinal disease (stomach, small intestine, colon), skin lesions (psoriasiform dermatitis, nail dystrophy), spinal lesions, peripheral arthritis, carditis and some develop anterior uveitis [150] . These rats generally develop bowel inflammation which is then followed by arthritis. However, if the HLA B27 transgenic rat is maintained in a sterile environment neither the inflammatory gastrointestinal disease, nor the arthritis develop [151].

Thus there is evidence that the link between these inflammatory conditions could be both genetic and environmental. How these diseases are linked will have an important impact on our understanding and treatment of AS and the associated risks to relatives of affected individuals [143] . This study explores the nature of the inter-relationship between inflammatory disease of the spine / joints, skin, eye and bowel [i.e ankylosing spondylitis (AS), psoriasis (Ps), iritis (I), inflammatory bowel disease (IBD)]. [Appendix 6]

Methods: The database

The Bath RNHRD AS database consists of 4953 spondylarthritis patients. These subjects are defined as those with symptomatic sacroiliitis diagnosed by x-ray, fulfilling the New York criteria for AS and meeting the Amor (1991) and the European Spondylarthropathy Study Group diagnostic criteria for the Spondylarthropathies. Subjects are either those referred to the Royal National Hospital for Rheumatic Diseases or are members of the National Ankylosing Spondylitis Society. Among the patients, 1915 (39%) have iritis, 811 (16%) psoriasis and 404 (8%) inflammatory bowel disease (IBD:158 Crohn's disease and

246 ulcerative colitis). The diagnoses for iritis and psoriasis were ascertained by the GP, rheumatologist, ophthalmologist or dermatologist. A gastroenterologist was used for the diagnosis of inflammatory bowel disease. In each case the diagnosis was recorded as representing one point in time. Two studies have been performed to validate the diagnosis of ankylosing spondylitis in those patients recruited through NASS. 146 subjects were assessed by a rheumatologist and 100% were confirmed as having AS according to the New York Criteria (personal communication, M. Brown, Oxford) The general practitioners of a further 240 NASS members were contacted to determine whether their patient's AS had been confirmed radiologically. In 229 (95.4%) cases, AS with radiological evidence of sacroliitis was confirmed. The GP's of 120 Ps-AS patients and 139 IBD-AS patients were contacted. Of these, 77 (64%) and 112 (81 %) replied confirming Ps in 65 (84%) of cases and IBD in 108 (96%) of cases.

Data processing and statistical methods

1. Of the 4953 subjects on the database, 3287 had a complete data set with full family and personal data. The prevalence of the three individual secondary disorders (psoriasis, IBD and iritis) was determined. From these data the expected numbers of patients with none, one, two or three of these conditions was established assuming independence between the diseases. The expected number was compared with the observed number using the χ^2 test. Patients with a secondary disorder were matched with an available and appropriate control (without the specific condition) for disease duration, age and sex (ie psoriasis patients were matched with those without psoriasis [600 pairs of 811 Ps patients], IBD patients with those without IBD [335 pairs of 404 IBD patients], and iritis patients matched with those without iritis [735 pairs]. These were compared using McNemar's χ^2 for prevalence of secondary disorders as outlined above.

2. Patients with a confirmed diagnosis for the reported secondary conditions and arthritis were asked to report when symptoms of each condition first began. Patient recollection was verified by GP reports.

3. Patients with a confirmed diagnosis of disease were asked 'to your knowledge do any members of your family have a diagnosis of psoriasis, inflammatory bowel disease, iritis or ankylosing spondylitis'.

4. Patients with multiple disease completed disease activity (Bath Ankylosing Spondylitis Disease Activity Index [BASDAI]) and function (Bath Ankylosing Spondylitis Functional Index [BASFI]) assessments. The radiographs of RNHRD patients were blindly scored by two independent observers using the Bath Ankylosing Spondylitis Radiology Index [BASRI] and then sub-grouped according to presence of secondary disorders.

Results: 1. Analysis of the prevalence of secondary disorders within the individual: There were more people with spondylitis and multiple co-diseases than expected and more with pure ankylosing spondylitis (ie without secondary diseases) but there were fewer with spondylitis and only one single co-disease than expected. The observed /expected numbers for patients with no co-diseases were 2069 /1973, with one co-disease 1722 /1872, with two co-diseases 454 /417 and with three co-diseases 42 /25. (χ^2 =32.2 $p<0.001$).

Psoriatic patients (n=600, M: F=3.2:1, disease duration=22 yrs, age = 47 yrs) **had a higher prevalence of iritis**. 48% vs 40% [O.R: 1.4 (1.1-1.7)] **and more IBD than controls** [O.R: 1.3 (1.1-2.0)] 12.5% vs 9.4%. (n=600, M:F=3.2:1, disease duration = 22 ys, age = 47)

Iritis patients (n=735, M:F=2.3:1, disease duration = 21 yrs, age = 45 yrs) **had a higher prevalence of psoriasis** [OR: 1.4 (1.1-1.8)] 18.2 % vs 14% **and IBD** [OR: 1.5 (1.1-2.2)] 10.6% vs 7.3% **than controls** (n=735, M:F=2.3:1, disease duration = 21yrs, age = 45 yrs).

IBD patients (n=335, M:F=2.3:1, disease duration =22 yrs, age = 47 yrs) **had a higher prevalence of iritis** [O.R: 1.4 (1.1-2.0)] 45.4% vs 36.7% **and psoriasis** [O.R: 1.9 (1.3-2.8)] 23.9% vs 14.3% **than controls** (n=335, M:F=2.3:1, disease duration =22 yrs, age = 47 yrs).

2. Temporal relationship between onset of the different conditions: Patient recollection of age of onset and diagnosis was confirmed using GP reports. The GP and patients recollection was comparable for age/year in 95% of cases of ankylosing spondylitis, inflammatory bowel disease and psoriasis and 100% of those with iritis. Onset of AS symptoms occurs significantly before advent of iritis ($p<0.001$) and IBD ($p<0.001$). Onset of Ps and AS symptoms occurs at comparable ages. Gut, skin and eye symptoms do not precede those of AS. [Table 5]

3. The prevalence of disease among first degree relatives: Relatives of patients with IBD-AS are at an increased risk of developing psoriasis and iritis. (Tables 6 and 7) Those with multiple disease predict the highest prevalence of co-diseases (ie Ps, IBD, I or AS) within family members, followed by those with IBD, psoriasis and lastly iritis.

4. The influence of secondary disorders on outcome of AS: Disease activity and function are worse for patients with psoriasis and/or IBD. However, those with iritis are comparable to those with primary disease. [Table 8] Radiological change was worse for those with iritis than for patients with pure AS [Table 9].

Interpretation of study: The data explore the nature of the inter-relationship between the spinal disease, AS, and the extra-spinal co-disorders. Particularly focusing on the relative role of environment and genetics in the inter-relationship of these inflammatory disorders:

1. in an individual with AS, the presence of one concomitant disorder enhances the probability of there being a second or third co-disease.

2. the symptoms of AS precede or are contemporaneous with the concomitant disorders

3. family members are at increased risk of secondary disorders even in the absence of their expression in the index case and finally

4. the expression of severity (ie BASRI, BASDAI, BASFI) in AS is influenced by the presence of the secondary conditions. Thus, it can be concluded that the susceptibility genes of these co-disorders overlap with each other and with AS and impact on disease severity.

In terms of the increased prevalence of secondary disorders within the individual, it is recognised that patients seen at a tertiary referral clinic may have more co-diseases than those seen in the general population. Similarly, patients who join a self-help group (NASS) may be more likely to have multiple disorders. This suggests that a bias towards multiple disease (Berkson's Bias) may be observed among the total sample. However, more patients than expected were found without a concomitant disease (pure AS) and fewer were found with AS and a single secondary disorder. This is not a finding predicted by the bias. In addition, the matched data used control and sample patients from the same population which were equally likely to have multiple disease as there were no selection differences between the subgroups (ie both entered the hospital/self help group system).

A link between the secondary disorders in the absence of AS has been previously described. Psoriatic patients suffer from IBD more than controls [152] [153] the prevalence of psoriasis is increased in Crohn's disease [154][155][156] and there is a higher incidence of iritis than expected among psoriasis patients [157]. Genes on chromosome 16 are associated with iritis [158], psoriasis [159] and Crohn's disease [160]. These inflammatory disorders are linked to chromosome 6, perhaps through HLA-Cw6, in the case of psoriasis [161], B62 in IBD [162] and B27 for AS [163] and iritis [164]. Thus, there is evidence to support

the finding in this study that the genetic susceptibility to inflammatory bowel, skin, eye and joint disease overlaps and may be additive.

The suggestion that the triggering agent for AS may enter the body through the inflamed gut or skin (as proposed in the HLA transgenic rat) is not supported by our human data. There was little overt (symptomatic) inflammation due to IBD prior to the onset of symptomatic AS. However, we do appreciate that occult bowel involvement may occur early in AS [165] and that the clinical expression of psoriasis varies, very mild disease (ie minor scalp involvement) could be overlooked by the patient and GP. In fact, it is not known when the actual onset of disease begins in any of these conditions. It is possible that the trigger is pulled in the womb and disease begins in the infant with age of symptom onset simply a reflection of severity of disease. However, this study has not found evidence that overt and symptomatic IBD or Ps are involved in triggering AS (ie allowing the conduit of environmental pathogens to induce AS). Conceivably, the results may be different in a population chosen from a gastroenterological or dermatological clinic. However, the finding that onset of bowel disease and the other inflammatory conditions do not follow a temporal pattern but may occur at any time (before or after the onset of arthritis), supports the hypothesis that susceptibility genes of inflammatory skin, eye and bowel disease may overlap with those for inflammatory joint disease.

The prevalence of disease among family members of the index case represents an estimate of the occurrence of disease within this population. The observation of familial aggregation has been previously identified. [153]. Herein, we reinforce the previous observations and suggest that the strongest genetic load can be seen among the IBD-AS sufferers with the least observed among the iritis-AS patients (Table 6). The data from the relatives of the pure AS subjects suggest that occult genes associated with IBD & Ps and/or the presence of iritis genes may be required for the development of ankylosing spondylitis (see Figure 14)

Patients with psoriasis and / or IBD had poorer function and greater disease activity. Psoriasis/IBD genes may have an additive effect on susceptibility to and severity of AS. Alternatively, an inflammatory/immunological response to a sizeable area of body (skin surface or bowel) may have an effect of potentiating inflammation elsewhere. Indeed, it is recognised that active inflammatory bowel disease can coincide with flares in peripheral joint disease [165]. However, this condition does not appear to increase disease specific AS changes (ie radiological change). Conversely, iritis appears to be a strong phenotypic marker for more severe radiological disease. Moreover, peripheral arthritis in a patient with ankylosing spondylitis enhances the likelihood that iritis will develop [166].

The secondary diseases do appear to have a genetic overlap with AS in terms of susceptibility genes. The susceptibility factors for these conditions may be additive or have a synergistic effect on each other. The presence of these conditions has a pronounced effect on the phenotypic expression. The patient is more likely to have multiple disorders, develop these disorders after the onset of AS and have a poorer outcome in terms of the spondylitis. These findings point to the striking overlap within the patient and their family of rheumatological, dermatological and gasteroentrological processes. The impact of a relevant family history is clearly demonstrated and the data enhance our understanding of how the shared gene hypothesis can have an impact on disease expression.

Table 5: Mean age of onset of the inflammatory disorders [Iritis (I), psoriasis (Ps), inflammatory bowel (IBD)] compared to onset of AS.

	AS (yrs)	I (yrs)	Ps (yrs)	IBD (yrs)	Mean difference (Confidence Intervals)
Pure AS (n=2221)	25				
AS I (n=151)	23	33			AS vs I : 10 (7.4-10.8)
AS Ps (n=40)	27		26		AS vs Ps : none
AS IBD (n=74)	24			30	AS vs IBD : 6 (3.4-9.7)
AS I Ps (n = 30)	28	39	32		AS vs I : 11 (6.6-15.2), AS vs Ps : 4 (0.5-8.8)
AS I IBD (n=66)	22	34		31	AS vs I : 12 (9.6-15.8), AS vs IBD: 9 (5.1-12.2)
AS Ps IBD (n=18)	23		26	31	AS vs Ps : none , AS vs IBD : 6.6 (1.7-11.6)
AS I Ps IBD (n=26)	23	32	33	33	AS vs I : 9 (4.5-14.8), AS vs Ps : 10 (2.5-16.9), AS vs IBD : 10 (3.6-16.1)

Table 6 First degree relatives

	Ps	IBD	Iritis	AS	Order
1. AS pure (n = 138)	10%	7%	6%	25%	1
2. AS + iritis (n=142)	5%	3%	13%	29%	2
3. AS + Ps (n=42)	33%	12%	0%	14%	3
4. AS + iritis + Ps (n= 26)	19%	8%	15%	19%	4
5. AS + IBD + Ps (n=17)	12%	24%	6%	24%	5
6. AS + iritis + IBD (n=63)	14%	14%	16%	29%	6
7. AS + IBD (n=76)	17%	24%	8%	24%	6
8. AS +IBD + Ps + Iritis (n=23)	17%	17%	26%	30%	8
Population Rate.	1.1%	0.07%	-	0.19 %	

Moll et al. Medicine 1974 vol

53 (5) 343-364)

Table 7 : Relatives of AS patients affected with inflammatory diseases not identified in the index case.

	+ IBD				- IBD		
	Relatives with:				Relatives with:		
	Ps	Iritis	Summary		Ps	Iritis	Summary
AS + IBD	13/76	6/76	16/76	AS pure	14/138	8/138	21/138
AS + iritis + IBD	9/63		9/63	AS + iritis	7/142		7/142
AS +Ps + IBD		1/17	1/17	AS + Ps		0/42	0/42
			26/156				28/322
			(17%)				(9%)

p=0.01

	+ Iritis				- Iritis		
	Relatives with:				Relatives with:		
	IBD	Ps	Summary		IBD	Ps	Summary
AS + iritis	4/142	7/142	11/142	AS pure	10/138	14/138	23/138
AS + iritis + IBD		9/63	9/63	AS + IBD		13/76	13/76
AS + iritis + Ps	2/26		2/26	AS + Ps	5/42		5/42
			22/231				41/256
			(10%)				(16%)

p=0.033

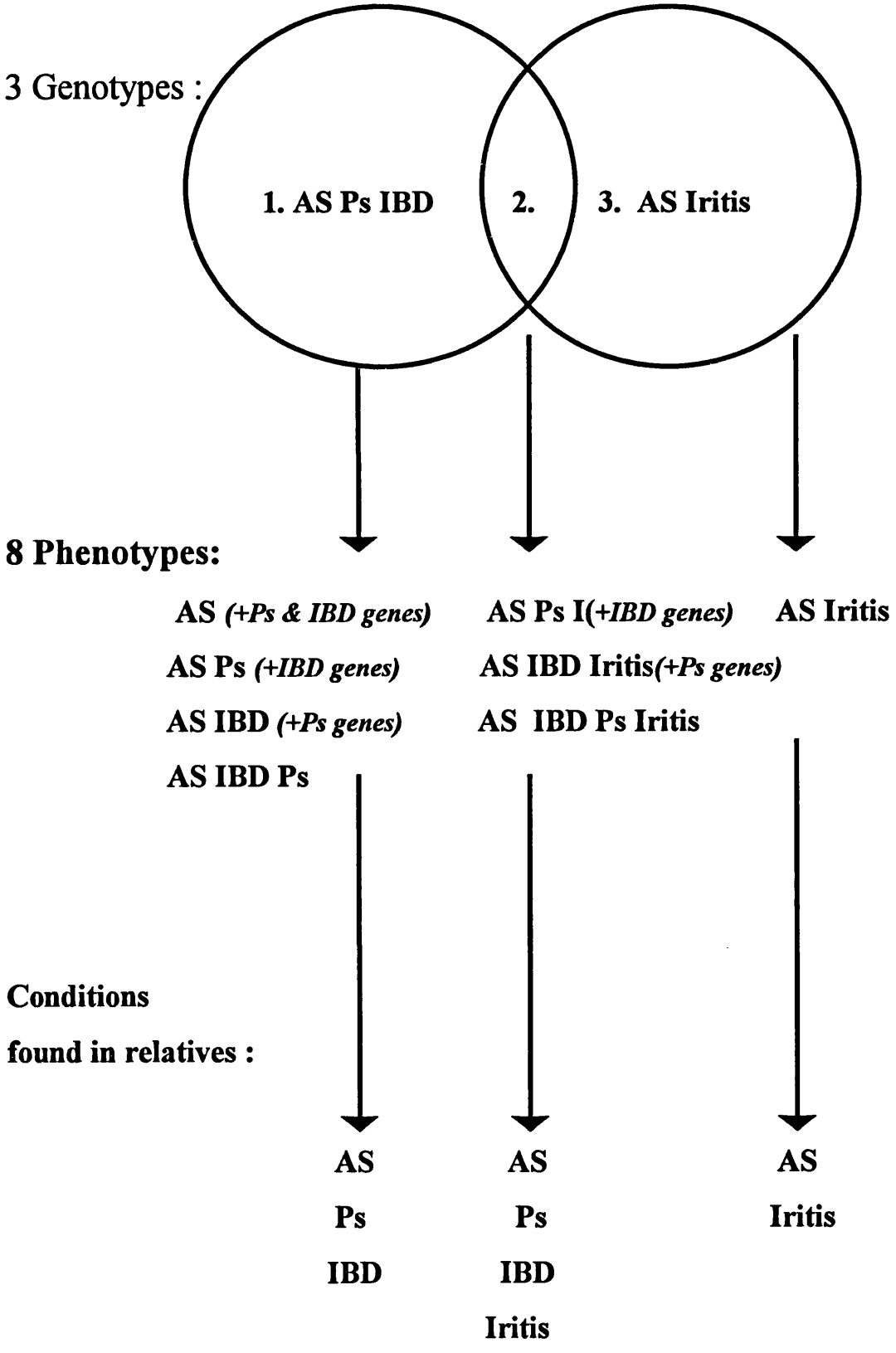
Table 8: Phenotypic expression of secondary disease

	AS duration	BASDAI	BASFI	Mean Difference between 1^0 AS vs 2^0 AS [BASDAI] (confidence interval)	Mean Difference between 1^0 AS vs 2^0 AS [BASFI] (confidence interval)
Pure AS (n=2221)	19.0 yrs	4.1	3.9		
AS I (n=1342)	23.7 yrs	3.9	4.0	ns	ns
AS Ps (n=389)	19.3 yrs	4.7	4.6	0.5 (0.3-0.7)	0.4 (0.2-0.7)
AS IBD (n=341)	25.7 yrs	4.5	4.8	0.4 (0.05-0.7)	0.6 (0.3-1.0)
AS I Ps (n=323)	25.6 yrs	4.6	4.8	0.4 (0.2-0.6)	0.8 (0.4-1.0)
AS I IBD (n=150)	25.3 yrs	4.3	4.6	ns	06=.6 (0.2-1.1)
AS Ps IBD (n=44)	20.5 yrs	5.9	5.4	1.7 (1.2-2.4)	1.6 (0.9-2.4)
AS I Ps IBD (n=46)	23 yrs	4.5	5.4	ns	1.5 (0.8-2.3)

Table 9: BASRI in primary vs secondary disease

	AS disease duration	BASRI	Mean difference 1^0 AS vs 2^0 AS (confidence intervals)
Pure AS (n=151)	**19.8 yrs**	**8.3**	
AS I (n=121)	**23.8 yrs**	**10.1**	**1.8 (0.8-2.7)**
AS Ps (n=60)	**22.3 yrs**	**9.6**	**1.3 (0.2-2.4)**
AS IBD (n=23)	**21.3 yrs**	**8.7**	**ns**

Figure 14: Relationship between AS and secondary disorders



b) Role of susceptibility in severity (established genes)

Objective: Studies in diverse populations have demonstrated that HLA B27 [46] and B60 [66] both influence susceptibility to ankylosing spondylitis (AS). B60 may increase susceptibility three fold in both B27 positive [66] and negative individuals [68]. However, this is not confirmed by all studies [69] [70][71].

In terms of severity effects, HLA-B27 has no effect on the phenotypic expression of progressive ankylosis in ank/ank mice. In patients with HLA B27 there is an earlier onset of disease, more hip involvement and peripheral arthritis [49] but no influence on radiological and clinical outcome measures. However, B27 (+) patients do suffer less psoriasis and inflammatory bowel disease [99].

The role of B60 in severity of disease has not been investigated. Therefore, the present study was undertaken specifically to assess the role of B60 and B27 (in patients who have inflammatory bowel disease) in determining disease severity of AS as measured by disease activity [BASDAI] and function [BASFI].

Methods: **Patients and control subjects**: AS patients were recruited from the Royal National Hospital for Rheumatic disease and the National Ankylosing Spondyltitis Society.

1. Four hundred and three (403) AS patients were typed for B60, DR1 and B27. Many had been originally selected and typed for family studies. Therefore, there was a bias towards those with a positive family history (ie those with a first degree relative with disease). Thirty five (8.7%) were identified as B60 positive (M:F 2.2: 1). These patients were matched with 35 B60 negative control subjects for sex, age and disease duration.

2. One hundred and forty three (143) AS patients with bowel disease were typed for B27. [Bowel disease patients were selected as they have a higher prevalence of B27 negative disease] Thirty three (23%) of these 143 patients were negative for B27. These subjects were matched with B27 positive patients (with IBD) for sex, age and disease duration.

All subjects were assessed for disease activity (BASDAI), Functional Status (BASFI) and Global well-being (BAS-G).

Confirmation of diagnosis for AS and all secondary conditions was obtained from the GP for all subjects.

Statistical Analysis All matching was performed by identifying all possible matches and using a random number table to select the appropriate control. McNemars χ^2 test was used to compare matched pairs. The SPSS package was used for the analysis.

Results

1. B60 (+) patients matched for sex, age and disease duration:

There was no difference in age of onset of AS among the B60(+) and (-) patients. [24.2 years & 22.9 years B60 (+) & B60 (-) respectively, p=0.4,] .Of the 35 B60 positive patients, 20 had familial AS (57%). Of the 35 B60(-) patients, 26 had familial AS (74%) (p=0.13). Iritis and psoriasis were more prevalent in the B60(-) control group (p<0.041 O.R 3 [1.3-6.7] & p<0.035 O.R: 4 [1.07-15] respectively). There was a 5.7% prevalence of IBD in the B60(-) group and no cases in the B60(+) group. However, the disease activity and functional status were comparable. [Table 10]

2. B27(+) patients matched for sex and disease duration (all patients have bowel disease)

The age of onset of AS was younger among those with HLA B27. [22.3 years & 29 years, B27(+) & B27(-) respectively, p<0.001,]. Disease activity and function were comparable between the two groups [p=0.2]. [Table 11]

Interpretation: The susceptibility genes B27 and B60 do not impact on disease activity or function.

HLA B60 was only present in 8.7% of patients typed and although sample numbers were relatively small but the power for the B60 sample was 95%. These patients were selected from a specific population. Most, were originally typed for family studies. This may imply that the AS would be inclined to be milder [167] [168] and may increase the prevalence of concomitant disorders observed.

It is known that in the HLA B27 negative individual, genes for psoriasis and inflammatory bowel disease take on a greater relevance in AS [168]. Patients with ulcerative colitis and ankylosing spondylitis are less frequently B27 positive (in this sample 23% were B27 negative) than those with ankylosing spondylitis alone [169]. The implication being that genes for psoriasis and IBD may be playing a role in the pathogenesis of spondylitis. The susceptibility genes B60 can be seen to reflect the findings observed with B27. The susceptibility genes for iritis, and psoriasis may be involved in susceptibility to ankylosing spondylitis. Without the influence of B60 and B27, other genes such as those for iritis, psoriasis and perhaps IBD may be additional susceptibility factors. B60 and B27 negative patients may have a higher frequency of non B60 and B27 pathogenic genes.

In conclusion the susceptibility genes HLA B60 and HLA B27 do not influence severity of AS. However, the phenotypic expression of AS in terms of prevalence of co-diseseas is significantly different for the B60(+)/B27(+) vs the B60(-)/B27(-) individual. AS patients without these susceptibility genes may need other pathogenic genes such as those for psoriasis, iritis and bowel disease to develop AS.

Table 10: Patients matched for sex, age and disease duration.

	B60(+)	B60(-)	
B27(+)	91% (n=35)	94% (n=34)	
DR1(+)	41% (n=34)	48% (n=33)	
Age (years)	46	46	
Age at onset (years)	24	24	
Disease Duration (years)	22	22	
Delay in diagnosis (years)	8.8	5.5	p=0.079
BASDAI. Scale 1-10	3.6	4.0	p=0.331
BASFI. Scale 1-10	3.5	3.6	p=0.882
BAS-G. Scale 1-10	3.8	4.3	p=0.456
Iritis	31%	60%	p=0.041 OR = 3
Psoriasis	11%	37%	p=0.035 OR= 4
IBD	0%	5.7%	

Table 11: AS-IBD Patients matched for sex, age and disease duration

	B27(+)	B27(-)	
Age (years)	45 years	46 years	
Age at onset (years)	25 yrs	27 years	
Disease duration (years)	20 yrs	19 years	
Delay in diagnosis (years)	4.7 yrs	8.0 years	3.3 [0.2-6.3] p=0.04
BASDAI. Scale 1-10	5.0	4.4	p=0.2
BASFI. Scale 1-10	4.7	4.5	p=0.7
Age onset IBD	30.2 yrs	27.9 yrs	p=0.4

(c) Interaction between age of onset and severity:

Objective: In ankylosing spondylitis a juvenile age at symptom onset (less than 16 yrs) has been found to correlate with increased disease severity [121][101][171]. In addition, hip involvement (and need for total hip replacement) is more often seen in those with juvenile onset [170] [171] and hip involvement itself is a marker for more severe axial involvement [101][172]. However, late onset, (after age 55) has also been reported to affect the clinical pattern of disease. Such patients are said to have more cervical pain, anterior chest wall involvement, aseptic osteitis [173] and shoulder involvement [174] . It can be assumed that the expression of AS results from a combination of severity and susceptibility genes. Therefore, patients with a younger age at onset may have an increased number of susceptibility factors and perhaps a different disease expression from those with late onset. Late onset individuals may carry fewer susceptibility and a different array of severity genes.

This aspect of the study aims to examine the influence of age at symptom onset on disease expression as measured by radiological change (BASRI), disease activity (BASDAI), function (BASFI), percentage undergoing AS-related surgery, and prevalence of secondary disorders (iritis, psoriasis, inflammatory bowel disease). [Appendix 7]

Methods : The Bath Ankylosing Spondylitis Database consists of 4741 patients (2.5:1 M:F). All were out-patients of the Royal National Hospital for Rheumatic Diseases (n=851) or were members of the National Ankylosing Spondylitis Society. Patients referred to the RNHRD had their diagnosis confirmed according to the New York Criteria. The NASS members are those who have received a positive diagnosis of ankylosing spondylitis from a specialist rheumatologist as a result of an x ray. To validate the diagnosis in those patients recruited through NASS, one hundred and forty-six consecutive subjects were invited to attend an assessment clinic and all 146 were confirmed as having AS according to the same criteria. In addition, for 240 patients a

confirmation was sought from the GP, and confirmed in 229 cases (95.4%) [ie AS with radiological evidence of sacroliitis]. We contacted the GP's of 120 Ps(AS) patients and 139 IBD(AS) patients. Of these, 77 (64%) and 112(81%) replied confirming the diagnosis of Ps and IBD in 65 (84%) and 108 (96%) cases.

Patients were divided into cohorts according to age of symptom onset and were controlled for a) age now and b) disease duration now (McNemar's chi-squared). In addition, cohorts of juvenile onset (<16 yrs), young onset (17-20 yrs), twenties (21-29 yrs), thirties (30-39) and late onset (40+ yrs) were compared. The primary outcome measure was radiological status as determined by BASRI. Secondary measures were disease activity (BASDAI), function (BASFI), numbers undergoing surgery and percentage with secondary disorders.

Results: Radiological progression [sacroiliac joints, hips, lumbar spine, cervical spine]; BASRI

Age at onset has no significant effect on radiological progression [Table 12 & Figure 15]. Radiological change is a factor of disease duration ie those with a young age at onset have more severe disease when compared to like aged late onset patients [Young onset : Late onset ; 10.0 : 8.0 respectively (p=0.02)]. However, disease duration matched pairs are comparable for radiological change [Young onset : Late onset ; 8.0 : 8.6]

Patients with hip disease and a young onset had comparable spinal disease to those with hip disease and a late onset [Table 12] [Young : Late ; 9.0 : 10.8 p=0.04 (corrected value not significant)]. Hip disease patients have more spinal change than non-hip patients [Table 12a] [Young onset – Hip disease : Non-hip disease ; 9.7 : 7.2 respectively (p=0.0001). Late onset – Hip disease : Non-hip disease ; 10.13 : 7.1 p= 0.0001].

Secondary outcome measures Age at onset had no significant effect on disease activity, function, prevalence of secondary disorders, or need for surgery [Table 13 & Table 14]. At comparable age (ie age now [Table 13]), those with longer disease duration (ie young onset) have lower disease activity [Young onset : Late onset ; 4.0 : 4.3 respectively $p<0.02$], more iritis [Young onset : Late onset ; 50% : 40% $p<0.01$] and more surgical intervention [14% : 7% respectively $p<0.01$]. However, when matched for disease duration [Table 14], the disease activity [Young onset : Late onset ; 4.4 : 4.4] , prevalence of secondary conditions and need for surgery [9% : 8%] were all comparable for those with a young age at onset vs late age onset. When matched for disease duration the function was worse for the delayed onset males (ie older aged men) [$p<0.01$]. However, at equivalent ages [Table 11] the function is comparable regardless of disease duration (ie between young age at onset and delayed onset individuals).

Cohorts of juvenile onset (<16 yrs), young onset (17-20 yrs), twenties (21-29 yrs), thirties (30-39) and late onset (40+ yrs) were comparable for disease activity [Figure 16], function [Figure 17] and surgery [Figure 18].

Interpretation of study: The data suggest that age at onset has no impact on disease severity. However, this must be seen in the context that hip disease is more prevalent among patients with juvenile onset [THR rate - Juvenile vs non-juvenile : 18% : 8% respectively, $p<0.001$, Figure 19] and this phenomenon is known to be a predictor of more severe spondylitis [101] [131].

This paradox [ie a) there is a link between age at onset and hip disease,

b) hip disease is linked to increased severity but

c) there is no link between age at onset and increased severity] may be explained on the basis that only a subgroup of young onset patients develop hip disease and only this cohort is at risk of more severe spondylitis. [In our study 21/68 (31%) of the young onset subjects and 13/68 (19%) in of the late onset patients had hip involvement as assessed by a radiograph and THR occurred in 9% and 4% respectively].

It is possible that the young developing hip may be more at risk of becoming affected than the adult hip. Thus, patients with young onset are more at risk of hip involvement (because of the juvenile hip). However, patients with hip disease and a young onset do not have more severe disease than subjects with a late onset and hip disease [Figure 20]. Hip involvement per se appears to be the relevant factor contributing to outcome.

The trigger for AS is thought to be a ubiquitous bacterium [175] . If this is so, then the age of onset of disease should be related to the genetic susceptibility load. Yet, this enhanced genetic susceptibly in young onset patients does not influence outcome, implying that the contributing genes for susceptibility and severity are independent of one another. If by contrast, the age of onset of a patient is governed by the timing of contact with an environmental trigger, the age when this happens appears to have no influence on the later disease development. Separate and unrelated severity factors must influence disease progression. In conclusion, there are three clearly distinct independent factors: the environment and both susceptibility and severity genes.

Table 12: Young onset compared to Late onset in terms of radiological progression

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	p value (corrected)
Age Now (& Sex) Matched n=56 pairs			
BASRI-total*	10.0	8.0	p=0.02 (ns)
BASRI-spine**	8.7	7.6	p=0.02 (ns)
With hip involvement (n= 19 , 10)	9.7	10.0	ns
Without hip involvement (n=37,46)	8.2	7.1	ns
Disease Duration (& Sex) Matched n=68 pairs			
BASRI-total*	8.0	8.6	ns
BASRI-spine**	7.1	8.1	ns
With hip involvement (n=21, 13)	9.0	10.8	p=0.04 (ns)
Without hip involvement (n=47, 55)	6.3	7.5	p=0.06 (ns)

*including hips **without hips

Table 12a : Hip disease vs non-hip disease patients : spinal severity score and age at onset of AS

	Hip disease	Non-hip disease	P value (corrected)
Young onset	n=81	n=148	
Disease duration	22.1 yrs	22.7 yrs	
Mean (sd)	9.7 (2.43)	7.2 (3.0)	p<0.001
Late onset	n=19	n=64	
Disease duration	15.2	14.1	
Means (sd)	10.13 (2.5)	7.1 (3.0)	p<0.001

Table 13: Influence of age at onset on outcome - Age Now and Sex matched

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	p value$
BASDAI			
Whole group (n=784)	4.0	4.3	p<0.02
Males (n=543 pairs)	3.7	4.2	p<0.01
Females (n=241 pairs)	4.5	4.4	ns
BASFI			
Whole group (n=829)	4.4	4.3	ns
Males (n=574 pairs)	4.3	4.3	ns
Females (n=255 pairs)	4.8	4.3	p=0.03
Secondary Disorders			
Iritis (n=829 pairs)	50%	40%	p<0.01
Ps (n=807)	20%	20%	ns
IBD (n=828)	7.5%	7.6%	ns
Surgery			
Total surgery n=991	140 (14%)	67 (7%)	p<0.01

$ Corrected

Table 14: Influence of age at onset on outcome - Disease Duration and Sex Matched

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	p valu
BASDAI			
Whole group (n=784)	4.4	4.4	ns
Males (n=543 pairs)	4.2	4.1	ns
Females (n=4.5)	4.6	4.5	ns
BASFI			
Whole group (n=810)	3.7	4.5	p<0.01
Males (n=546 pairs)	3.6	4.5	p<0.01
Females (n=216)	4.1	4.5	ns
Secondary Disorders			
Iritis (n=799 pairs)	40%	41%	ns
Ps (n=792 pairs)	20%	19%	ns
IBD (n=810 pairs)	7.5%	8.9%	ns
Surgery			
Total surgery n= 924	86 (9%)	71 (8%)	ns

$ Corrected

Figure 15

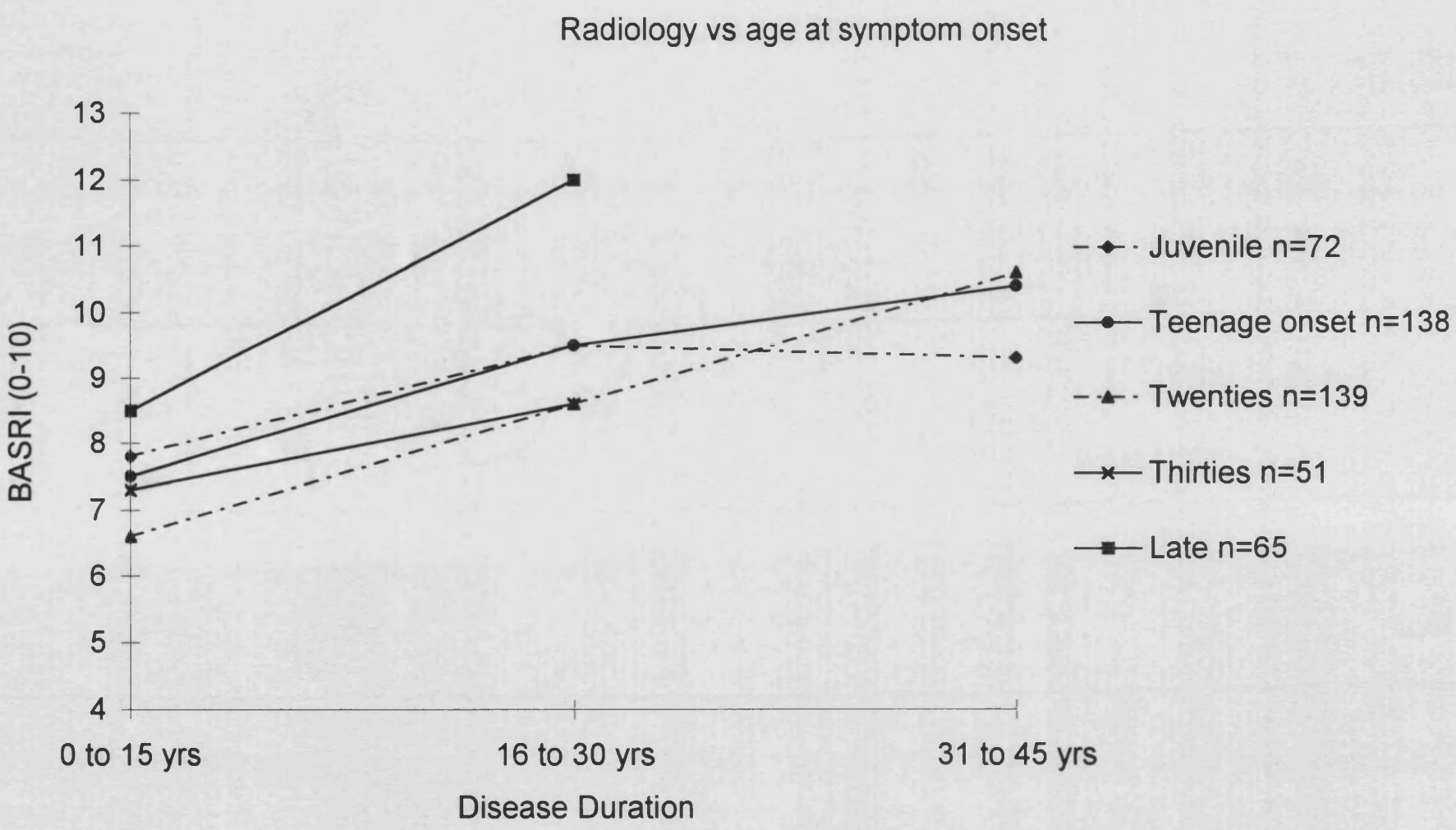

Radiology vs age at symptom onset
BASRI (0-10)
13
12
11
10
9
8
7
6
5
4
0 to 15 yrs
16 to 30 yrs
31 to 45 yrs
Disease Duration
Juvenile n=72
Teenage onset n=138
Twenties n=139
Thirties n=51
Late n=65

Figure 16

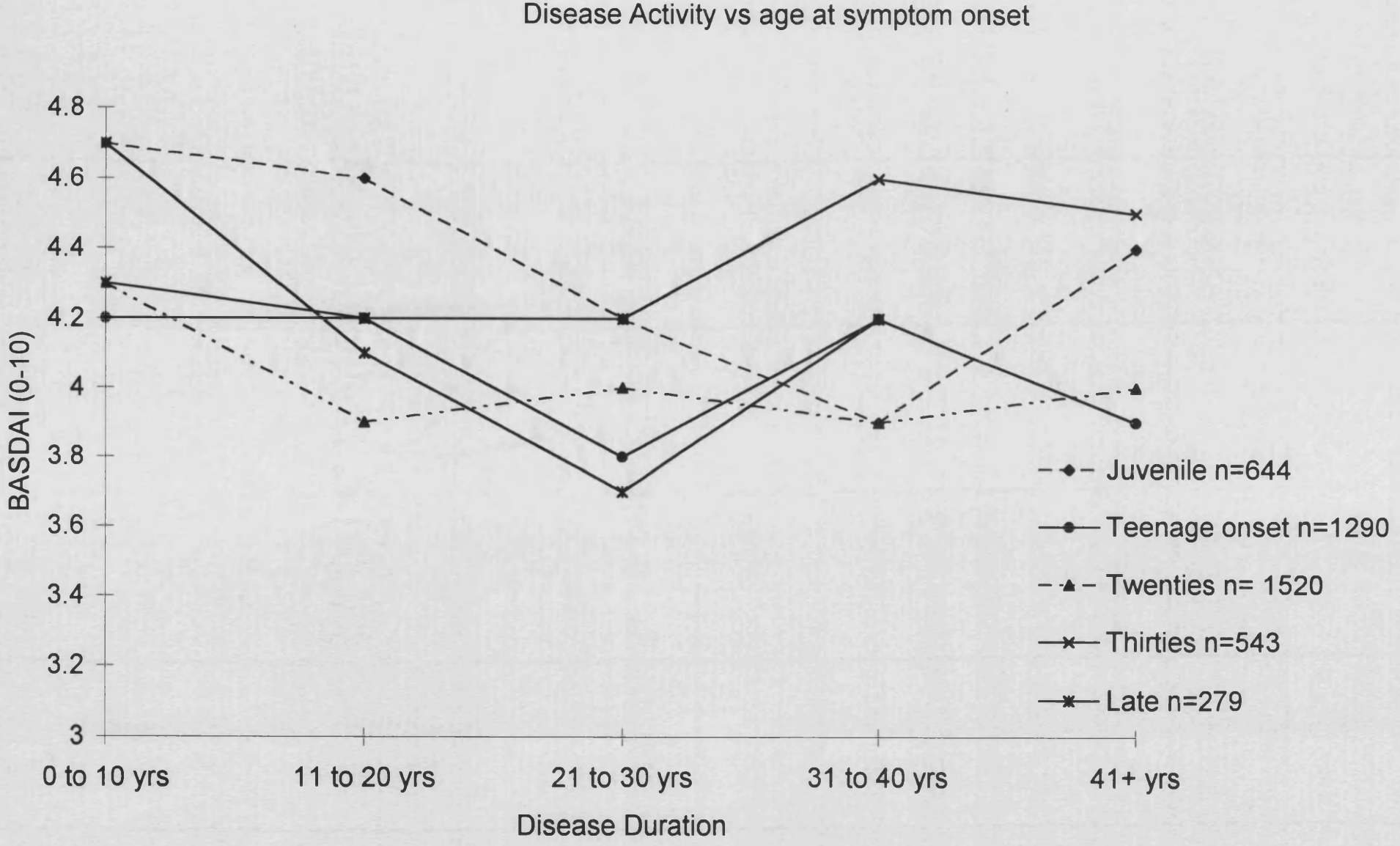

Disease Activity vs age at symptom onset
BASDAI (0-10)
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
3.2
3
0 to 10 yrs
11 to 20 yrs
21 to 30 yrs
31 to 40 yrs
41+ yrs
Disease Duration
Juvenile n=644
Teenage onset n=1290
Twenties n= 1520
Thirties n=543
Late n=279

Figure 17

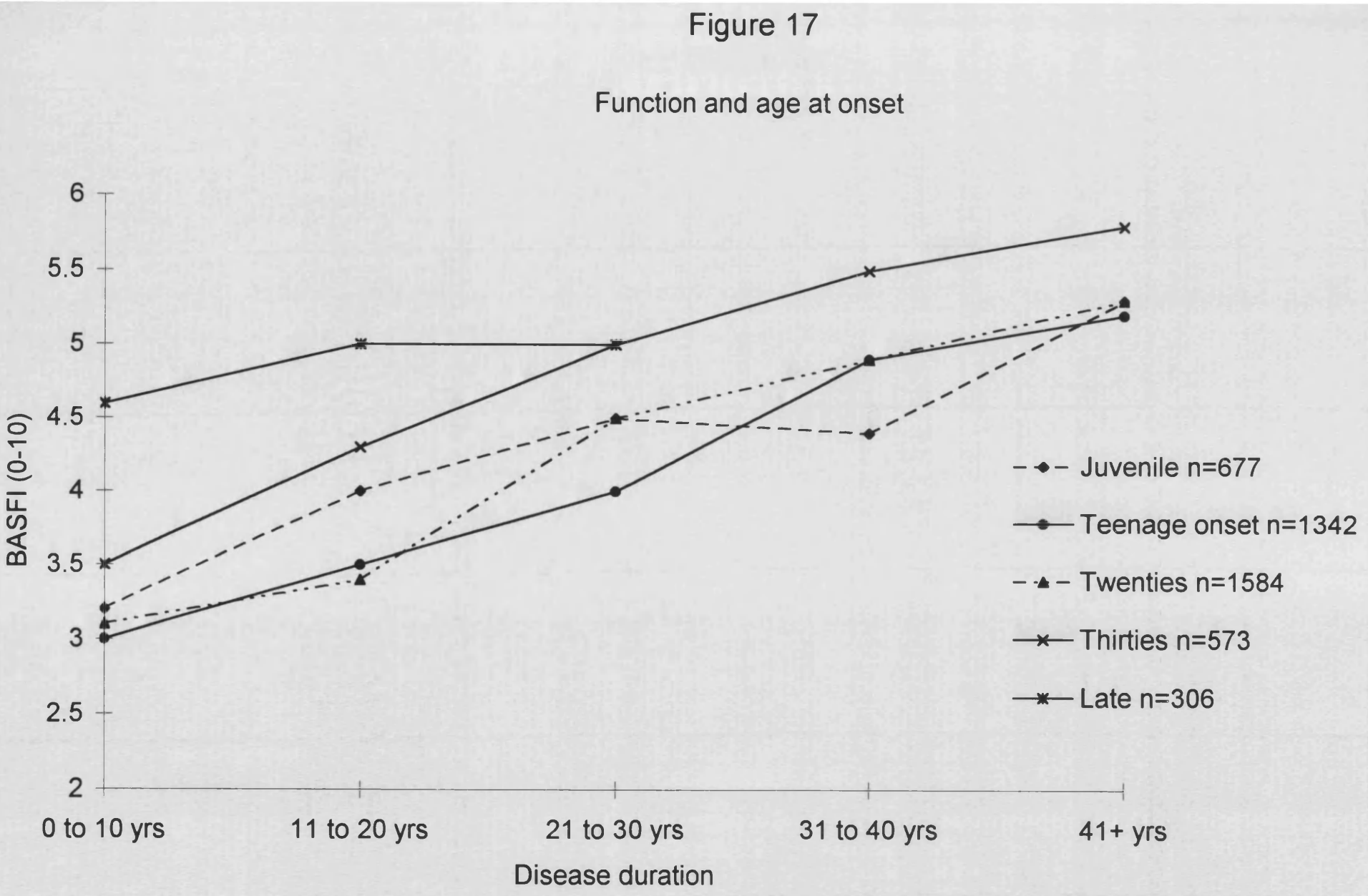

Function and age at onset
6
5.5
5
4.5
4
3.5
3
2.5
2
BASFI (0-10)
0 to 10 yrs
11 to 20 yrs
21 to 30 yrs
31 to 40 yrs
41+ yrs
Disease duration
Juvenile n=677
Teenage onset n=1342
Twenties n=1584
Thirties n=573
Late n=306

Figure 18

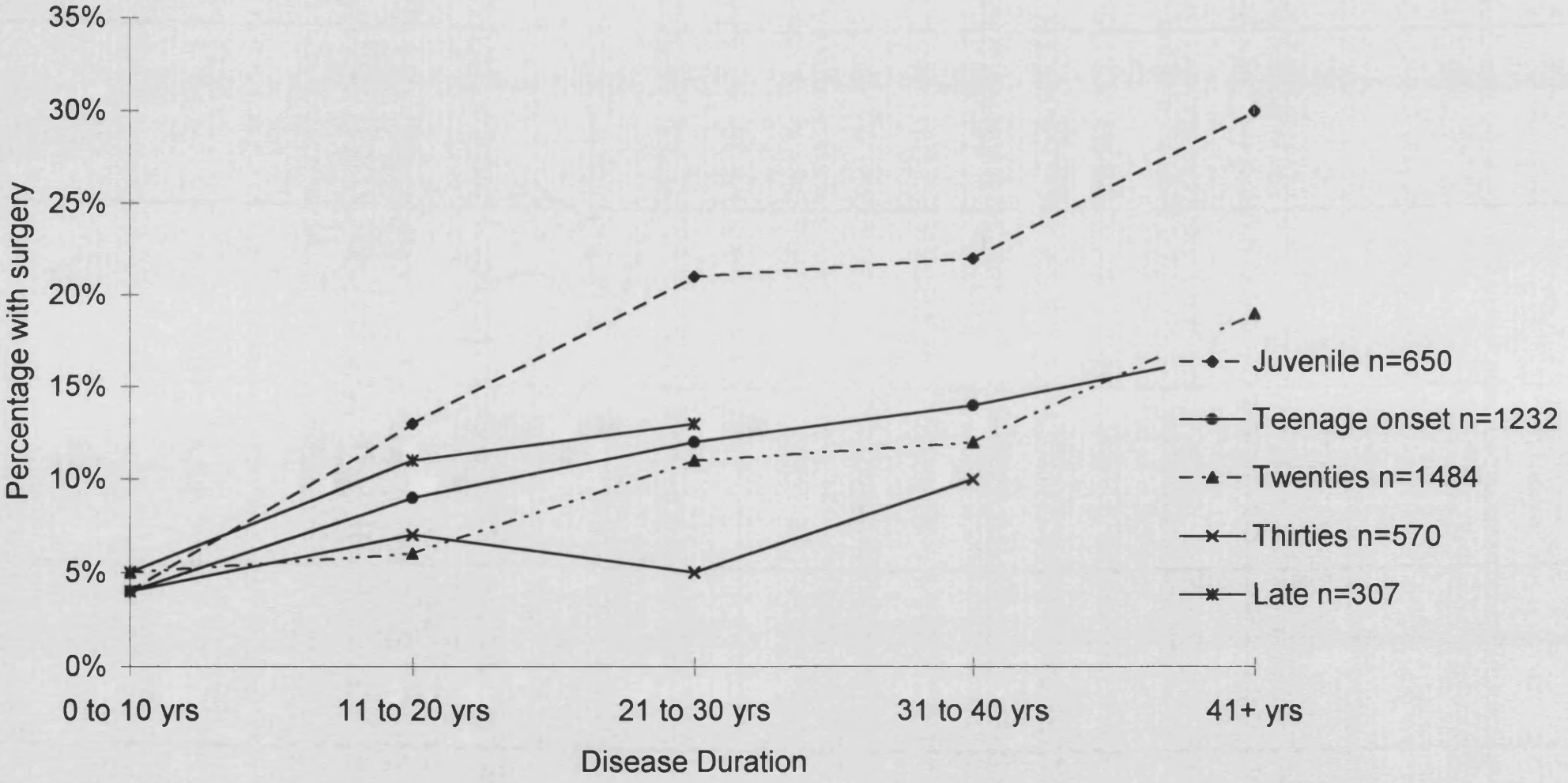

Surgery vs Age at symptom onset
35%
30%
25%
20%
15%
10%
5%
0%
Percentage with surgery
0 to 10 yrs
11 to 20 yrs
21 to 30 yrs
31 to 40 yrs
41+ yrs
Disease Duration
Juvenile n=650
Teenage onset n=1232
Twenties n=1484
Thirties n=570
Late n=307

Figure 19

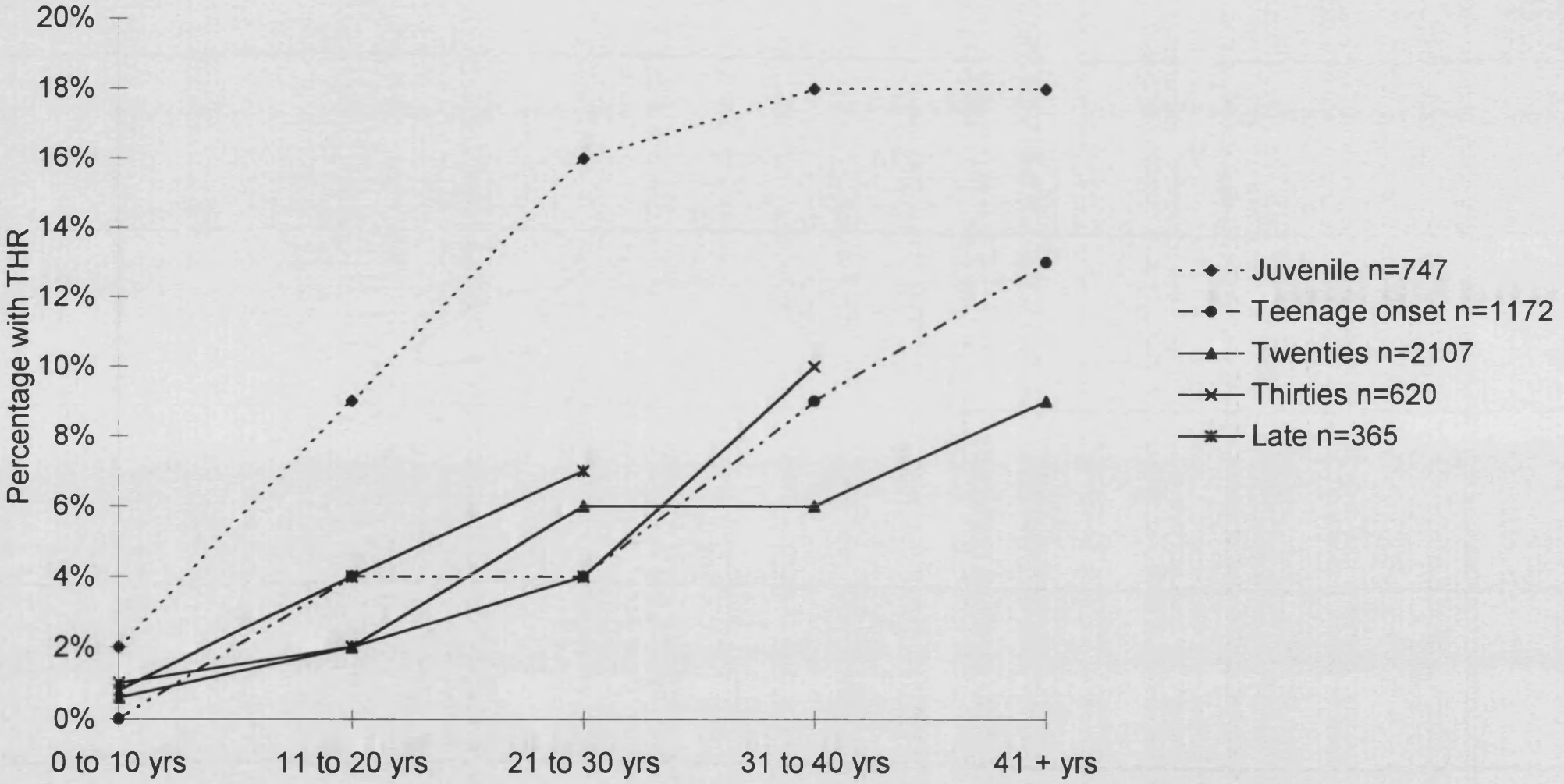
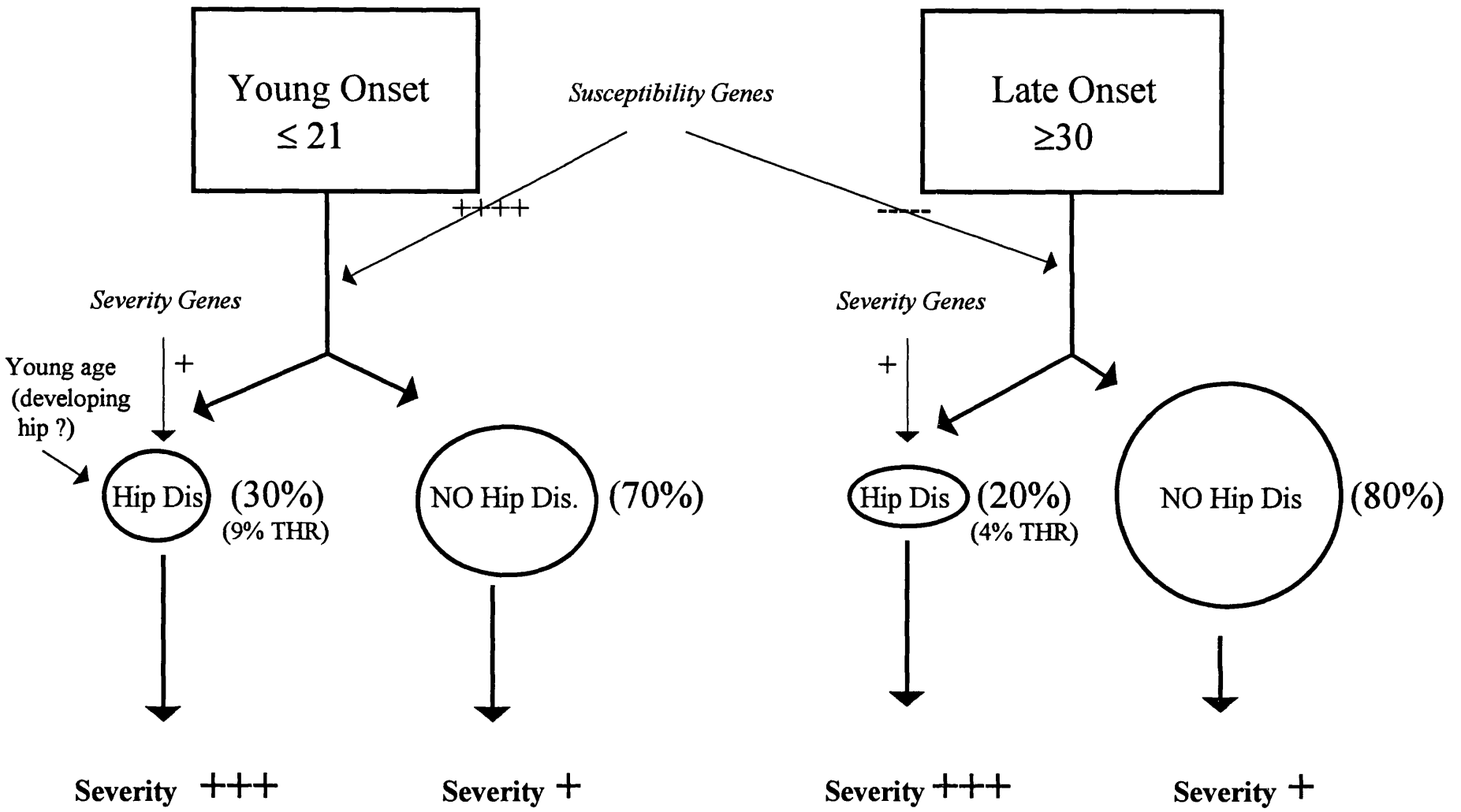

Total Hip Replacement vs age at symptom onset (p<0.001)
Percentage with THR
20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
0 to 10 yrs
11 to 20 yrs
21 to 30 yrs
31 to 40 yrs
41 + yrs
Juvenile n=747
Teenage onset n=1172
Twenties n=2107
Thirties n=620
Late n=365

Figure 20 : Relationship between severity, age at symptom onset and hip disease.



5.2 (b) . The impact of sex on inheritance of AS

Objective: Disease expression differs in men and women, with about 2.5 men affected for every woman with disease [176]. Men develop more severe spinal disease [177]. Women have more peripheral joint involvement [178] . These differences may be due to genetic factors in the sex chromosomes [179] , which would lead to a difference in the sex ratio of children and siblings who inherit the disease. The influence of the sex of the index case of ankylosing spondylitis on disease inheritance among offspring and siblings, irrespective of HLA-B27 status is investigated. [Appendix 8]

Methods: The frequency distributions of age of onset and age of diagnosis of the 4400 individuals were calculated so that we could identify the ages at which 50% (ie 30 yrs) and 75% (ie 39 yrs) of patients should have obtained a diagnosis of ankylosing spondylitis (Figure 21) The study then focused on 1192 individuals (879 men, 313 women) who had children and were older than 50 yrs , and 2222 individuals (1615 men, 607 women) with siblings older than 39 yrs. Where there were several family members with ankylosing spondylitis, only one sibling per family was selected. All patients who had children older than 39 yrs who had ankylosing spondylitis were contacted to ensure the child had a radiographicaly validated diagnosis. Of 29 families with a child older than 39 yrs with AS, all the affected offspring provided confirmation of diagnosis. 100 (25%) patients who report a sibling with ankylosing spondylitis were contacted to provide medical confirmation of diagnosis. We selected these patients using a random number table. Of those chosen, 18 could not be contacted (moved or died). 82 individuals were traced, of whom 78 (95%) had AS confirmed, two had no formal diagnosis but were still in the process of being investigated for AS, and two did not have the disease.

The age of disease onset of fathers and mothers with AS was compared with the percentage of children with disease. To investigate the possibility of a uterine effect (eg passage of pathogens or other agents across the placenta) in mothers with AS, we assessed whether the

affected child was born before or after disease onset in the parent. The results for affected fathers were used as control for when no uterine effect is present.

We analysed the data using χ^2 tests with Yates's continuity correction of SPSS (version 7.0). We used Fisher's exact test for small entries.

Results: The frequency distribution of age of diagnosis showed a mean 10-year delay from onset of symptoms to diagnosis (Figure 21). Men and women did not differ in mean age at onset [24.5 (+/- 10.1) vs 24.4 (+/-10.5) years] or mean age at diagnosis [31.7 (+/- 11.5) vs 33.2 (+/- 11.4)]. The sex ratio of sporadic (simplex family) cases (74%) was 2.8, whereas the male/female ratio of familial (first degree relatives, multiplex family) cases (22%) was 1.7 ($p<0.0001$). Further relatives (ie second and third degree; 4%) had a ratio of 2.3.

The inheritance of ankylosing spondylitis among children of affected parents is shown in Table 15. Overall, sons of men with ankylosing spondylitis were 2.5 times more likely than daughters of men to inherit the disease. Children of women were more likely to develop ankylosing spondylitis than were children of male patients (Table 15). Women with a young age at onset were more likely to have children with disease than were men or women with older age at disease onset (Table 15). Women aged 21 yrs or less at disease onset had a much higher proportion of affected children (38%) than women who were at least 25 years at disease onset (13%; odds ratio 4.0 CI: 1.1-14.0; Figure 22).

For women with an affected child, 74 (43%) mothers had onset of ankylosing spondylitis before birth of the affected child, whereas 48 (28%) women had onset of disease after birth of child and 26 (15%) had child and onset of disease in the same year (onset unknown in 25 (15%) cases). For men with an affected child, 205 (58%) had onset of ankylosing spondylitis

before birth of an affected child, and 46 (13%) developed ankylosing spondylitis at the same time as the birth of their child (p=0.7).

For siblings older than 39 yrs of men with ankylosing spondylitis, brothers were 1.6 times more likely to have inherited the disease than sisters [Table 16]. For siblings older than 39 years of men with ankylosing spondylitis with young age of onset (≤21 yrs), brothers were twice as likely to have inherited the disease as sisters. In all cases, siblings of women were more likely to have the disease than siblings of men [Table 16].

Interpretation: One bias in the study is patients who have a relative with AS may be more likely to join a self help group, resulting in a greater number with a positive family history. However, this would apply to patients regardless of the sex of their relative and should not influence the comparison of ratios for each sex affected by AS. In addition, the percentage of affected relatives in this study (11 %) is comparable to that of published data [180] [181] [182] . A further issue relates to the inevitably small size of certain subsets of individuals (ie young age at onset maternal cases).

Patients and relatives were not systematically typed for HLA B27 status. Nevertheless, of the children randomly reviewed for B27 status used in this study 92% (34/ 37) and 96% (113,118) of first degree disease concordant relatives of AS patients were B27 positive. Moreover, over 95% of AS patients carry this antigen and B27 is inherited as a co-dominant factor [183] . About 50-56% of siblings and children of patients will be B27 positive. Thus, the risk for those carrying B27 will be twice that of the given data. The prevalence of AS among relatives may be a conservative estimate. Clearly, subjects with AS can not be included within the study if they are undiagnosed, misdiagnosed or have clinically silent disease. In addition, although we did ascertain that patients had a diagnosis from their rheumatologist following a pelvic x-ray, we were not always able to review source material. With rheumatoid disease, female susceptibility predominates. The patient's gender appears to be an important risk factor, with relatives of male patients having the greatest cumulative

risk of RA [184] . We now demonstrate that the reverse appears to be true for ankylosing spondylitis, a mainly male susceptibility disease with relatives of female patients being more at risk. Women have a greater family history of disease than do men and this finding is reflected in data from our database showing the sex ratio of sporadic (simplex family) cases compared to the sex ratio of familial disease. Where the index case is male, female relatives are less susceptible to AS than are males. By contrast, there is no significant difference in prevalence of AS among the relatives of either sex of female AS patients. The genetic load passed on by women with a young age at onset appears to be even more dramatic. However, this finding is not seen among siblings. There are at least 4 possible explanations for these findings, none of which are mutually exclusive:

1. Genetic Load Effect: Generally, females are at less risk of developing AS. Thus, it remains possible that those with AS have a higher genetic load in order to develop the disease. A woman with AS clearly carries sufficient genetic susceptibility factors for disease expression. This could explain why both her male and female children will have an equal chance of developing disease. By contrast daughters of AS males may not inherit enough genetic material to present with clinical symptoms.

2. A uterine effect: In general, the overall sex ratio for AS is 2.5 :1 (M:F). However, eighty percent of these individuals have sporadic disease. For those with familial disease, a similar distribution is observed among the offspring of AS fathers. By contrast, the sex ratio for offspring of maternal cases is equal. Moreover, children of AS mothers have a higher risk of AS than do children of men. AS mothers may pass an environmental disease inducing factor across the placenta, given the intimate contact the child has with the mother for nine months. Such a uterine effect (ie. environmental overlay) may enhance the risk of disease susceptibility in children who would not normally have developed AS. It remains possible that this phenomenon is particularly relevant for inducing disease in female offspring. Women with a young age at onset may have a greater genetic susceptibility load than

women developing the disease later in life. Therefore, children exposed to a uterine effect and carrying this larger genetic susceptibility factor would have an even higher prevalence of disease. Indeed, in the small subset of women with a young age of onset, no less than 38% of children developed disease (ie approximately 70-80% of B27 positive subjects [see Table 17]). Nevertheless, we showed that for 28% of women the onset of AS had not occurred before the birth of the AS child. Indeed, there is no difference in numbers of AS children born before/after/during onset of AS among fathers and mothers. However, it remains unknown when the triggers for AS development is 'pulled' given there may be many years of latency between precipitation of disease and symptom onset.

3. Influence of cytoplasmic/mitochondrial DNA: The children of AS fathers inherit disease at the 'normal' rate of 2.5:1 (M:F). That mothers with AS have more diseased offspring than do men could be explained in part by additional non-nuclear cytoplasmic/mitrochondrial genetic material that is operative only in females. Sons and daughters of AS mothers inherit susceptibility to AS equally as they both receive maternal mitochondrial DNA.

4. Susceptibility genes on X or Y chromosome: The X chromosome may carry susceptibility or protective genes and influence disease. Regardless of B27 status the sons of young disease onset AS mothers have a 50% chance of developing the condition. Possibly, the healthy 50% are those who have not inherited their mother's B27. Of those with the antigen, there may be closer to 100% penetrance of disease. These sons have all inherited their mothers X chromosome. Daughters who develop disease may receive an X chromosome from their healthy father that carries the same recessive susceptibility gene. However, with an X-linked disease the daughters of affected fathers should have a higher risk than sons but our data do not support this finding.

Alternatively if the Y chromosome was involved this would mean that some of the susceptibility to AS may come from the Y chromosome. Therefore males carrying this

factor have an extra susceptibility gene which can not be inherited by women. The sons of males would be more likely to develop disease and the children of affected females would be equally likely to develop disease. This suggestion is supported by the finding that in mice the Y chromosome has been shown to interact with the HLA B27 gene [185] . Peptides encoded by the Y chromosome are believed to be presented by the HLA B27 gene.

Environmental and genetic factors are required in disease pathogenesis [186] . No doubt genes impact on both susceptibility and severity and may influence onset and course of disease by way of negative or positive effects (protective genes). Both HLA and non-HLA genes are relevant. Herein, we demonstrate an important influence of the sex of the index case and suggest that additional factors are operative. These could include a uterine (environmental) effect and an influence from either or both the X and Y chromosomes. Meanwhile, the role of protective genes, particularly in the female, and the inter-relationship between such putative genes and sex remains unknown.

Figure 21

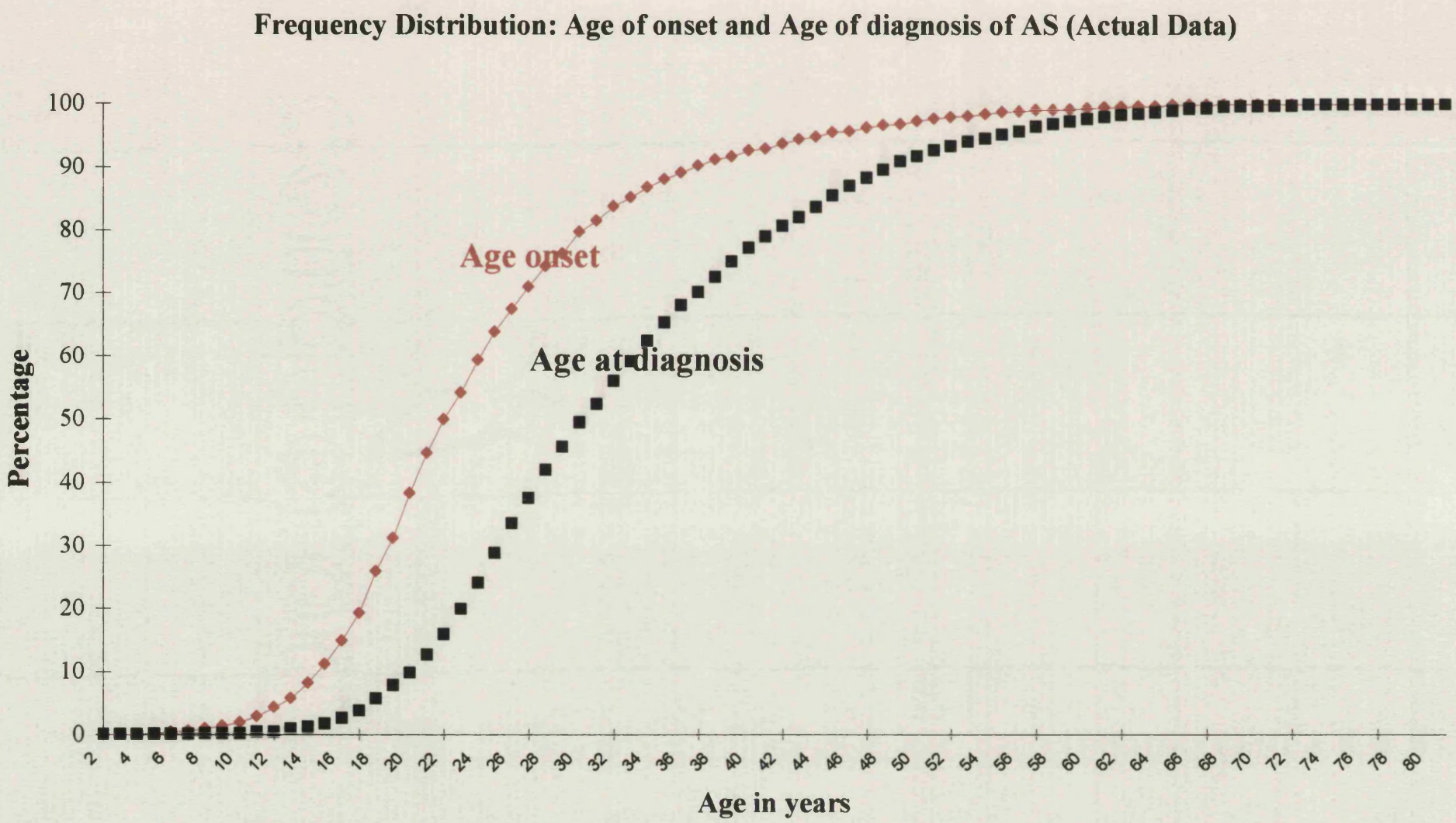

Frequency Distribution: Age of onset and Age of diagnosis of AS (Actual Data)
Age onset
Age at diagnosis
Percentage
100
90
80
70
60
50
40
30
20
10
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80
Age in years

Table 15: Inheritance of AS among sons and daughters of AS patients.

Index case	N	Sons	Daughters	Rate of inheritance (sons vs daughters)		Total	Rate of inheritance (father vs mother)		Ratio (M/F)
				Odds ratio (95% CI)	p		Odds ratio (95% CI)	p	
For children > 30 years (50% diagnosis)									
Father	354	35/396 (8.8%)	13/376 (3.5%)	2.6 (1.4-5.2)	0.003	48/772 (6.2%)	..	..	2.5
Mother	173	23/185(12.4%)	17/190 (8.9%)	1.4 (0.7-2.8)	0.3	40/375 (10.7%)	1.9 (1.2-3.0)	0.005	1.4
For children > 39 years (70% diagnosis)									
Father	101	11/90 (12%)	5/92 (5%)	2.4 (0.8-7.2)	0.17	16/182 (8.8%)	..	..	2.25
Mother	53	9/45 (20%)	7/48 (15%)	1.5 (0.5-4.3)	0.6	16/93 (17%)	2.1 (1.0-4.5)	0.063	1.4
Age of onset ≤21years and children >30 years									
Father	90	11/105 (10%)	4/99 (4%)	2.8 (0.9-9.0)	0.1	15/204 (7.3%)	..	..	2.6
Mother	41	7/49 (14%)	6/44 (14%)	1.0(0.3-3.4)	1.0	13/93 (14%)	2.0 (0.9-4.5)	0.086	1
Age of onset ≤ 21 years and children >39 years									
Father	22	2/20 (10%)	1/19 (5%)	2.0(0.2-2.4)	0.58	3/39 (8%)	..	..	1.9
Mother	9	4/7 (57%)	2/9 (22%)	4.7 (0.5-40.4)	0.3	6/16 (38%)	7.2 (1.5-34)	0.013	2.6

Table 16: Inheritance of AS among brothers and sisters of patients.

Index case	n	Brothers	Sisters	Rate of inheritance (brothers vs sisters)		Total	Rate of inheritance (father vs mother)		Ratio M/F
				Odds ratio (95% CI)	p		Odds ratio (95% CI)	p	
Siblings >39 years									
Male	1615	153/168 (9.46%)	90/1559 (5.77%)	1.7 (1.3-2.2)	<0.0001	243/3177 (7.65%)	..	..	1.6
Female	607	85/673(12.6%)	57/613 (9.3%)	1.4 (1.0-2.0)	0.62	142/1286 (11.04%)	1.5 (1.2-1.9)	0.0001	1.4
Age of onset ≤21 and siblings >39 years									
Male	598	76/564 (13.5%)	40/594 (6.7%)	2.2 (1.4-3.2)	<0.0001	116/1158 (10.02%)	..	..	2.0
Female	220	43/260 (16.5%)	21/201 (10.4%)	1.7 (1.0-3.0)	0.07	64/461 (13.9%)	1.5 (1.0-2.0)	0.029	1.6

Table 17: Data and extrapolation in inheritance of ankylosing spondylitis to children.

	Father to son	Father to daughter	Mother to son	Mother to daughter
Actual data				
50% diagnosis *	9%	4*	12%	9%
75% diagnosis +	12%	5%	20%	15%
Extrapolated data				
100% diagnosis Ξ	~15%	~6%	~30%	~20%
HLA-B27 positive Φ	~30%	~12%	~60%	~40%

* Age at which 50% of patients are assumed to have received a diagnosis of ankylosing spondylitis. + Age at which 75% of patients are assumed to have received a diagnosis of ankylosing spondylitis. Ξ Percentage of sons or daughters who will develop ankylosing spondylitis eventually based on linear extrapolation from actual data of 50% and 75%. Φ 100% diagnosis based on HLA-B27 positive and negative cases; 100% diagnosis underestimates the value of HLA-B27 positive children by 50%.

(e) The interrelationship between sex, susceptibility factors and outcome in ankylosing spondylitis.

Objective: Men and women are affected with spondylitis differently. A greater number of men develop the disease (2.5:1 Males: Females) and they develop more severe spinal disease [126] . Women have more disease activity (pain, fatigue, discomfort) [187] and the children of female patients get the disease more frequently than children of male patients [188] . Also, children of mothers with ankylosing spondylitis with an onset of disease at a younger age get ankylosing spondylitis more frequently than children of mothers with later onset of disease [188] . One explanation for this is due to a genetic load effect. In general, women are less likely than men to develop ankylosing spondylitis. Thus, it remains possible that women with ankylosing spondylitis have to have a higher genetic load to develop disease. This higher genetic load (ie susceptibility genes) is passed on to the children causing them to develop spondylitis. Alternatively, factors like uterine environment and breast feeding may impact on susceptibility to disease.

This study is based on the hypothesis that women need a higher susceptibility gene load to develop the condition. The son of a father with disease may inherit enough genes to predispose a man to the disease, but any daughter of a father with disease who does not inherit enough susceptibility factors will not suffer from AS. [Figure 22 (a)].

Any female who develops disease must by definition carry enough susceptibility factors to show clinical symptoms of disease. [Figure 22 (b)]. This means that although mothers carry more susceptibility genes, the daughter of a man with disease must be comparable to the daughter of a women with disease in terms of genetic susceptibility load because by definition both daughters must carry enough genetic load to predispose them to develop disease.

This study will examine further evidence that families where the mother has disease carry more heritable factors (as opposed to environmental factors influencing susceptibility such

as breast milk of uterine environment) than families where the father has AS. It will examine the effect of maternal inheritance of disease on severity (as measured by disease activity, function and radiology) as opposed to paternal transmission.

Methods: All patients with a family history were selected from the database and a confirmation of the diagnosis was sought from the GP for all these subjects. Only those patients with a confirmed diagnosis were used within the study.

1. Susceptibility : The number of affected second generation relatives (ie grandparents, aunt/uncle) of AS children with an AS affected mother was calculated and compared to number of affected relatives for AS children with and AS affected father. This analysis should highlight if mothers do carry more genetic factors related to susceptibility to ankylosing spondylitis.

2. Severity: (a) The outcome measures for children with an AS mother compared to an AS father were compared. Measures used were disease activity [BASDAI calculated on a 0-10 scale], function [BASFI calculated on a 0-10 scale] and radiology [BASRI calculated on a 2-16 scale].

(b) Sons of AS mothers compared to AS affected fathers for prevalence of secondary disease (iritis, psoriasis, inflammatory bowel disease [IBD]). This analysis was repeated for daughters of AS mothers compared to daughters of AS fathers.

Statistical methods: SPSS was used for all analysis. T-tests or χ^2 were used.

Results: There were 328 children of AS parents with a confirmed diagnosis of AS. Of these the B27 status was available on 44 (43/44 were positive) and SI joint radiographs were

requested and scored on 90 subjects, all were found to have evidence of sacroliitis of grade 2 or more.

1. Susceptibility: The offspring of women with disease had more additional AS relatives (grandparents, uncle/aunt) than the offspring of men with disease [p=0.012, odds ratio : 2.3 (1.2-4.5)]. There were 203 patients with an AS father and 125 patients with an AS mother, these cohorts were comparable for disease duration, age and sex [Table 18]. There were 25 (20%) of patients with an AS mother and 19 (9%) of patients with an AS father who had a previous family history. For children of AS mothers, among daughters there were 13/58 (22%) who had other relatives compared to 12/68 (18%) of sons. For children of AS fathers, 10/76 (13%) of daughters and 9/125 (7%) of sons had additional relatives. [Figure 23]

2. Severity: (a) The children of a mother with AS were comparable in terms of disease activity, function and radiology to the children of a father with AS [Table 18]. For children of AS mothers, among daughters the average disease activity and function was 4.3 (sd: 2.9) and 3.7 (sd:3.2), while among sons it was 3.5 (2.7) and 3.0 (3.4) respectively [Daughter compared to son - BASDAI: 0.8 (0.2-1.6) and BASFI: 0.7 (-0.2-1.8)] For children of AS fathers, among daughters the average disease activity and function was 4.9 (2.3) and 3.8 (2.7), while among sons it was 3.2 (3.3) and 2.8 (2.6) respectively [Daughter compared to son - BASDAI: 1.7(0.6-1.8) and BASFI: 1.0(0.3-1.8)]

(b).The prevalence of iritis is comparable between children of female AS patients compared to male patients [33%-39%]. However, inflammatory bowel disease is more prevalent among children of AS mothers [19/123(15%)] than AS fathers [10/196 (5%)] [p=0.009 OR: 2.9(1.3-6.3)]. Psoriasis is less prevalent among sons of AS mothers than among sons of AS fathers [Table 19] [p=0.03 OR 0.4(0.2-0.9)].

Of the 29 children with IBD the records of the parents were available in 14 (48%) cases. Only 1 of the 14 (7%) affected parents of IBD-AS children also suffered from inflammatory bowel disease. Of the 38 children with psoriasis and an AS father the records of the parent were available in 19 (50%) cases. There were 6/19 (32%) cases of psoriasis among the AS affected fathers.

Interpretation of study: The study examines 1. Susceptibility: whether the increased inheritance of AS among the children of affected mothers could be due to environmental factors (e.g. uterine environment or breast feeding) or whether there is evidence for a higher susceptibility gene load within these families.

2. Severity: the effect of maternal transmission of disease compared to paternal transmission.

However, the assumption in this study is that AS affected daughters of AS parents (either fathers or mothers with disease) carry comparable susceptibility loads. The sons of AS fathers should on the whole carry fewer susceptibility genes than sons of AS mothers [Figure 22(a)]. If the transmission of AS to sons/daughters from the father is 2:1 then we expect that 2 out of 3 sons of men carry fewer susceptibility genes than the sons of women.

Susceptibility: Families where the mother and child have disease do appear to carry more heritable factors predisposing the family to disease, than father-child families. Approximately 20% of patients with an AS mother compared to 10% of children of AS fathers, had second generation relatives with disease.

Impact of susceptibility factors on outcome: In terms of disease activity, function and radiology, there is no difference between disease transmitted from the maternal side as opposed to that from the paternal side. The level of susceptibility should be comparable between daughters of mothers with AS and fathers with AS. However, $^{2}/_{3}$ of the sons of

AS fathers should carry fewer susceptibility genes than son of AS mothers. The finding that AS sons inheriting disease from the maternal side do not differ from sons inheriting disease from the paternal side might suggest that susceptibility effects (predisposition to disease) do not impact on the outcome or severity of disease. That susceptibility factors are not associated with altered severity may be supported by findings that in the transgenic ank/ank mouse the susceptibility gene HLA-B27 plays no part in the phenotypic expression of ankylosis (ankylosing spondylitis) [189] . Among patients, HLA B27 is associated with younger age at onset but not with severity measures as determined by clinical and radiological parameters. [190]

Inflammatory bowel disease is inherited more by the children if the mother had AS. However, only 7% of the mothers traced of AS-IBD patients had concomitant bowel disease. Psoriasis was inherited by the sons of a father with AS more than a mother. Among patients with psoriasis it has been previously shown that paternal transmission of PsA is higher than maternal transmission [191] [192]. In this case, 32% of the fathers also had psoriasis. The sex ratio in uncomplicated psoriasis and uncomplicated inflammatory bowel disease is virtually the same [193] . However, more women with AS have bowel disease than expected and more men with AS have psoriasis. Among this sample of patients women do not appear to be protected from psoriasis (as 15%-17% have the disease) but male offspring of male AS patients appear more at risk, simply because the father may be more likely to have the condition. However, offspring of women with AS do develop more IBD even when the mothers do not seem to suffer from the condition. Iritis is inherited equally from mother and father, this may be because this disorder is strongly linked to the HLA B27 gene and most subjects with AS are carrying this gene.

For women to develop AS some of the susceptibly genes they need may over lap with those for inflammatory bowel disease. These genes are passed on the children. Thus, women appear to carry more of these occult or expressed bowel disease genes.

Therefore, the number of susceptibility genes inherited by the children are very strongly linked to the sex of the parent. Women carry a higher susceptibility load and this load contains factors linked to inflammatory bowel disease. There is a male sex effect on susceptibility to psoriasis when AS is present. However, there is no evidence that susceptibility load has an effect on outcome and severity of disease (as measured by disease activity, function and radiology) or that outcome is influenced if the transmission is maternal as opposed to paternal.

In summary, 1. Families where the mother has AS contain more heritable factors than families with an AS father. 2. Disease transmitted from the maternal side does not differ from disease transmitted from the paternal side in male patients in terms of severity. (In terms of susceptibility however, disease among female patients may differ, as there are fewer susceptibility genes from the paternal side and daughters are less likely to develop the condition). 3. Offspring of AS women develop more inflammatory bowel disease and sons of male patients develop more psoriasis. Thus, there is a sex effect on inheritance of secondary conditions associated with AS. [Figure 23]

Table 18: Impact of sex on inheritance of disease expression

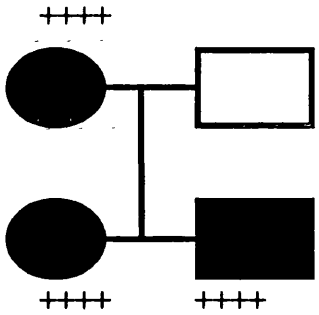
AS women	**with AS mother**	**with AS father**	**T test**
N=	57	76	
Disease duration	15.9 (+/-11.5)	16.2 (+/- 9.1)	0.3[-3.0-3.8] ns
Age	38.6 (+/- 11.3)	37.2 (+/-10.8)	1.4[-2.7-4.9] ns
Disease activity [BASDAI]	4.3 +/- 2.9	4.9 (+/-2.3)	0.7[-0.2-1.6] ns
Function [BASFI]	3.7 +/- 3.2	3.8 +/- 3.4	0.2[-0.9-1.4] ns
AS man	**with AS mother**	**with AS father**	
N=	68	127	
Disease duration	18.8 (+/-11.3)	15.9 (+/-9.9)	2.9 [-0.2-6.0] ns
Age	40.5 (+/- 12.7)	38.1 (+/- 11.0)	2.5 [-0.9-6.0] ns
Disease activity [BASDAI]	3.5 +/-2.7	3.2 +/- 3.3	0.3[-0.7-1.2] ns
Function [BASFI]	3.0 +/- 3.4	2.8 +/- 2.6	0.2[-0.6-1.1] ns
Patient	**with AS mother**	**with AS father**	
Radiology	n=22, dis.dur=15, age = 37	n=39, dis.dur=17, age =41	
[BASRI]	5.8	6.2	0.4[-1.6-2.6] ns

Table 19: Daughters and sons of AS affected mother and AS affected father compared for prevalence of secondary disease [iritis, psoriasis and inflammatory bowel disease].

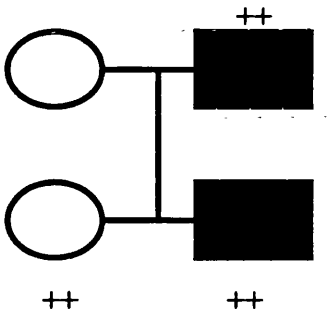
AS woman	**with AS mother**	**with AS father**	**Odds ratio**
Number	57	76	
Disease duration	15.9 (sd:11.5)	16.2 (sd 9.1)	
Age	38.6 (sd 11.3)	37.2 (10.8)	
Iritis	33% (19)	39% (30)	0.8 [0.4-1.6]
Psoriasis	15% (9)	17% (13)	0.8[0.3-2.1]
Inflammatory bowel disease	18% (10)	6% (4)	3.7 [1.1-12.6] p=0.0
AS man	**with AS mother**	**with AS father**	**Odds ratio**
Number	68	127	
Disease duration	18.8 (11.3)	15.9 (9.9)	
Age	40.5 (12.7)	38.1 (11)	
Iritis	36 % (24)	34%(43)	1.1[0.6-2.1]
Psoriasis	9% (6)	22% (28)	0.4[0.2-0.9] p=0.03
Inflammatory bowel disease	13% (9)	5% (6)	2.9[1.0-8.7] p=0.05

Figure 22: Inheritance of susceptibility factors [If women need a higher susceptibility load than men to develop AS]

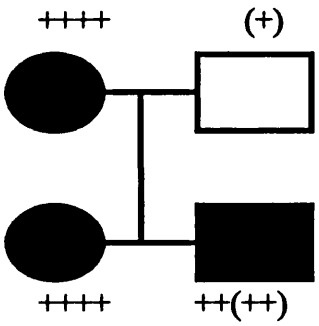
(a) AS mother carry enough susceptibility to pass on disease to son and daughter

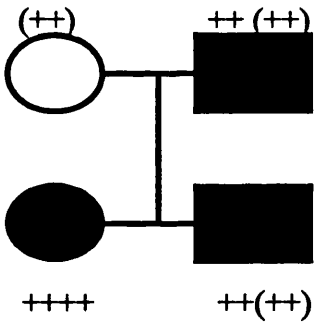


Many AS fathers do not carry enough susceptibility to pass on disease to daughters



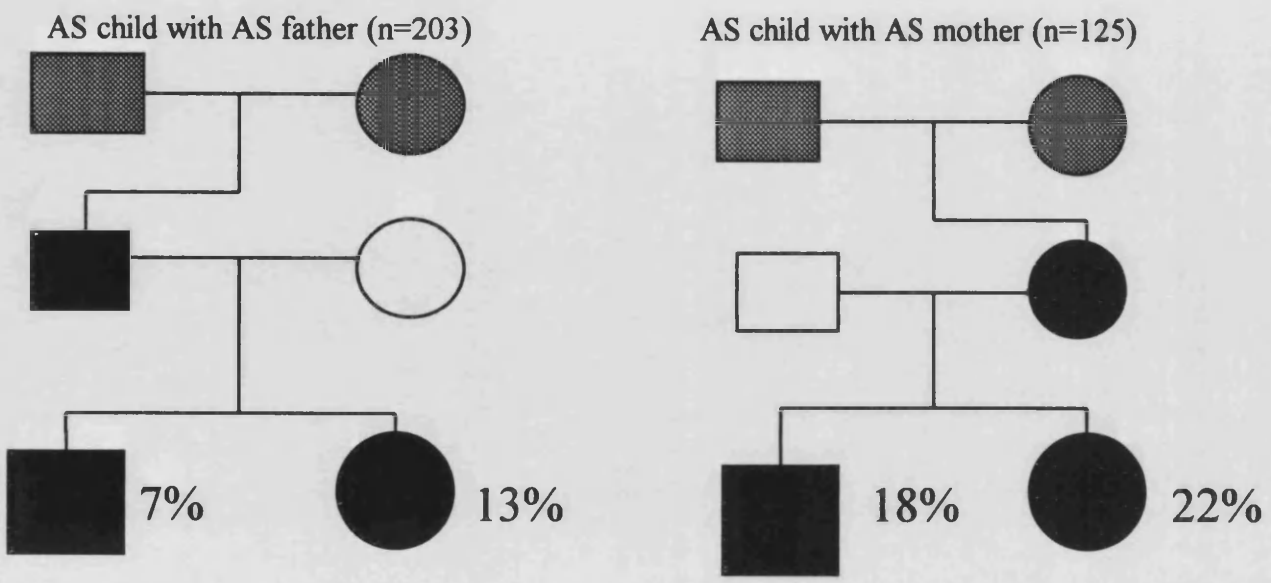
(b) Any female who does develop disease (whether inherited from the father or the mother) will carry enough (and equal) susceptibility factors to show disease.





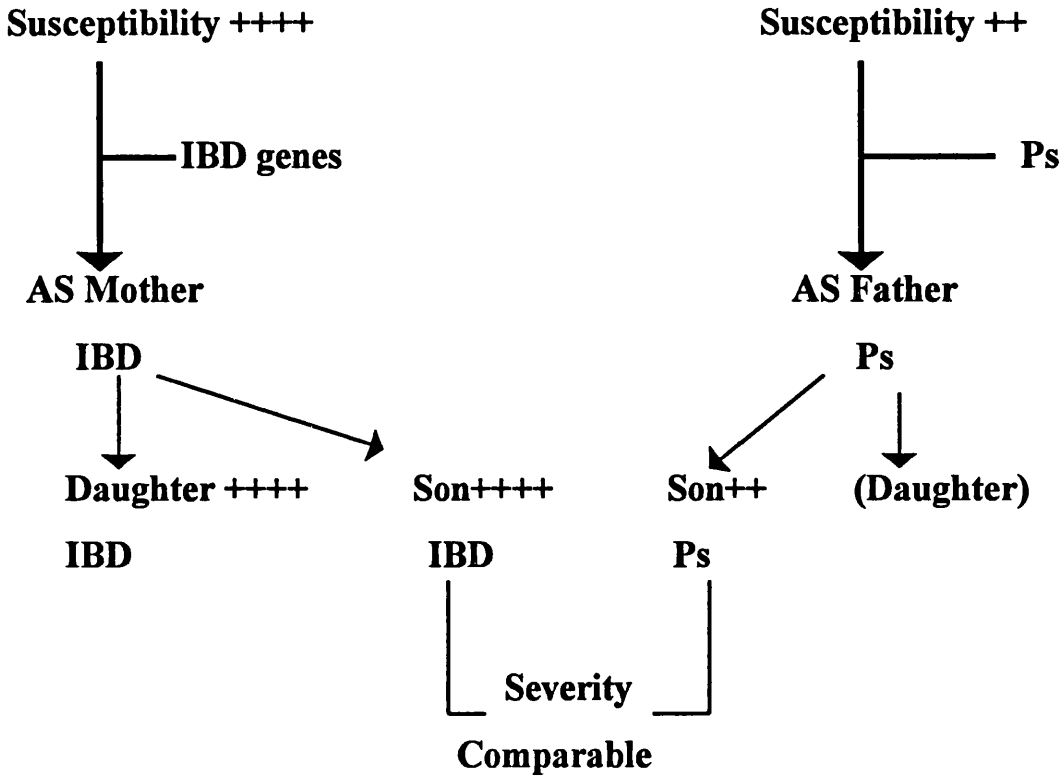
+++ = susceptibility factors **Male** **Female** **Affected**

Figure 23: Percentage of index children (mother/father with disease) with a second degree relative with AS.



Overall: 9% p=0.012 20%

Figure 24 :


Susceptibility ++++
IBD genes
AS Mother
IBD
Daughter ++++
IBD
Son++++
IBD
Susceptibility ++
Ps
AS Father
Ps
Son++
Ps
(Daughter)
Severity
Comparable

(f) Ankylosing spondylitis: Concomitance of disease among affected relatives

Objective Susceptibility to ankylosing spondylitis depends on two factors. Firstly, a genetic background involving many interlinking and overlapping genes and secondly factor is an external trigger [1]. However, little is known about factors which influence outcome in this condition. It can be expected both in the determination of susceptibility and severity that there will be a great deal of interaction both between the different genetic components as well as between the genes and environmental factors.

Previous work has suggested that women carry more susceptibility genes than men and these translate into a higher prevalence of disease among offspring of AS women [188]. Children of a women with disease are more likely to suffer from inflammatory bowel disease and sons of a man with disease are more likely to suffer from psoriasis. Thus, number of susceptibility genes inherited by the child is very strongly determined by the sex of the parent.

However, known susceptibility genes such as HLA B27 have not been found to impact on severity. In the transgenic ank/ank mouse the gene B27 plays no part in the phenotypic expression of ankylosis (ankylosing spondylitis) [189]. Among human subjects, HLA B27 is associated with younger age at onset but not with severity measures as determined by clinical and radiological parameters [190].It is possible that severity genes exist which are independent and separate from susceptibility genes. For example, polymorphism in the manganese super oxide dismutase (MnSOD) gene is not associated with susceptibility to RA but is associated with more severe radiological outcome [194].

This study aims to explore how genetic, sex and environmental factors interact to influence the disease course. 1. A comparison of the expression of disease in parent-child and sibling-sibling pairs may provide evidence of a genetic effect on severity. 2. A comparison of

familial and sporadic disease will further examine the effect of susceptibility factors (ie those carried by familial patients) on determining severity.

Methods The Bath Ankylosing Spondylitis Database consists of 5507 (2.5: 1 M:F) patients. All were out-patients of the Royal National Hospital for Rheumatic Diseases or were members of the National Ankylosing Spondylitis Society. Patients referred to the RNHRD had their diagnosis confirmed according to the New York Criteria. The NASS members are those who have received a positive diagnosis of ankylosing spondylitis from a specialist rheumatologist as a result of an x ray.

All patients with a family history were selected and a confirmation of the diagnosis was sought from the GP for all these subjects. Only those patients with a confirmed diagnosis were used within the study:

A. Parent / Child :i] Parent compared to child versus parent compared to control [There were 2 control groups : 1 A **Familial** control ie the affected child of another AS-parent 2. A **Sporadic** control ie a patient with no family history].

A matched pair t-test was carried out on : The absolute difference between each parent and child versus the absolute difference between each parent and control .

1) Parent compared to child versus Parent compared to **Familial** control (ie matched child of another AS parent)

2) Parent compared to child versus Parent compared to **Sporadic** control (ie match patient with no family history)

ii] Analysis of variance used univariate analysis and random effects first to establish the extent to which the child's disease expression can be predicted from the parental disease

level. Then, a model of factors affecting BASDAI and BASFI was established using multivariate analysis.

B Sibling/Sibling i] Sibling compared to Sibling versus Sibling compared to Control (1. **familial** control ie affected sibling of another AS-patients, 2. **sporadic** control ie patient with no family history)

A matched pair t-test was carried out on : The absolute difference between each patient and sibling versus the absolute difference between each patient and control.

ii] Analysis of variance using univariate analysis and random factors were used to establish the extent to which the patients disease expression can be predicted from the siblings disease level. Multivariable analysis was then used to examine factors influencing BASDAI and BASFI.

Matching was performed by identifying all possible matches [Sex, disease duration and age] and using a random number table to select the control.

Results: All subjects used had a confirmed diagnosis of ankylosing spondylitis from their GP [89 % of those typed (293/329) were B27 positive] :

1) i] Parent/child versus parent/control matched pairs: The level of disease activity and function was not closer between parent -child pairs than between parent - control pairs. [Table 20]. Also, same sex comparisons [mother-daughter & father-son] were not closer than opposite sex comparisons [mother-son & father-daughter] (Absolute difference between same sex : opposite sex - BASDAI : 2.2 : 2.7 p=0.1, BASFI : 3.1 : 3.5 p=0.3) .

The parent and child did however had comparable disease in terms of prevalence of iritis (p=0.005) and psoriais (p=0.01) [Of the 50% of parents with a history of iritis, [70/141] : 39% of their children also had iritis, as opposed to 29% of familial and 26% of sporadic controls]. Of the 22% of parents who had psoriasis (32/143); 19% of the children also had psoriasis as opposed to 9% (Fam and Spor) of controls]. Numbers with IBD were too small for analysis.

Finally, the child of the AS parent had lower disease activity [BASDAI ; p=0.005] and better function [BASFI ; p=0.001] than their sporadic matched control. [Table 21]

ii] Analysis of variance of child disease expression: The functional level [BASFI] of 142 patients was related to: their disease activity (p<0.001), educational level (p=0.004), presence of bowel disease (p=0.01) and age of patient (p=0.04). These variables together explain 64% of the variation seen in BASFI (p<0.001). [BASDAI alone explains 46% of the variation seen in BASFI and age, education and bowel disease account for 17% of the variation]. Factors which are not related to patients functional level was the functional level of the parent (with AS) (p=0.2), the disease activity level of the parent with disease (p=0.8), the sex of the patient (p=0.1), the family (p=0.3) [as a random effects variable, patients were not closer in severity to members of their family than to non-related individuals].

The level of disease activity [BASDAI] was not related to the level of disease activity in the parent (p=0.7), the functional level of the parent (p=0.5), the age of the patient (p=0.4), or sex of patient (p=0.12), or the family (p=0.08) [as a random effect]. However, it was affected by presence of bowel disease (p=0.041) and educational level (p=0.004). These two variables explain 15% of the variation seen in BASDAI (p<0.001).

2) i] Sibling - Sibling versus Sibling - Control : A patient is closer to their diseased sibling in disease activity level and function than to a control matched with their sibling for sex, age and disease duration [Table 22].

However, there was no difference between sibling-sibling and sibling-control in terms of inheritance of psoriasis or iritis (numbers were too small for bowel disease) [Iritis : 39.9% of probands (87/218) had iritis or these 47% (40/84) sibs and 43% (34/79) controls also had iritis. Psoriasis: 16.7% (36/215) of probands had psoriasis of these 18% (6/33) of sibs (5 Brother / brother, and 1 brother/sister) and 17.6 % of controls (6/34). IBD 9.9% (21/212) probands had IBD of these 9.5% (2/21) of the sibs and 5.6% (1/18) of the controls had IBD].

The sibling had a trend to lower disease activity [BASDAI : 3.8 versus 4.3 respectively; p=0.07 , BASFI : 3.8 versus 4.2; p= 0.1] (but not function) than their sporadic matched control. [Table 23]

b] Analysis of variance of disease expression level of siblings: The functional level of 204 subjects was related to: their siblings functional level (p=0.05 describing 8.8% of the variation seen in function), the patients disease activity level (p=0.001) and education (p<0.001). This model describes 50% of the variation seen in function (p<0.0001). Family (p=0.004) had an effect ie siblings are closer in severity than to individuals outside the family [random effects].

The disease activity level is related to the siblings disease activity level (p=0.05) and education (p<0.001). A model of these two variables describes 21.4% of the variation seen in BASDAI (p<0.0001). Siblings were closer in BASDAI [p=0.005] than to non related individuals [random effects].

Interpretation of study: This study explores the interaction between sex, environment and genetics in determining outcome in ankylosing spondylitis. It suggests that:

1. Severity between parent and child is not comparable.
2. Severity between affected siblings is comparable
3. Children suffer from the same secondary disorders as their parents (iritis and psoriasis) but not as their affected siblings.
4. Offspring of AS-parents have milder disease than sporadic AS cases.
5. Important determinants of disease activity [BASDAI] and function [BASFI] were - sibling severity measures, education and prevalence of secondary disorders.

Some areas of the study which may be improved in the future relate to measures of assessing severity. This study examined disease activity and function in patients but a radiological assessment of severity among relatives may provide more subjective results. However presently numbers of radiographs are too few to use. The use of radiographs may better examine the effect of sex on outcome as axial involvement is more marked among men with AS.

However, using disease activity and function as the measure of outcome suggests that there is evidence for severity genes in AS. These genes must be carried by both affected and non-affected parents. Therefore, severity genes in the affected parent are modified by the non-affected partner. Siblings can inherit the severity genes from both parents and therefore are more likely to have comparable disease.

Alternatively, it is also possible that severity is a factor of the environment and that siblings share more environmental variables (ie educational level) than perhaps offspring and their parents. Prevalence of iritis and psoriasis are clearly genetically determined outcome measures and these are inherited directly from the affected parent. However, severity could

be determined by a range of variables such as uterine environment, nutrition, childhood disease and education level which may be closer in siblings than parental-child relationships. However, some support that severity genes may play a role in this condition comes from the finding that offspring of a diseased parent have milder disease than sporadic controls. Patients with milder disease may be more able to stay on in education (hence higher level of education associated with milder BASDAI) and perhaps more willing have children. [Parents of AS children have milder disease than sporadic controls (Table 21)]. The children may inherit some of this milder expression of the disease. The difference between familial and sporadic disease is not as clear between siblings. Siblings resemble sporadic cases as they do not have a 'previous' generation family history. Thus, to some extent milder disease may be inherited from the parents.

An alternative explanation could be that offspring of parents with AS are more often diagnosed with AS even if symptoms are mild. Sporadic cases with mild symptoms may not be diagnosed. However, this hypothesis does not account for the lower BASDAI and BASFI scores among the parents of AS children.

Severity in terms of function and disease activity (pain, fatigue etc) may be in part genetically determined and may be influenced by heritable factors carried by the non-AS affected parent. Familial disease which should carry a higher load of susceptibility factors appears to be milder than sporadic disease. This might in part be explained be the hypothesis that the most severe 'sporadic' patients may choose not to have children and therefore do not have the opportunity to become 'familial disease' cases.

In summary, this study suggests that siblings have comparable disease activity and functional levels but this similarity is not seen between parents and children. Secondary disorders are inherited from the parents. Children of affected parents have milder disease than sporadic controls. These findings enhance our ability to determine outcome for the

individual (ie advice to patients that they will not resemble their parent but may follow the disease pattern of their sibling), and may provide evidence that severity genes are involved in this condition.

Table 21: Parent-child versus parent-control.

	Mean (sd)	
Disease activity [BASDAI]		
Difference in BASDAI between Parent compared to Child [n=150]	2.4 (1.8)	
Difference in BASDAI between parent and Familial control [n=137]	2.5 (1.9)	
Difference in BASDAI between parent and Sporadic Control [n=150]	2.6 (1.6)	
	Mean difference [C.I]	
Matched paired T-test of difference between parent and their child versus parent and matched control for their child	Familial : 0.1 [-0.3-0.5] Sporadic : 0.2 [-0.1-0.8]	Not signif.
Function [BASFI]		
Difference in BASFI between Parent compared to Child [n=150]	3.0 (2.2)	
Difference in BASFI between parent and Familial control [n=137]	3.3 (2.3)	
Difference in BASFI between parent and Sporadic Control [n=150]	3.1 (2.5)	
	Mean difference [C.I]	
Matched paired T-test of difference between parent and their child versus parent and matched control for their child	Familial : 0.3 [-0.3-0.9] Sporadic:.0.1[-0.4-0.6]	Not signif.

Table 22: Familial vs sporadic disease

	BASDAI (sd)	BASFI (sd)
Parent [n=195]	3.8 (2.3)	4.6 (2.7)
Sporadic match	4.3 (2.1)	5.2 (2.6)
(for parent)	p=0.034	p=0.021
Child [n=150]	3.6 (2.2)	2.9 (2.3)
Sporadic match	4.4 (2.3)	3.9 (2.5)
(for child)	p=0.004	p=0.001
Sibling [n=221]	3.8 (2.3)	3.7 (2.8)
Sporadic match	4.2 (2.3)	4.2 (2.6)
(for sibling)	p=0.072	p=0.1

Table 23: Sibling-Sibling vs Sibling - Control.

	Mean (sd)	
Disease activity [BASDAI]		
Difference in BASDAI between Sibling compared to Sibling [n=221]	2.2 (1.6)	
Difference in BASDAI between Sibling and Familial control [n=137]	2.7 (1.9)	
Difference in BASDAI between Sibling and Sporadic Control [n=150]	2.6 (2.0)	
	Mean difference [C.I]	
Matched paired T-test of difference between parent and	Familial : 0.5 [0.1-0.7]	p=0.008
their child versus parent and matched control for their child	Sporadic : 0.4 [0.1-0.7]	p=0.038
Function [BASFI]		
Difference in BASFI between Parent compared to Child [n=150]	2.6 (2.2)	
Difference in BASFI between parent and Familial control [n=137]	2.9 (2.3)	
Difference in BASFI between parent and Sporadic Control [n=150]	3.1 (2.3)	
	Mean difference [C.I]	
Matched paired T-test of difference between parent and	Familial : 0.3 [0.004-0.7]	p=0.05
their child versus parent and matched control for their child	Sporadic:.0.4[0.04-0.8]	p=0.045

(g) Identification of severity loci in ankylosing spondylitis:

Objective: Little is known about the genetic control of disease severity in ankylosing spondylitis. Greater similarity of disease severity is observed in monozygotic compared to dizygotic twins, although no individual severity measure achieved statistical significance [57]. In collaborative work with The Wellcome Trust Oxford [Appendix 9] a segregation analysis was performed to examine the best model describing the method of inheritance of severity in terms of disease activity (BASDAI) and function (BASFI). This analysis suggested that environmental factors play little role in determining disease severity, instead both BASDAI and BASFI were found to be highly familial. This segregation study suggests that a single recessive or codominat locus influences level of BASDAI and BASFI.

Thus, this study aims to perform a genome screen of autosomal factors (ie chromosomes 1-22 not examining sex chromosomes X and Y) in order to examine evidence for genetic severity loci. A whole genome screening has been performed for genetic susceptibility loci [Appendix 10] and this will be used to compare the overlap of possible susceptibility and severity loci.

In order to do this study, variance -components analysis will be performed, this assesses the relative contribution of random (environmental) factors, major genes and polygenes at individual marker locations to the overall trait variance being studied [ie to severity]. The major effect in the presence and absence of linkage are compared using the likelihood ratio test to provide a measure of linkage of the marker and the trait. Likelihood test ratio is the odds ratio that the area (locus with marker) is linked to severity vs the probability it is not linked. The LOD score (log of the odds ratio) gives the probability that they are linked (as opposed to chance result when not linked). Families are studied as single units resulting in significantly greater power.

Methods: All patients were seen by a qualified rheumatologist to confirm a diagnosis of AS. Four hundred and fifty six (456) had been previously genotyped (see appendix 10) of these 382 individuals from 184 families completed disease activity and functional measures (BASDAI, BASFI).

Statistical analysis: Multi-point non-parametric linkage analysis was performed using the ALL statistic of the program GENEHUNTER version 2.0 (Pratt et al 2000). This method should identify evidence of additive genetic variance (ie effect of individual alleles on BASDAI and BASFI). Two-point parametric analysis was then performed assuming a 1. co-dominant 2. dominant or 3. recessive interaction between alleles using the LINKAGE CONTROL PACKAGE. The parameters used (ie standard deviation for genotype means, frequency of major gene alleles etc) were taken from the estimates derived using the segregation analysis (appendix 10).

Results: Of the 382 individuals studied, 246 were male and 136 were female (M:F 1.8:1). Average disease duration was 24.3 years (sd: 11.7) and age of onset was 22.7 years (sd 7.8 years).

Disease Activity [BASDAI]: 'Significant' Linkage (>3.6, ie expected to occur once by chance per 20 whole genome screens) was found using multipoint linkage analysis at position 18cM from the p-telomere on chromosome 18p [LOD score =4.3].

'Suggestive' linkage (≥ 2.2 , ie expected to occur once by chance per whole genome screen) was observed in 21 markers on chromosome 18 (ie markers in position 111.9 -123.9 cM all had LOD scores of 2.2-2.6] using non-parametric analysis and on one marker in Chromosome 20 (position 96.5 cM) and one marker on chromosome 21 (position 8.6 cM) using parametric analysis. LOD scores of >1.5 were observed on chromosomes 6 [LOD = 1.7 (parametric), LOD = 1.4 (non-parametric) at position 129.8 cM] , chromosome 16

[LOD = 1.6 (parametric) at positions 60 cM and 76 cM]. All chromosomes are represented in Figure 25.

Function [BASFI] : LOD scores ≥ 2.2 was observed in one marker on chromosome 2 [position 248.3 cM (parametric)] and one marker on chromosome 13 [position 7.4 cM (parametric)]. LOD scores of >1.5 were observed on chromosomes 8 [5 markers at positions 8.4 - 18 cM], chromosome 9 [one marker at position 17.5 cM (parametric)], chromosome 11 [23 markers at positions 132.24 - 139.86 cM and 143.04 - 149.8 cM (non-parametric) and at position 71.1 (parametric)], and chromosome 18 [one marker at 17.65 cM (parametric)]. Figure 26.

Interpretation of study:

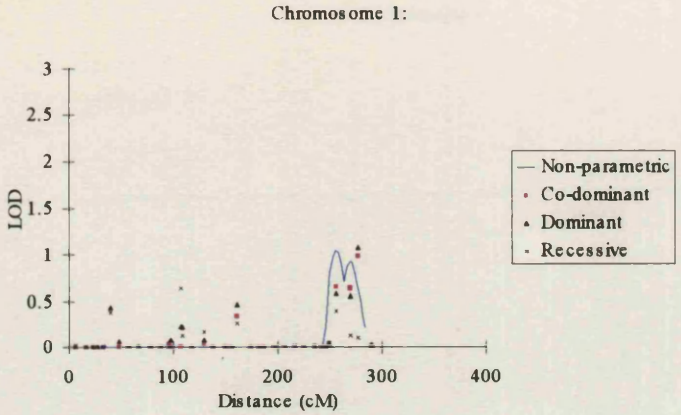
This study provides strong evidence supporting the existence of genes involved in severity of AS, and points to their likely location. One region on chromosome 18 was found to be significantly linked to disease activity and regions on chromosomes 18,20,21 & 2, 13, 11 were identified as having suggestive linkage.

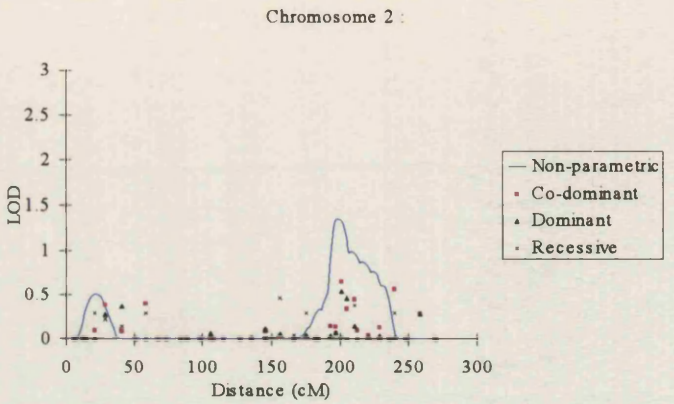
The lack of linkage of the MHC region on chromosome 6 with severity supports the hypothesis that susceptibility genes and severity genes are independent and separate. However, the LOD score of 1.2 at position 100.66 cM on chromosome 16 is in agreement with the susceptibility genome screen (appendix 10) which found a LOD score of 4.7 at 101cM on chromosome 16. This region may be involved in both susceptibility and severity.

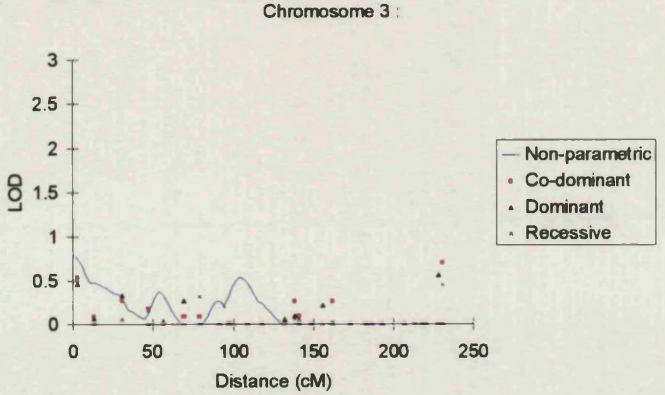
No other genome-wide scans have been performed in severity aspects of disease. A genome screen for loci involved in controlling normal variation in bone mineral density and osteoporosis identified chromosome 11 (position 12-13) as important in linkage. However, this is not the same region as identified on chromosome 11 as affecting BASFI in AS.

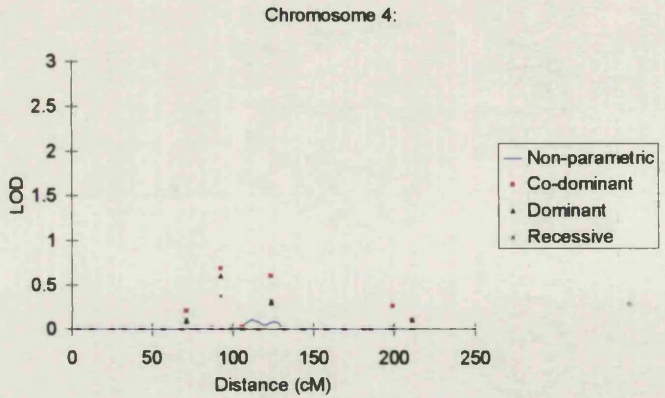
Thus, there is clear evidence for a genetic influence on severity of AS.

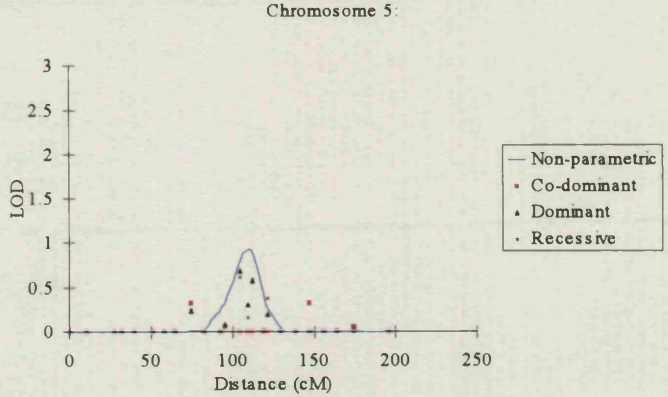
Figure 24 : Loci on chromosomes 1-22 associated with disease activity

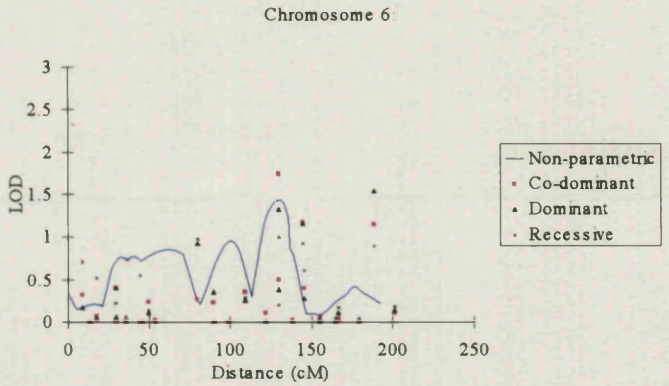




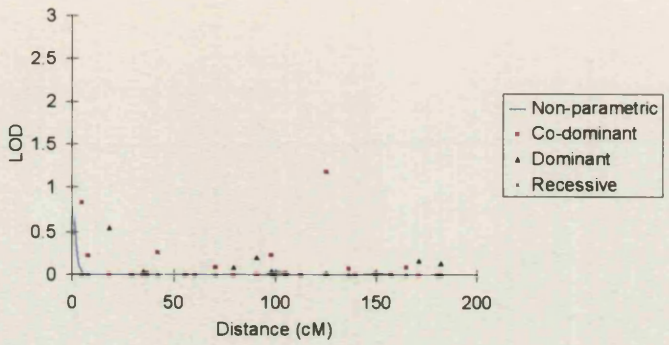




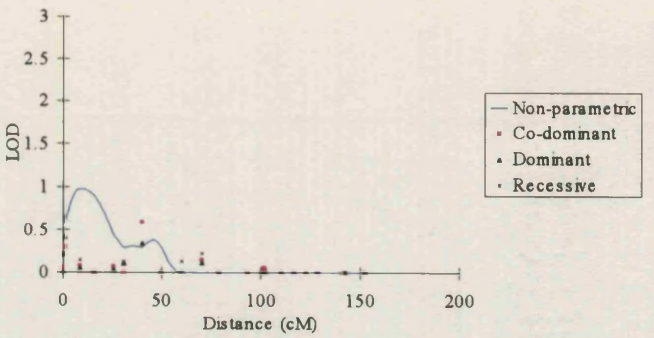




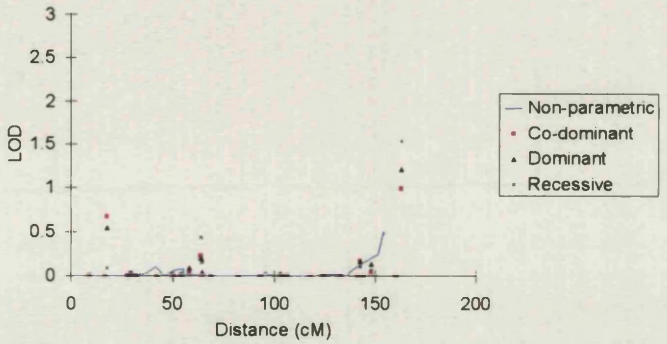
Chromosome 7 :



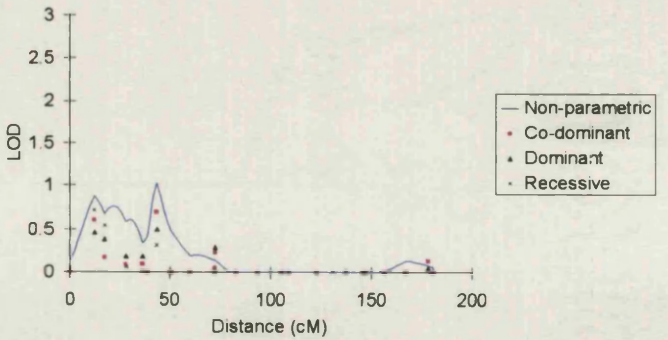
Chromosome 8 :



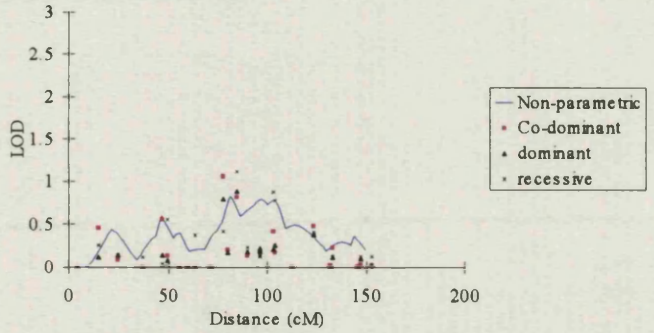
Chromosome 9 :



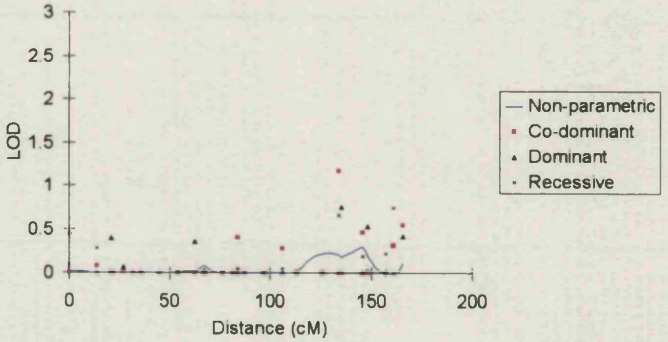
Chromosome 10 :



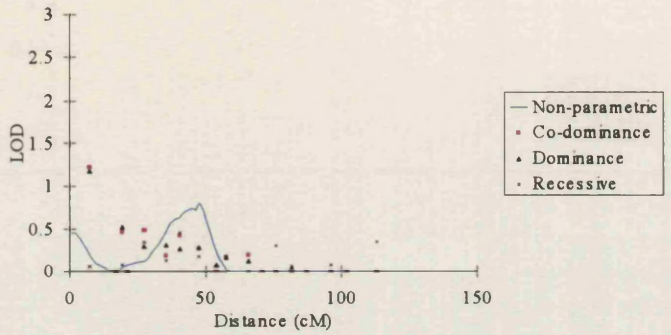
Chromosome 11 :



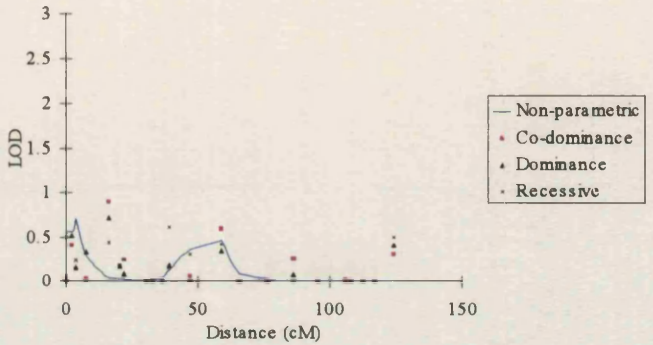
Chromosome 12 :



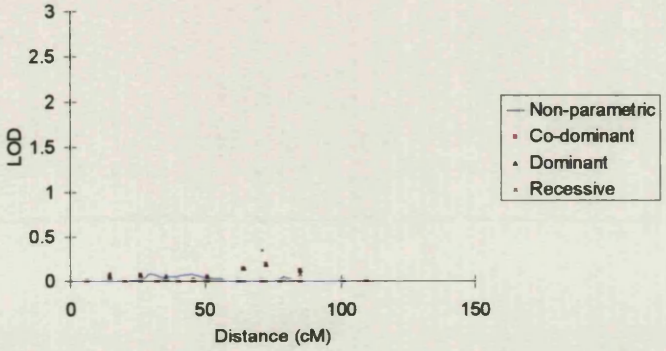
Chromosome 13 :



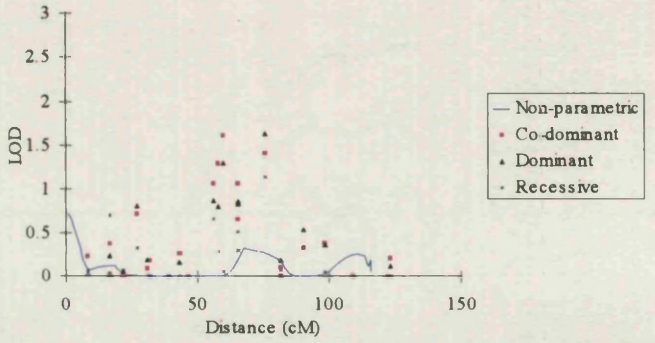
Chromosome 14 :



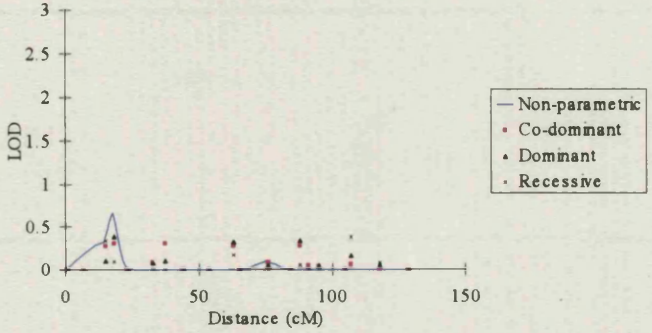
Chromosome 15 :



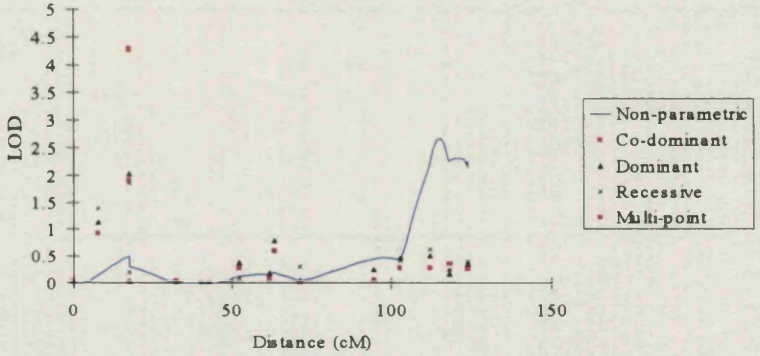
Chromosome 16 :



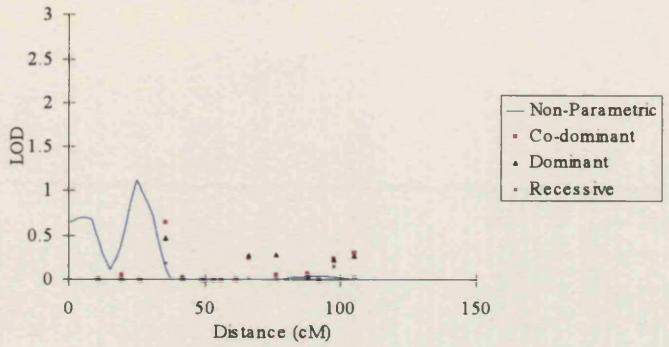
Chromosome 17 :



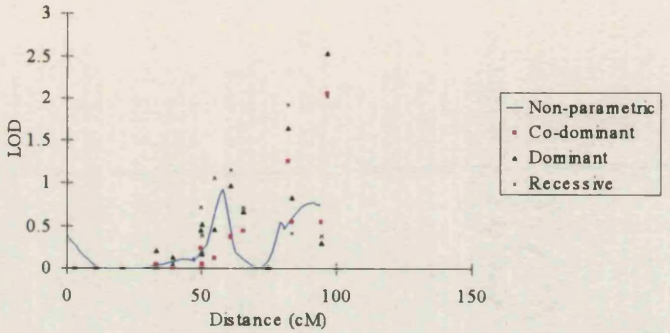
Chromosome 18 :



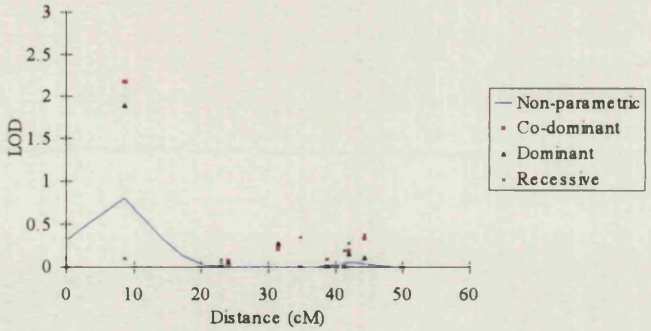
Chromosome 19 :



Chromosome 20 :



Chromosome 21 :



Chromosome 22 :

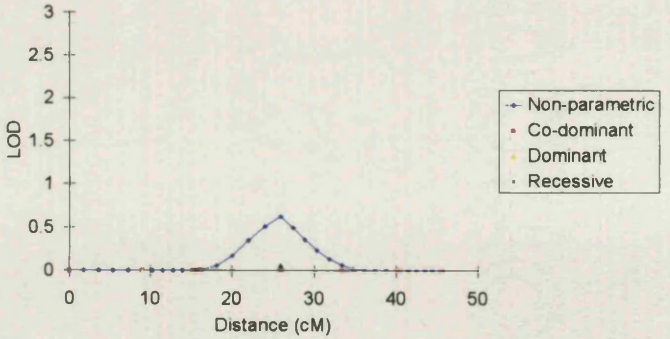
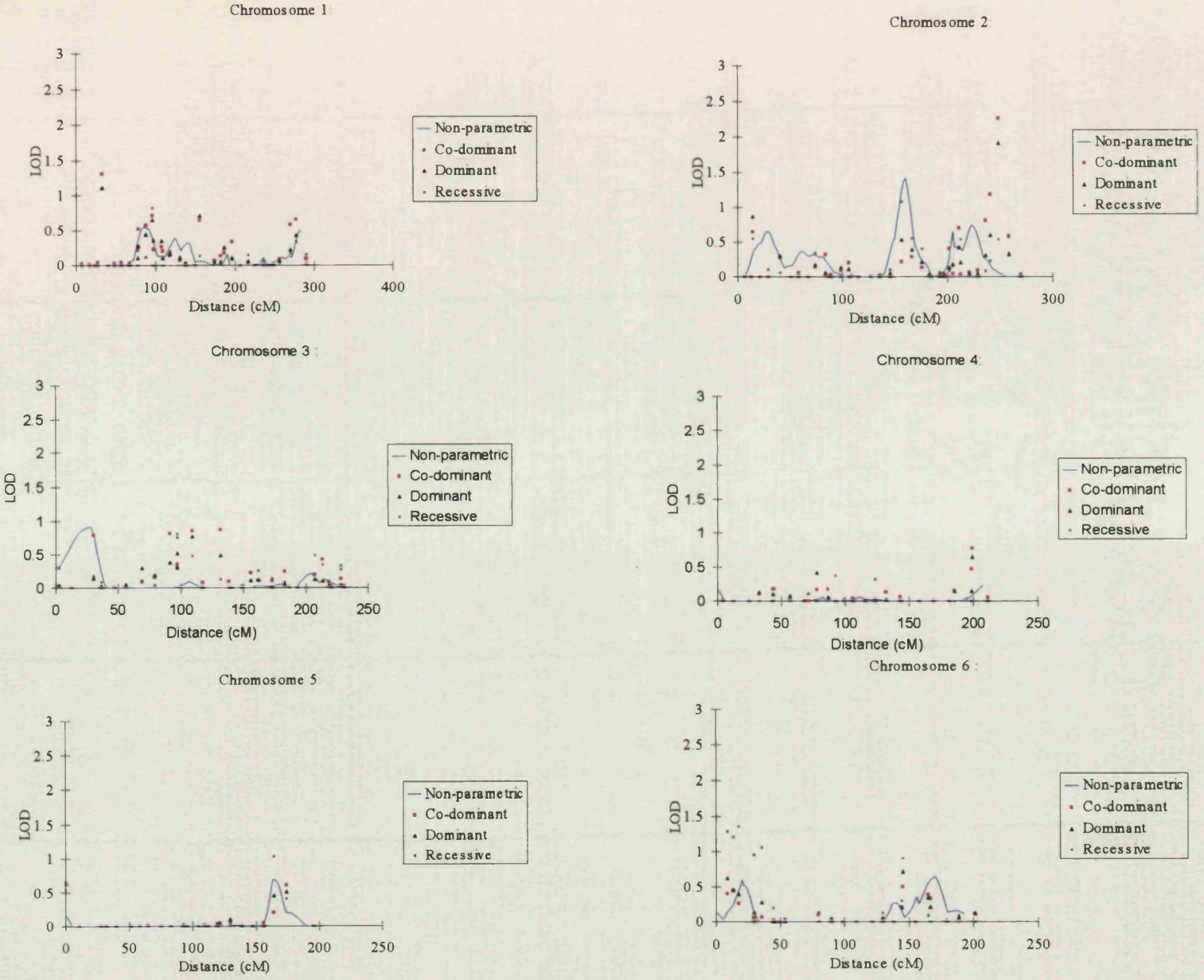
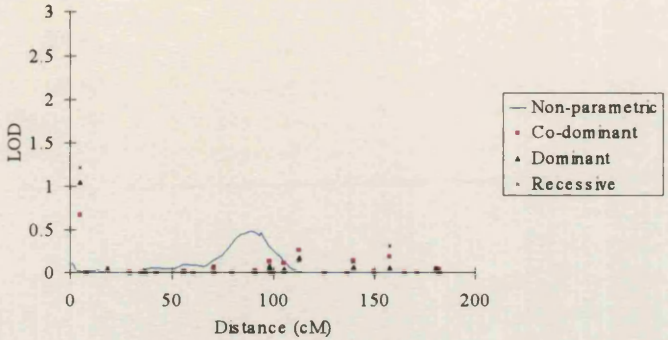


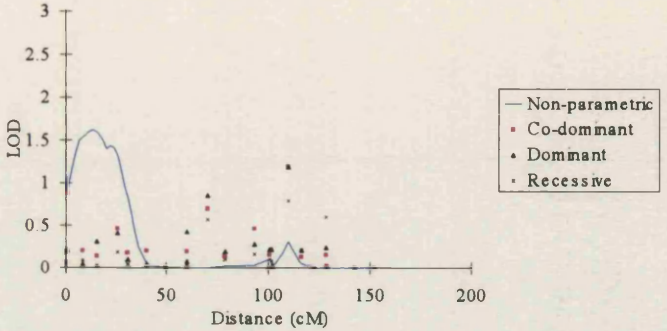
Figure 25 : Loci on chromosomes 1-22 associated with function



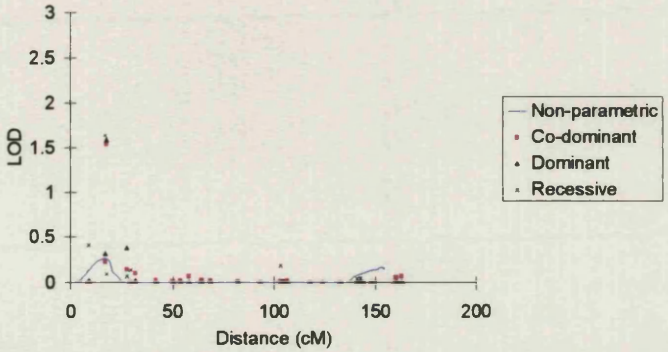
Chromsome 7 :



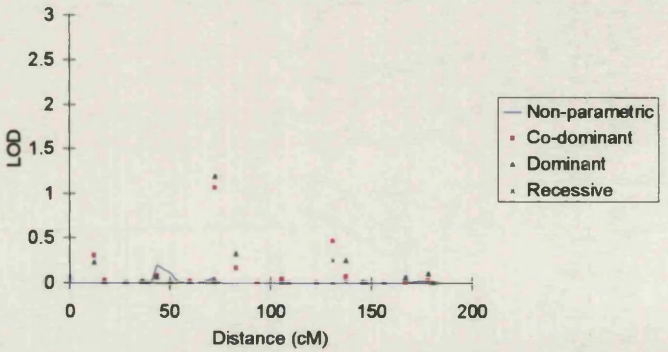
Chromosome 8:



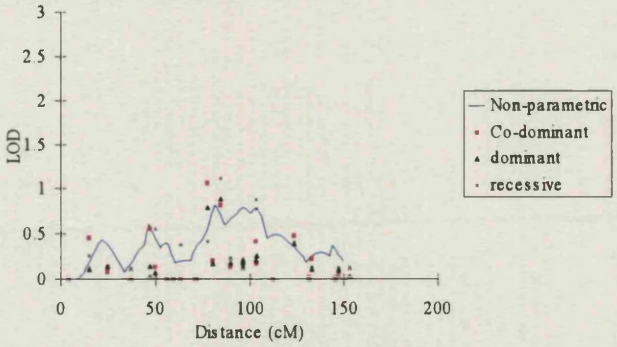
Chromsome 9 :



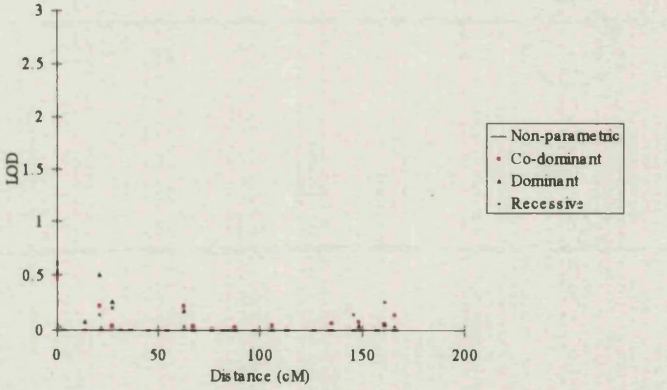
Chromsome 10 :



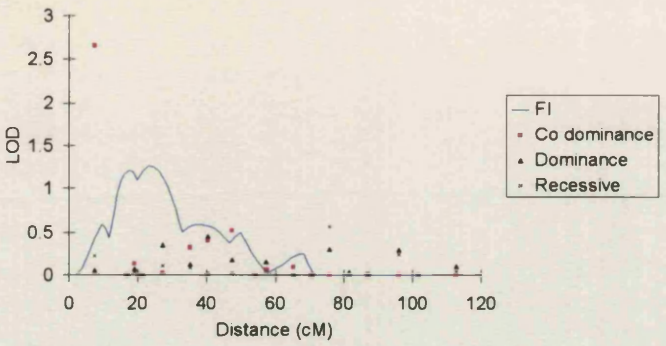
Chromosome 11 :



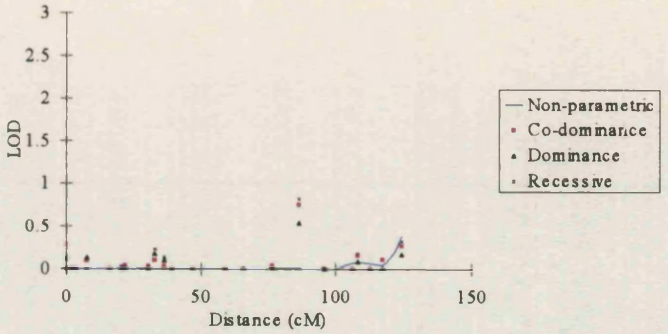
Chromosome 12 :



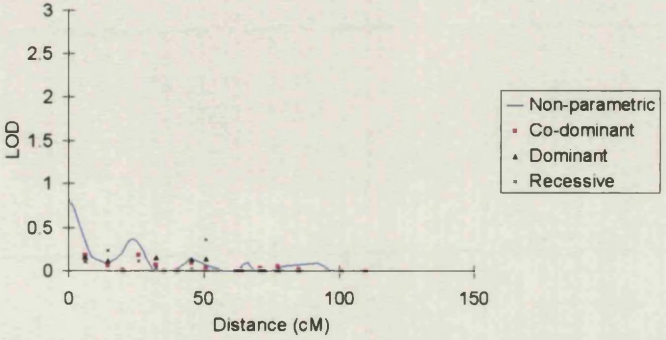
Chromosome 13 :



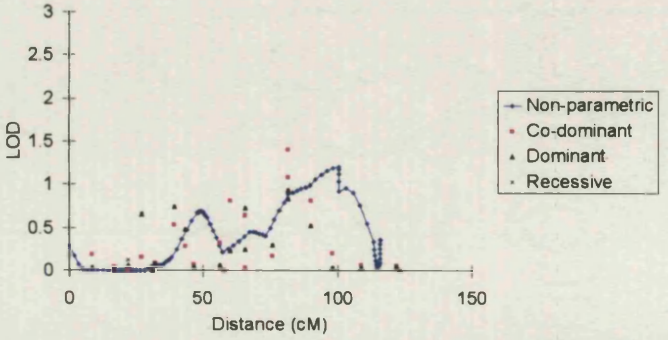
Chromosome 14 :



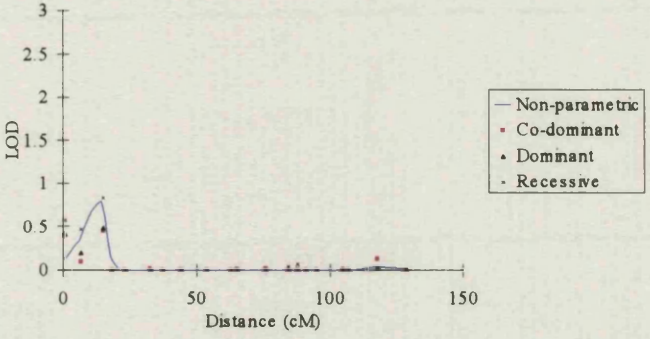
Chromosome 15 :



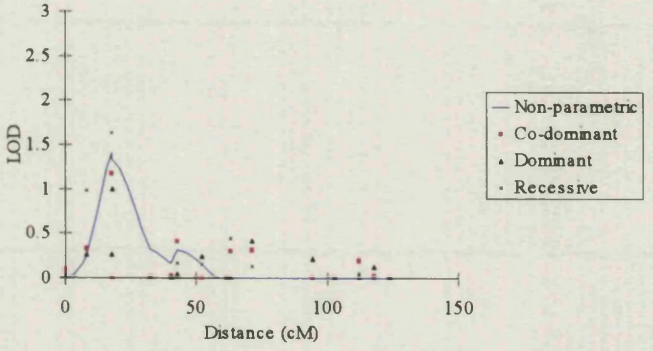
Chromosome 16 :



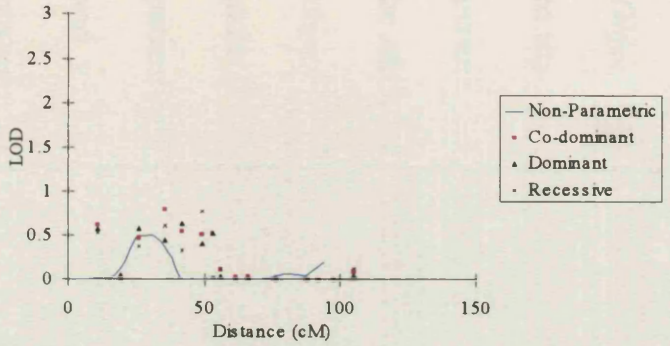
Chromosome 17 :



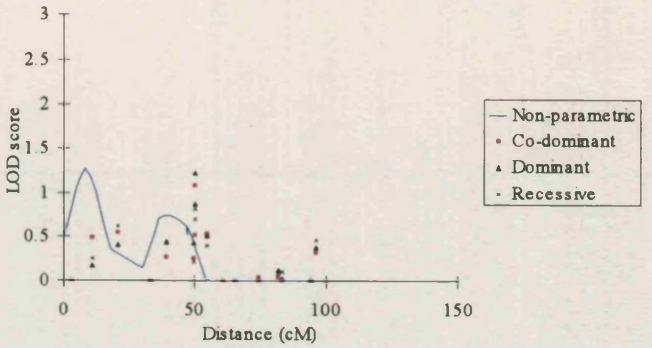
Chromosome 18 :



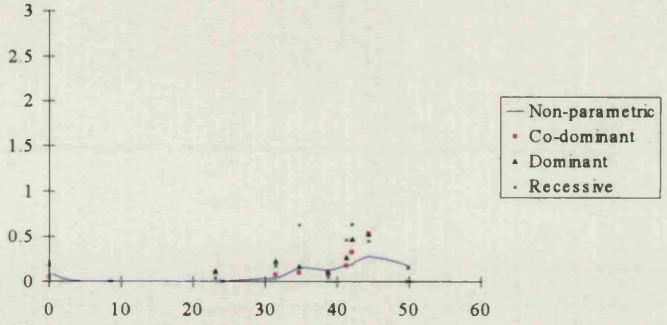
Chromosome 19 :



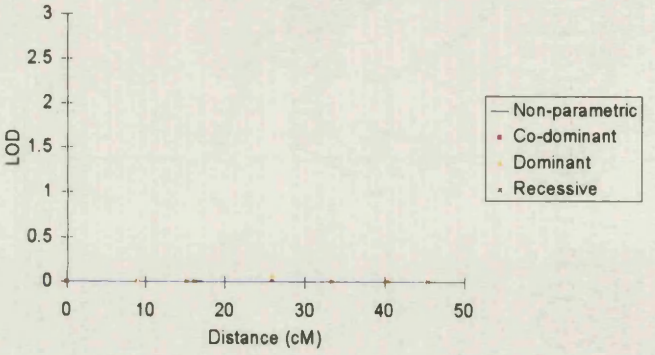
Chromosome 20 :



Chromosome 21 : FI



Chromosome 22 :



(b) The influence of environmental factors:

Identical twins both suffer from ankylosing spondylitis in only 70 % of cases [57]. Thus, 30% of pairs are not concordent for disease even though the genetic load carried by both twins is comparable. This means that the environment must play role in susceptibility to spondylitis. However, very little is known about the role of environmental factors in determining outcome and seveirty of disease. Herein, the focus is on treatments for AS, specifically (a) exercise and (b) physiotherapy in order to evaluate their effectiveness as long term modifiers of disease development. (c) Entry variables such as clinical measures and demographic details are analysised to see if they can be used to help predict outcome in terms of radiological change and functional ability. Finally (d) to evaluate if birth order has an effect in AS as has been reported in mice [195].

(a)Exercise in Ankylosing Spondylitis : How much is optimum ?

Objective : Drug therapy for symptom control in patients with AS has largely been confined to the use of NSAIDs and second line agents like sulfasalazine, which have some activity on peripheral joints but less effect on the spine itself. Although drug therapy with NSAID can be effective, the cornerstone of medical management is therapeutic exercise with the prime objective of maintaining normal posture and activity. This is now an essential part of a patients' treatment [196]. Many kinds of physical therapy can be considered for AS patients: supervised individual therapy, unsupervised self-administered individual exercise, and supervised group therapy [197] . Short-term improvement, following in-patient and out-patient treatment regimes, has been demonstrated in a number of centres [118][198] [199-207]. There remains some doubt whether this improvement is sustained in the longer

term. In addition there is the bias that the act of follow-up may create the Hawthorne Effect [208] encouraging patients to do more exercise.

This study aimed to evaluated how much exercise patients with ankylosing spondylitis (AS) perform on a regular basis, to analyse demographic and clinical variables that might influence adherence to exercise, and to determine the effect of exercise on disease activity and function.

Methods : Study Population

A self-administered questionnaire regarding physical exercise was completed by 4282 patients (M:F = 3057:1221) Patients were asked "For how many hours per week on average have you taken part in sports, AS exercises or hydrotherapy during the last three months ?". Patients were given five options: 0 hours (Group 0), 1 hour (Group I), 2-4 hours (Group II), 5-9 hours (Group III), 10 or more hours (Group IV). Those who did not answer the question were excluded from the study (n = 83).

Data processing and statistical methods

To determine the relationship between physical exercise, demographic data and clinical variables, patients who exercised 2 to 4 hours and 10 hours were compared to the non-exercising group. Group II (2 to 4 hours exercise) was matched for sex, age and disease duration with Group 0 (n = 553). Group IV (10 or more hours exercise) was matched for sex, age and disease duration with Group 0 (n = 272). The matched pairs were analysed for demographic and clinical status. This included: marital status, employment status, education level, medical follow-up (rheumatologist or general practitioner), delay in diagnosis, medication/no medication and family history of AS. Beliefs in the benefits of exercise were assessed by asking the patients to record on a separate 10 cm visual analogue scale (VAS) the effectiveness which exercise has on reliving their symptoms. Clinical status was assessed by self-administered validated instruments: 1) The Bath Ankylosing Spondylitis Disease

Activity Index (BASDAI) 2) The Bath Ankylosing Spondylitis Functional Index (BASFI) 3) The Bath Ankylosing Spondylitis Global Score.

Statistical analysis was performed using the SPSS package. Paired t-tests or McNemar's chi-squared tests were used in analysis.

Results : In total, 915 (21%) patients were in Group 0, 836 (20%) patients were in Group I, 1491 (35%) were in Group II, 647 (15%) were in Group III and 393 (9%) patients were in Group IV (see Figures 25 and 26). There were proportionally more men in the higher exercise groups and more women in the lower exercise groups.

Group II (2 to 4 hours exercise):

Group II [M:F = 408:145 (ratio 2.8:1)] when compared to matched controls (n = 553 pairs) had improved function and lower disease activity ($p < 0.001$ and 0.015 respectively, Table 24). There was no difference in the global well-being of the two groups.

Delay in diagnosis, family history, marital and employment status were comparable (Table 25). A significantly greater number of patients from Group II were followed by a rheumatologist ($p < 0.001$) , had a higher education level ($p = 0.005$), and took more medication ($p < 0.001$). Controlling for medication, disease activity and function were still significantly better for group II ($p = 0.017$ and $p = 0.001$ respectively).

Group IV (10 or more hours exercise):

Group IV [M:F = 220:52 (ratio 4.2:1)] had improved functional status when compared to matched controls ($p = 0.033$, table 26). The delay in diagnosis was significantly greater in Group IV ($p = 0.003$), as was the number of non-married individuals ($p < 0.04$), and the number of patients followed by a rheumatologist ($p < 0.001$, table 27)

Employment, education level, family history , medication, disease activity and global well-being were comparable.

Interpertation of study: This study assessed physical exercise of patients with AS and explored the impact in terms of disease activity, function and well-being. It identifed factors associated with adherence to long-term exercise.

One potential defect of the study is the cross-sectional design allowing assessment only at one point.

The definition of exercise was very general, including sports, specific AS exercises and hydrotherapy. However the accuracy of the self-reported exercise frequency is hard to judge, and the quality of the exercise is unknown. Some of the patients reporting no exercise would have performed regular housework, walked short distances, and helped with all the usual household tasks. All these activities, although not strictly classified as formal exercise, are in themselves forms of exercise and patients would in fact be stretching, bending and lifting. However, all the groups would be performing these daily activities.

Most of the subjects reported either 2 to 4 hours of exercise per week (35%) or no exercise (21%). There were more men in Group IV and predominantly more women in Group 0.

Most of the research reported in the literature relating to exercise in AS has been carried out in Europe using in-patient programmes of 2-4 weeks [200], [202], [119],[204], [205], [206],[207]. The majority of these studies did not include functional measurements. However, they reported improvement in metrology and short-term benefits in symptom control . Those that used functional measures reported that after the in-patient programs those who continue exercising had improved function [180].

The present study found that individuals who do moderate daily exercise have lower disease activity and improved function. Patients who performed intensive daily exercise had improved function but the disease activity did not improve. This suggests an optimum exercise duration with most benefit being derived from moderate consistent exercise. This

finding is encouraging as the majority of the AS patients were already in this moderate group.

Exercise has been reported to improve well being [210]. However this study did not find an improvement in the patients global well-being with increased exercise. This was possibly a reflection of a difference in study design. Previous studies used an intensive course and assessed well being post treatment. Therefore there is a point of intervention against which patients can be assessed to improve or deteriorate. This study on the other hand asked patients for their subjective opinion of their day to day well being. Patients who exercise regularly will have a better function, but they may not report any improvement or change in global well-being.

It has been suggested that compliance to exercise may be influenced by unmarried status [211]. This study did not find marital status to influence regular moderate exercise, however the intensive exercise group were generally unmarried.

In our study, employment status and family history of AS did not influence adherence to exercise.

Adherence with a regular exercise regimen is associated with rheumatologist follow-up, beliefs in the benefits of exercise, and a higher education level (Degree, diploma, High National Diploma).

Rheumatologists may be more aware than general practitioners of the benefits of exercise and therefore encourage and motivate the patient in the importance of regular moderate exercise.

Patients who practice regular exercise had strong believe in its efficacy. This finding is supported by previous studies that suggest beliefs in the benefits of exercise would differentiate exercisers from non-exercisers [211].

Patients who performed moderate exercise had a higher education level. It has been suggested that higher occupational status, which is associated with higher education,

correlates with lower disease severity. Education may have an effect on an individual's sense of self-efficacy, encouraging the patients to play a more active role in the control and the treatment of their disease, by exercising, rather than relying purely on medical intervention [212].

Moderate, regular exercise is beneficial for both functional status and disease activity. However intensive long term exercise is not as valuable for function or disease activity. Therefore this study suggest there is an optimum to exercise. Consistency, not quantity is of most importance. Those individuals most likely to follow this regime attend a rheumatologist, believe that exercise is of benefit and are in the higher educated bracket. Therefore, the less educated and those followed by general practitioners, should be preferentially targeted.

Figure 25 - Distribution of the number of hours of exercise in the all cohort (%).

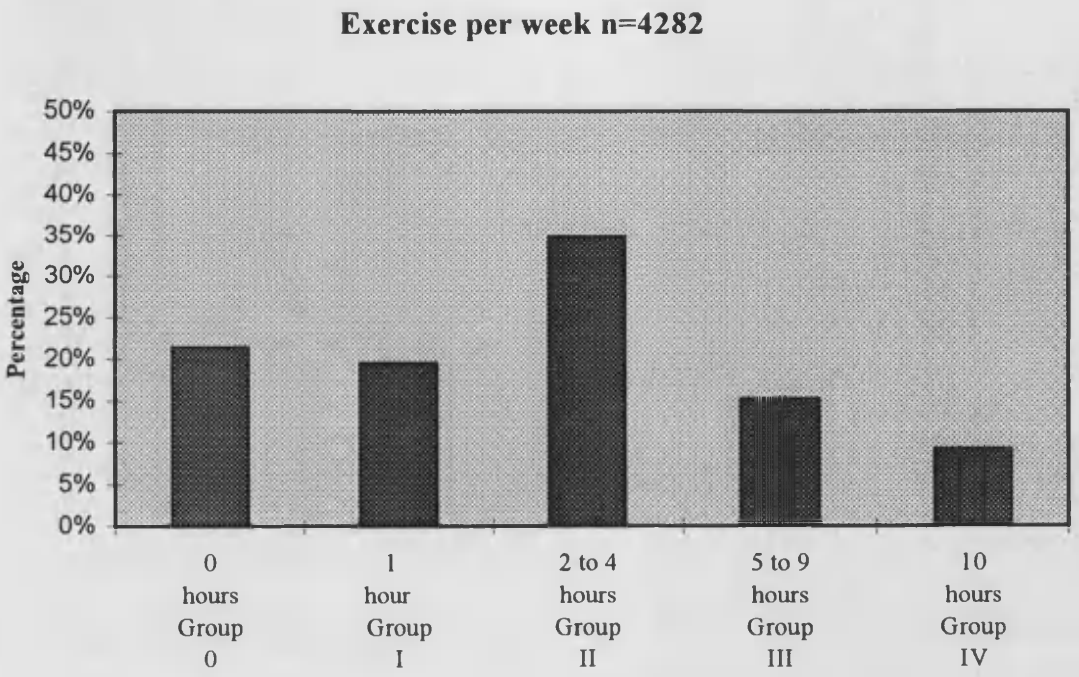


Figure 26 - Distribution of the number of hours of exercise per week in men and women (%).

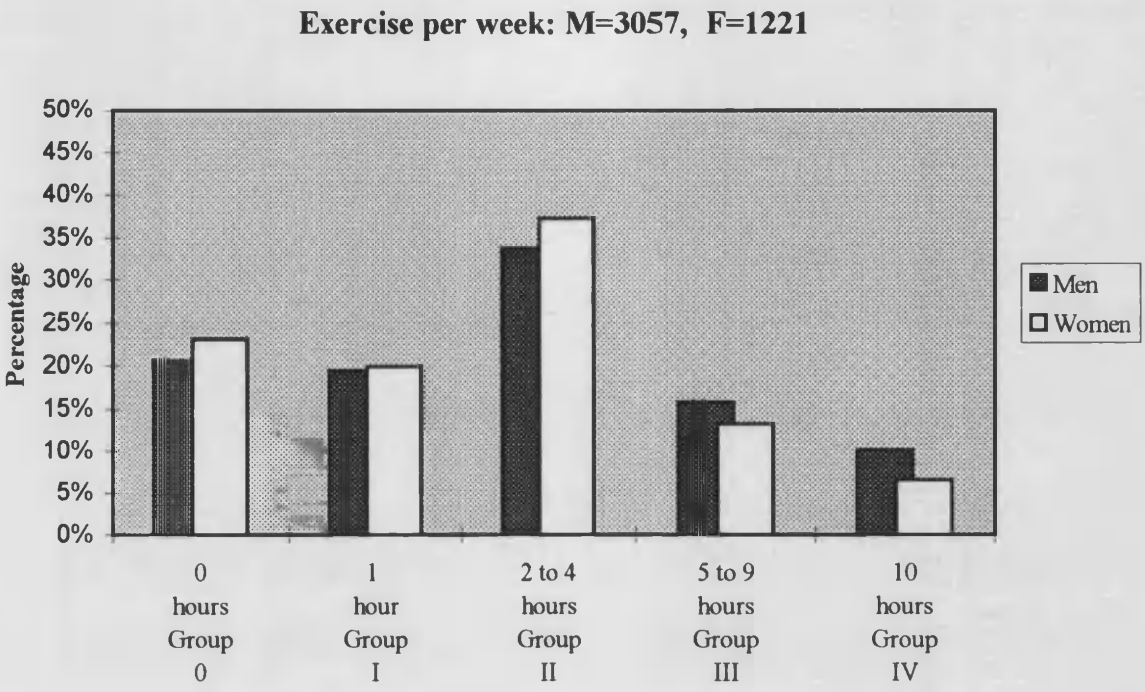


Table 24 - Comparison of self-assessment outcome measures between Group O and Group II.

	Group II*	Group 0*	
Total	n = 1460	n = 893	p
Study Group	n = 553**	n = 553**	
BASDAI	3.8 ± 2.0	4.2 ± 2.3	0.015
BASFI	3.9 ± 2.5	4.5 ± 2.8	< 0.001
BAS-G (1 w)	4.2 ± 2.6	4.3 ± 3.6	0.686
BAS-G (6 m)	4.8 ± 2.5	4.8 ± 2.9	0.880

* Group 0: no exercise; Group II: 2 to 4 hours of exercise per week.

* * Matched for current age, sex, and disease duration.

Table 25 - Comparison of demographic and clinical variables between Group II and Group 0.

	Group II*	Group 0*	
Total	n = 1460	n = 893	
Study Group	n = 553**	n = 553**	p
Current age, yrs Mean ± SD	47.3 ± 11.6	47.2 ± 11.6	0.150
Age at onset, yrs Mean ± SD	23.8 ± 7.9	23.6 ± 7.9	0.111
Duration of disease, yrs Mean ± SD	23.0 ± 11.9	23.0 ± 11.9	0.157
Delay in diagnosis, yrs Mean ± SD	9.6 ± 9.7	8.8 ± 9.6	0.141
Married, n	391	368	0.200
Employed, n	332	330	0.725
Education level - High, n	231	151	0.005
Rheumatologist, n	515	474	< 0.001
Family history of AS, n	139	158	0.222
Co-medication, n	454	404	0.001
Efficacy of exercise Mean ± SD	5.2 ± 2.4	3.4 ± 3.0	< 0.001

*Group 0: no exercise; Group II: 2 to 4 hours of exercise per week.

** Matched for current age, sex, and disease duration.

Table 26 - Comparison of self-assessment outcome measures between Group O and Group IV.

	Group IV*	Group 0*	
Total	n = 381	n = 893	p
Study Group	n = 272**	n = 272**	
BASDAI	4.3 ± 2.2	4.1 ± 2.3	0.394
BASFI	4.0 ± 2.6	4.5 ± 2.8	0.033
BAS-G (1 w)	4.7 ± 2.7	4.3 ± 3.0	0.148
BAS-G (6 m)	5.1 ± 2.6	4.8 ± 2.9	0.284

* Group 0: no exercise; Group IV: 10 or more hours of exercise per week.

* * Matched for current age, sex, and disease duration.

Table 27 - Comparison of demographic and clinical variables between Group IV and Group 0.

	Group IV*	Group 0*	
Total	n = 381	n = 893	
Study Group	n = 272**	n = 272**	p
Current age, yrs Mean ± SD	46.4 ± 12.6	46.4 ± 12.6	1.00
Age at onset, yrs Mean ± SD	24.4 ± 8.5	24.1 ± 8.5	0.422
Duration of disease, yrs Mean ± SD	22.0 ± 12.9	22.0 ± 12.9	0.706
Delay in diagnosis, yrs Mean ± SD	9.6 ± 10.1	7.4 ± 7.8	0.003
Married, n	174	189	0.040
Employed, n	141	159	0.118
Education level - High, n	88	84	0.389
Rheumatologist, n	250	227	0.001
Family history of AS, n	64	65	1.00
Co-medication, n	210	193	0.137
Efficacy of exercise Mean ± SD	6.8 ± 2.3	3.4 ± 3.2	< 0.001

* Group 0: no exercise; Group IV: 10 or more hours of exercise per week.

* * Matched for current age, sex, and disease duration.

(b) Impact of intensive physiotherapy and education program on ankylosing spondylitis: A longitudinal study.

Objective: Ankylosing spondyltis is a chronic systemic and progressive disorder. To date there are no disease modifying drugs to halt the advance of disease. Short term improvement in function along with reduction in pain and disease activity can be achieved by inpatient and outpatient exercise programmes [192-203] However, it is unknown if these benefits remain over the long term. In addition to physical therapy, patient education is believed to benefit the patient in terms of symptoms, mood , physical and social activities.

The RNHRD runs a treatment programme which combines inpatient physiotherapy with a comprehensive patient education package. Over the short term this programme reduces disease activity and improves function by on average twenty five percent. This study aims to examine the long term affect of this course on disease progression over a 2 year follow-up period.

Methods:

Study population: Annually, the RNHRD runs 24 two week inpatient programmes, with on average 10 patients attending each course. The programme consists of daily group sessions in hydrotherapy, circuit training, neck and trunk exercises, stretches and relaxation. Patient education includes; pathology, physiology, anatomy relevant to the disease process, pain management , medication, posture and coping strategies. The course should increase understanding of the nature of the disease and encourage adherence to exercise therapy.

To examine the longitudinal benefits of the course patients who attended [n=216] were asked to complete functional, disease activity and global well-being assessments at baseline (before course), 3 months, 6 months, 12 months and 24 months. In addition, a random

sample of patients who did not attend the course [n=216] were asked to also complete the questionnaires. These non-course subjects were members of NASS (National Ankylosing Spondylitis Society).

Statistical analysis: Matched pair t-tests were used to evaluate the status of the individual after 24 months. The baseline score of the subject was compared with the 24 month score. A scenario analysis was then performed to account for drop-outs from the study. In this analysis the scenario is assumed that at the 24 month time period, the drop-out patient remains at same disease activity and functional score as given in last questionnare recieved. Thus, last available questionaire is used as the final two year measure for all drop outs. Finally, univarent analysis was used to evaluate the effect of baseline level of BASDAI an BASFI (ie severity before course) on average change in score at the 3 month, 6 month and 12 month time period.

Results:

Full records for a follow-up period of twenty four months was avaliable on 181 (84%) course patients and 179 (83%) non-course patients. Patients attending the course were referred by a rheumatologist. Thus, at baseline the course subjects were more severe for disease activity and functional scores than the non-course individuals [Table 26]. These patients had an older age at onset and shorter disease duration than the non-course subjects.

1. Change over 24 months - Individual measures [matched pair T-Tests] :

For patients attending the course, function, disease duration and global well-being were comparable at baseline and after two years. There was no significant disease progression over a two year period.

For non-course patients, function [p=0.006], disese activity [p<0.001} and global well-being [p<0.001] scores were worse after 2 years.

Scenario analysis: To determine if patients droped out of the study because disease had deteriorated (failure) or improved substancially, the last available score received from the drop outs patients [from both the course and non-course groups] was used to represent the 24 months score. Match-test analysis on this new data showed that results did not differ when drops-out patients were included. [Table 27]. The mean time point when patients failed to return the questionnaire was at 18 months in both the course and non-course cohorts.

2. Analysis of variance:

In univariant analysis attendence to the course was associated with amount of change in funtional (p=0.01) , disease activity (p=0.002) and global well-being scores (p=0.012). At 2 years those not attending the course poorer BASFI, BASDAI and BAS-G scores . However, base line severity score was also a factor in determining amount of change in outcome measures. When these factor was included in multivariable analysis the effect of the course was no longer significant. (Figures 27, 28, 29). Thus, milder non-course patients appear to deteriorate over a two year period while more severe course patients do not change.

Interpertation of study:

Intensive physiotherapy and education has a long term benefit in slowing disease progression of ankylosing spondylitis. However, it is recognised that due to the nature of the referral process for the course only the more severe patients were allowed to attend the programme. It is possible that those patient who have already progressed to this level of severe disease may not alter greatly over a two year period. While, the milder non-course

subjects have the potential and scope to get worse. Regression to the mean may explain the findings of this study.

Yet, the patients who attended the course had the disease for a shorter duration than the non-course cohort, and in this time the severity was worse. Therefore, it is likely that these subjects represent a group with more active and aggressive disease. The disease activity of AS is normally constant throughout the patients life [42] and function deteriorates with age and disease duration. It is unlikely that the progression and activity of the disease would slow down and change unless the intervention programme had an effect.

Thus, an intensive physiotherapy and educational programme can have a long term (2 year) effect on disease progression. With the lack of any disease modifying drugs or therapy to combat this condition the intervention programme represents an effective and long lasting form of treatment for those patients with aggressive and advancing disease.

Table 26: Demographic Data

	Course (n=181)	Non-Course (n=179)	p value
Age	47.3 (+/-11)	48.4 (+/-11)	ns
Age onset	25.9 (+/-10)	23.1 (+/-9)	p=0.003
Disease duration	21.4 (+/-9)	26.3 (+/-12)	p<0.001
Delay in diagnosis	8.3 (+/-8)	7.8 (+/-7.9)	ns
M:F	142 : 39	147 : 32	ns
Iritis	38%	42%	ns
Psoriasis	19%	17%	ns
IBD	9.4%	7.8%	ns

Table 27 : Outcome of course vs non-course patients.

	Course (n=187)	Non-course (N=179)
BASDAI		
Baseline	4.7 (+/- 2.0)	3.5 (+/-2.1)
6 months	4.7 (+/- 2.2)	3.6 (+/-2.1)
12 months	4.5 (+/- 2.1)	3.8 (+/- 2.0)
24 months	4.5 (+/- 2.0)	3.9 (+/-2.3)
Scenario analysis	4.52 (2.25)	3.98 (2.26)
p value	**ns**	**0.001**
BASFI		
Baseline	4.8 (+/-2.1)	3.6 (+/-2.3)
6 months	4.9 (+/-2.5)	3.8 (+/-2.4)
12 months	4.8 (+/-2.4)	3.8 (+/-2.4)
24 months	5.0 (+/-2.4)	3.9 (+/-2.5)
Scenario	5.0 (2.4)	3.95 (2.5)
p value	**ns**	**0.006**
BAS-G		
Baseline	5.1 (+/-2.3)	3.8 (+/-2.6)
6 months	5.2 (+/-2.2)	4.1 (+/-2.5)
12 months	5.1 (+/-2.6)	4.2 (+/-2.8)
24 months	5.0 (+/-2.5)	4.5 (+/-2.7)
Scenario	5.1 (2.45)	4.5 (2.7)
p value	**ns**	**0.001**

Figure 27

Change in disease activity over 24 months (course versus non-course)

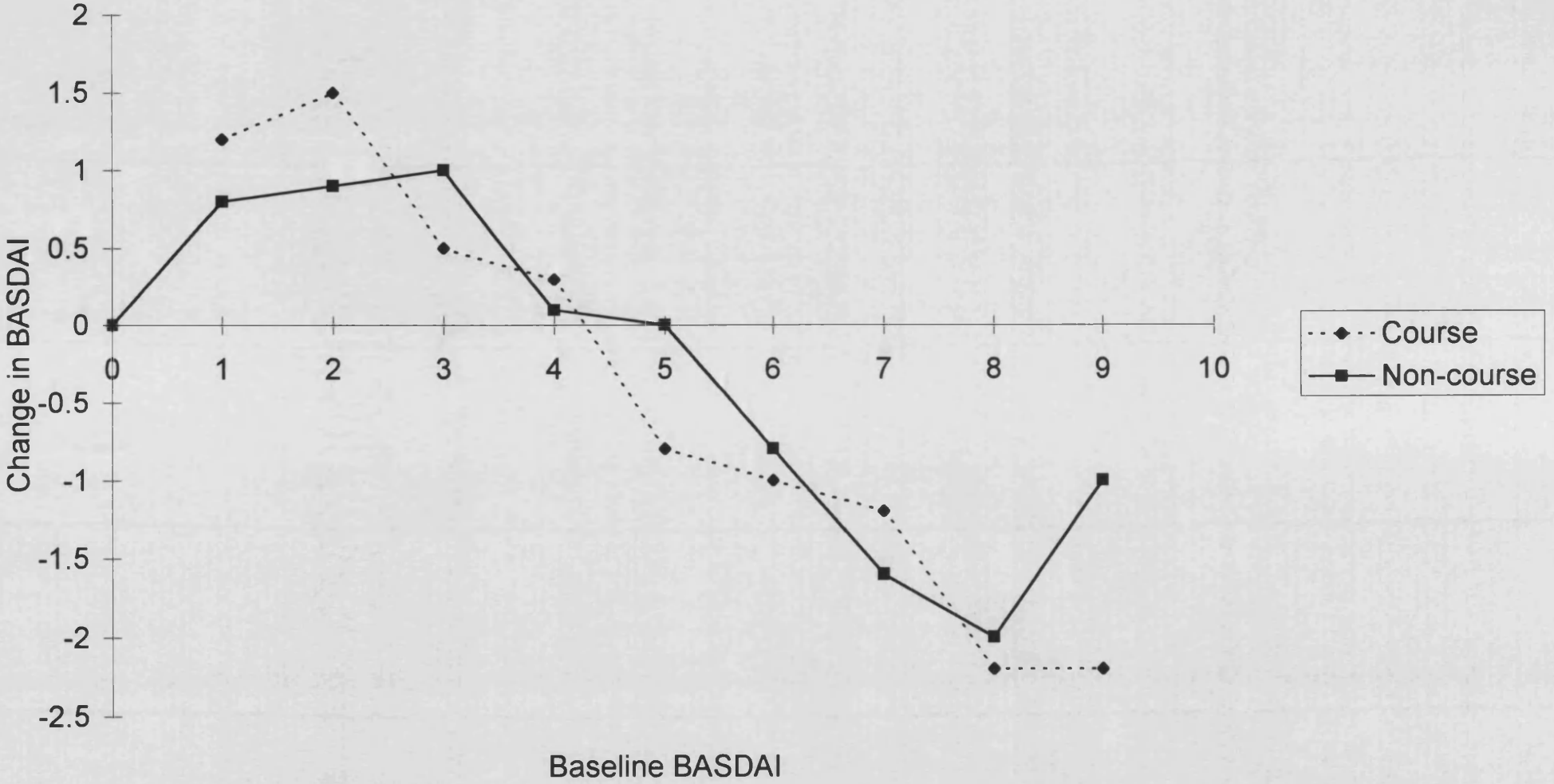


Figure 28

Change in function over 24 months (course vs non-course)

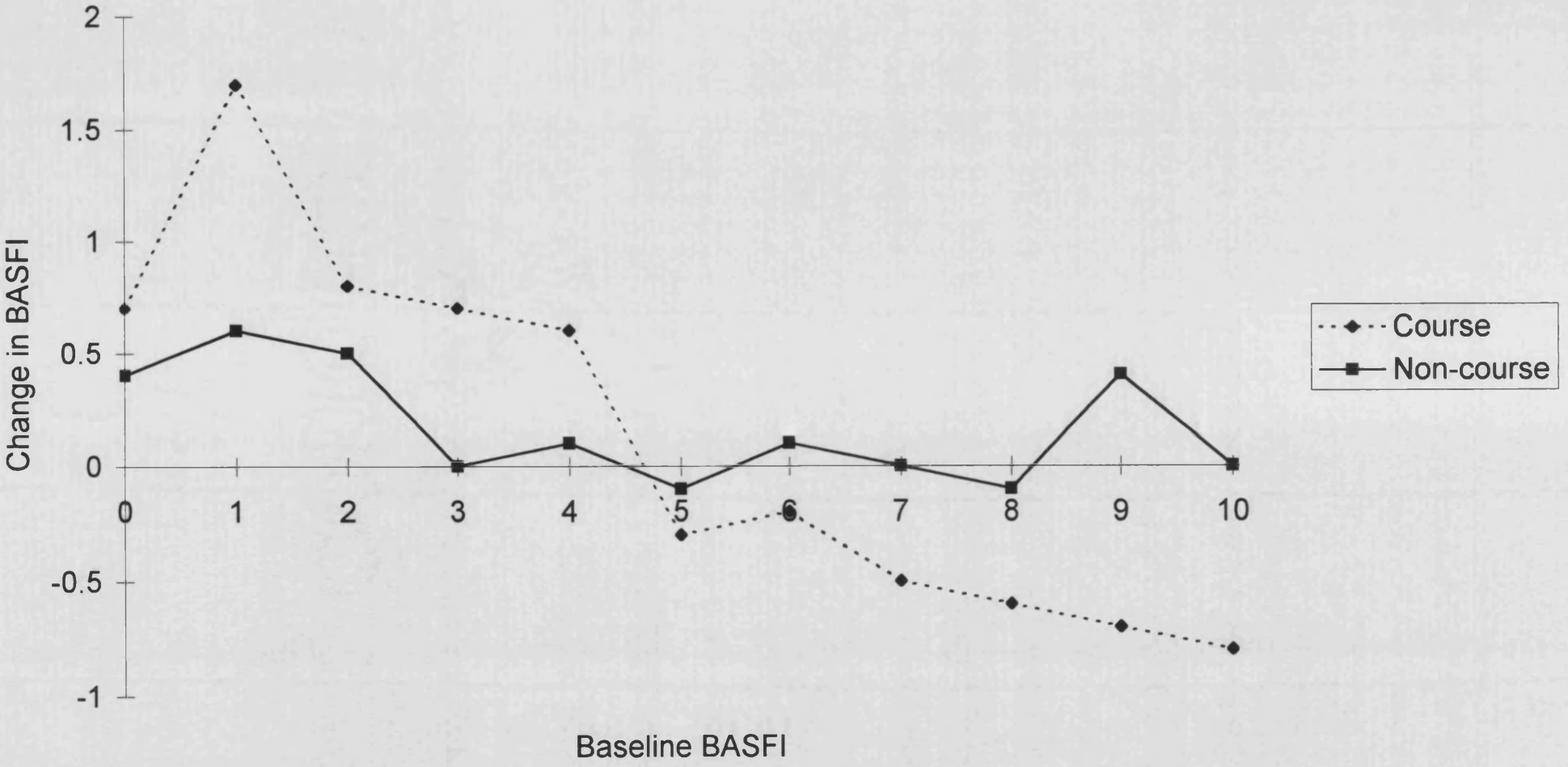
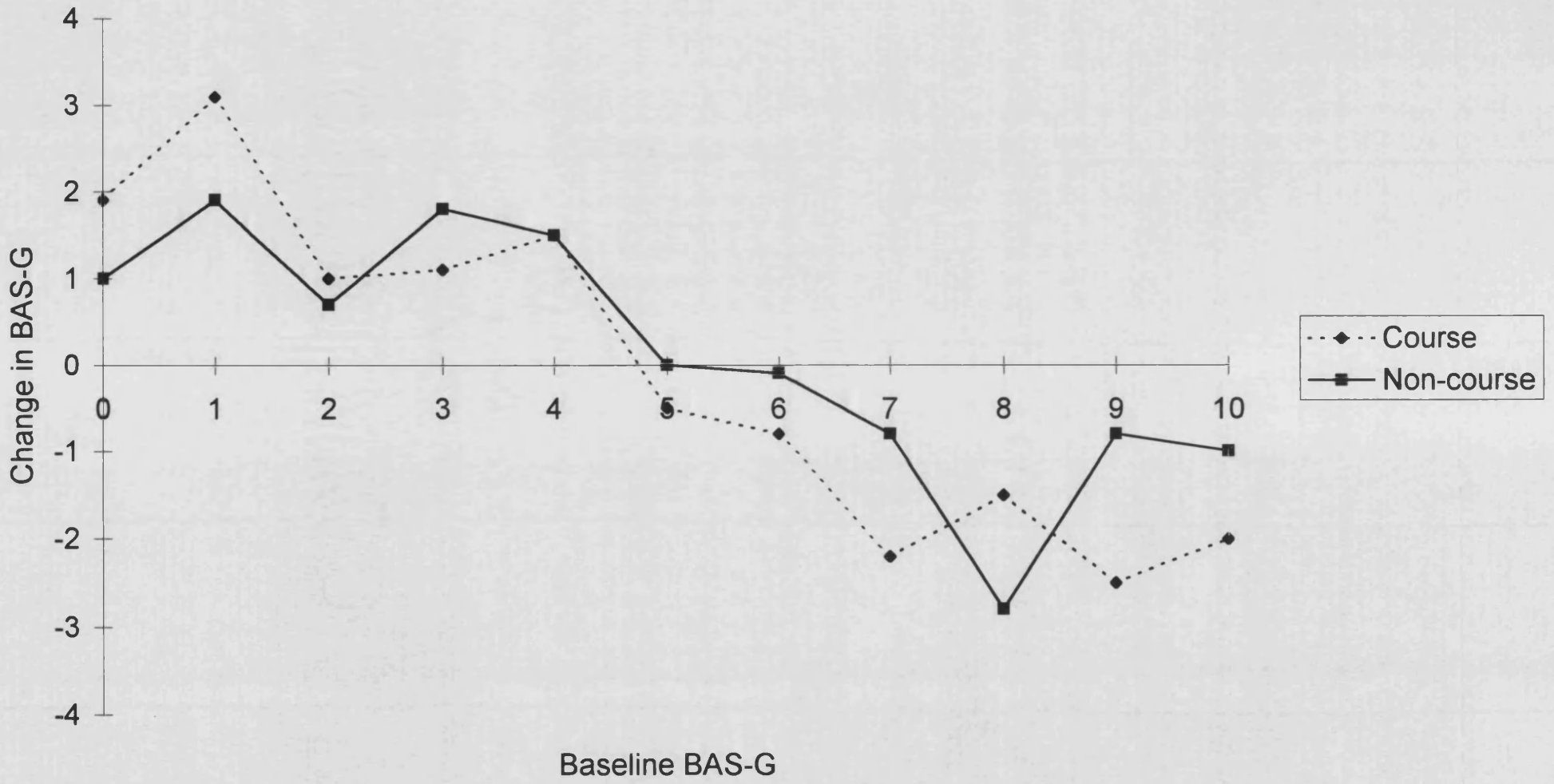


Figure 29

Change in global well-being over 24 months (course vs non-course)



(c) Predictors of outcome in ankylosing spondylitis

Objective: Many overlapping and interlinking variables have been shown to affect severity in AS. It has been suggested that disease severity is at least partially determined by genetic factors [Appendix 9] [186]. The relative contribution of genetic and environmental factors to disease severity has not, however, been elucidated.

In terms of environmental factors identified, it is difficult to separate the effects of one variable from another. For example, cigarette smoking has been associated with worse clinical, functional and radiological outcome [114], patients in the lower educational / socio-economic groups have a poorer prognosis [115][116] and daily exercise has been shown to retard the progression of disease. However, it is unclear weather variables like smoking is actually associated with poorer outcome or instead is a reflection of social status and lack of exercise. Amor et al [121] have shown that clinical entry variables predicting severity include hip arthritis, ESR>30mm/h, sausage like finger toe, oligoarthritis, onset <16 years, limitation of the lumbar spine and poor efficiency of nonsteroidal antiinflammatory drugs.

In this study radiology was seen to represent an unbiased, fixed, 'gold standard' for the measurement of disease status. Damage is irreversible and thus can reflect the cumulative natural history of disease in the individual patient. However, it was felt that radiological score may miss much of the change important in AS such as pain and soft tissue inflammation. Therefore, function was also used for the measurement of outcome as this should be a more accurate reflection of the quality of life of the individual and the ability to remain in employment.

Thus, this study soought to determine patient characteristics which might explain the variability in outcome in AS.

Methods: All subjects (n=311) were patients at the Royal National Hospital for Rheumatic Disease and had been diagnosed according to the New York Criteria. Patient hospital notes were used to confirm all variables such as diagnosis of secondary diseases (psoriasis, iritis, inflammatory bowel disease), peripheral involvement, smoking etc. All patients had full sets of radiographs (Lx, Cx, SI and hip) scored using BASRI by 2 trained independent readers. Discrepancies between scores were rescored by a third trained reader and a consensus was reached. All patients completed disease activity (BASDAI) and function (BASFI) assessments in the same year as radiological assessment (BASRI is sensitive to change over 2 years) .

Univariate analysis followed by multivariate regression analysis using backward selection was performed using SPSS to identify and analyse the factors correlated with severity. Correlations were performed using Spearman rank correlation coefficient. SPSS was used for all analysis.

***Results*:** Demographic data and distribution of the variables among the 311 patients are showen in Table 28. Of the 311 patients in the study, 254 (82%) were male and the average age at disease diagnosis was 22.9 years (SD 8.3).

1. Univariant analysis of factors influencing radiological status [BASRI] showed : Disease duration, sex, marital status, education level, iritis, psoriasis (and hip involvement in the case of spinal score) [Table 29] all impact on radiological change. Using these variables in multivariable analysis showed that disease duration , sex, marital status and iritis together explain 22.8% ($p<0.001$) of the variation seen in BASRI. [Hip involvement, disease duration, sex and iritis explain 29% ($p<0.001$) of variation seen in the spinal involvement]. These findings indicate that level of radiological change progresses with disease duration, men have more advanced change than women except when iritis is present (Figure 30) and

married or widowed patients have less radiological change than single or separated subjects. The presence of hip involvement is associated with more severe spinal radiological change.

2. Univariant analysis showed that factors influencing BASFI were: Radiological status, disease activity, disease duration, smoking, peripheral involvement, psoriasis and hip involvement. [Table 30] Using these factors in multivarable analysis showed : radiological status, disease activity and smoking were all associated with function ($p<0.001$) accounting for 50% of the variation seen in BASFI. There was a relationship between smoking and psoriasis ($p=0.001$) . Those psoriasis patients who smoked had poorer function than either patients with psoriasis who did not smoke or smokers without psoriasis [Figure 31].
Factors not associated with outcome included: variables such as first symptom of AS, age at onset, delay in diagnosis, family history, NSAID ineffective, family history and exerciser vs non-exerciser.

Interperation of study: Inherent characteristics such as iritis and the sex of the patients determine radiological outcome in AS. However, variables impacting on function such as smoking and disease activity (pain, fatigue, stiffness and discomfort) may be more easily altered or treated. Function is perhaps the most important outcome measure in allowing patients to maintain a quality of life at home and at work. Two of the three factors associated with functional outcome in this study are modifiable - disease activity and cigarette smoking. Thus, by targeting those individuals with peripheral involvement, psoriasis and hip involvement with aggressive treatment early on [such as physiotherapy, education (ie advice on smoking, drug treatments, pain management) and drug therapy] we may be better able to maintain function in the high risk individuals.
The criteria used by Amore et al to predict outcome were not all examined within this study (ie ESR was not included). However, this study support the previous findings that hip

arthritis predicts a poor outcome both in terms of radiology and function. Only 16 patients (of 311) were identified who had evidence that NSAIDs were ineffective and no subjects with sausage-like fingers or toes were found. Age at onset was not found to be a predictor of outcome.

Some environmental variables impacting on radiological outcome may in fact be due to reverse correlation. Marital status and education level may be a reflection that those subjects with mild disease have less disruption to their life than those with severe arthritis. Alternatively, social support (from spouses) may play a role in outcome. An education beyond school leaving level may allow patients to manage their disease better.

The patients used in this study are those referred to a tertiary referral centre and therefore will be more severe than patients managed by their GP. It is therefore possible that environmental variables like occupation and exercise would become important in subjects who have milder disease. However, the population we examined were by selection (ie referral) a more inherently severe cohort.

Much of the variability in outcome is not accounted for by the models within this study. There still remains 80% of the variation in radiology and 50% of the variation in function unexplained. It is possible that genetic factors (as suggested by influence of iritis and psoriasis) and hormonal (as suggested by predictive value of sex) have a greater influence on outcome than environmental factors particularly in terms of radiology.

In conclusion, smoking, secondary diseases (iritis and psoriasis), hip involvement and male sex are important predictors of a poor outcome in ankylosing spondylitis. Individuals with these characteristics should be targeted for aggressive treatment early on in the disease.

Table 28: Characteristics of AS study subjects/Demographic data (n=311)

Variable	Mean years (sd)
Disease Duration	23.5 (11.3)
Delay in Diagnosis	7.7 (7.7)
Age at onset	22.9 yrs (8.3)
	Number (% of total)
Male sex	254 (82%)
Iritis present	120 (39%)
Psoriasis present	69 (22%)
IBD present	23 (7%)
Hip involvement	81 (26%)
Peripheral joint involvement	130 (42%)
Affected family member	70 (23%)
Ever cigarette smoker	120 (39%)
Area first symptomatic	Low back 191 (61%) Hip /groin 46 (15%) Knee/ankle 31 (10%)
Exercise level(%)*	1 57 (18%) 2 48 (15%) 3 101 (32%) 4 65 (21%) 5 36 (7%)
Education level (%)	Primary school 11/ 217(5%) O level 78/217 (36%) A level 30/217 (14%) 3rd level 98/217(45%)
Occupational activity level (%)	Active 66/283 (23%) Sedentary 164/283(58%) Manual 53/283 (19%)
Marital Status (%)	Separated/Divorced 20 (6%) Married/Widowed 220 (70%) Single 71 (23%)
Social Status**	A=15, B=105, C=74, D=23, E=35, F=59

*1=None; 2=<2 hours per week; 3=2-4 hours per week; 4=5-9 hours per week; 5=10+ hours per week

** A=director; B=profesional; C=administrative; D=skilled; E=unskilled; F=farmer

Table 29: Factors influencing outcome as defined by radiology [BASRI] .

Predictor variable	Correlation's and Mean BASRI for each subgroup		Univariant Analysis
	Correlation's		
Disease activity [BASDAI]	-0.008		p=0.9
Disease duration	0.286		p<0.0001
Delay in diagnosis	-0.093		p=0.121
Age at onset	-0.024		p=0.2
	Means BASRI score	Mean difference in BASRI (95% CI)	
Sex	Male 9.3, female 7.8	1.5 (0.3-2.6)	p=0.022
Iritis	Present 10.2 absent 8.4	1.8 (0.9-2.8)	p<0.0001
Psoriasis	Present 9.6 absent 8.8	0.8 (0-1.9)	p=0.051
IBD	Present 8.7 absent 9.0	0.3 (-1.5-2.1)	p=0.7
Peripheral involvement	Present 9.0, absent 8.9	0.1 (-1.1-0.9)	p=0.9
Hip involvement	Present 10.3 absent 7.4	3.0 (2.2-3.7)	p<0.001
Family history	Present 8.9 absent 10.5	0.6 (-0.4-1.4)	p=0.6
Smoking	Yes 9.1, no 8.9	0.3 (-0.7-1.2)	p=0.6
NSAID ineffective	Yes 9.5, no 9.0	0.5 (-1.5-2.6)	p=0.6
First symptom	LBP 9.1, hip 8.5, knee/ankle 9.2, groin 8.3		p=0.7
Sedentary/active/manual job	Sed 9.4, active 8.5 manual 8.4		p=0.2
Marital status	Married 8.7, single 9.7, separated/divorced 10.26		p=0.025
Education level	School 11.0, O'level 8.0, A'level 9.5, univ. 9.5		p=0.034
Social status	Director 11.5, Teacher 9.2, admin. 8.5, skilled labour 9.6, unskilled labour 6.9, Farmer 8.6.		p=0.2
Exercise	1. 10.5, 2. 8.6, 3. 8.4, 4. 9.5, 5. 8.9		p=0.3

1= None, 2 = 1 hour per week, 3 = 2-4 hours per week, 4 = 5-9 hours per week, 5 = 10 + hours per week.

Table 30: Factors influencing outcome as defined by function [BASFI]

Predictor variable	Correlation's and Mean BASFI for each subgroup		Univariant Analysis
	Correlation's		
Radiology	0.4		p<0.001
Disease activity [BASDAI]	0.6		p<0.001
Disease duration	0.1		p=0.06
Delay in diagnosis	0.1		p=0.2
Age at onset	0.1		p=0.4
	Means BASFI score	Mean difference in BASFI (95% CI)	
Sex	Male 4.8, female 4.8		p=0.8
Iritis	Present 4.9 absent 4.7	0.2 (-0.4-0.8)	p=0.5
Psoriasis	Present 5.8 absent 4.5	1.3 (0.6-2.0)	p<0.0001
IBD	Present 5.0 absent 4.8	0.2 (-0.9-1.3)	p=0.7
Peripheral involvement	Present 5.4, absent 4.1	1.2 (0.7-1.8)	p<0.001
Hip involvement	Present 5.9 absent 4.4	1.6 (0.9-2.1)	p<0.0001
Family history	Present 4.5 absent 4.9	0.4 (-0.3-1.0)	p=0.3
Smoking	Yes 5.4, no 4.3	1.0 (0.5-1.6)	p<0.001
NSAID ineffective	Yes 5.7, no 4.7	0.4 (-0.9-1.6)	p=0.6
First symptom	LBP 4.7, hip 4.9, knee/ankle 4.9, groin 4.7		p=0.9
Sedentary/active/manual job	Sed 4.8, active 4.4 manual 5.3		p=0.1
Marital status	Married 4.5, single 4.8, separated/divorced 5.4		p=0.03
Education level	School 5.7, O'level 4.7, A'level 5.0, univ. 4.4		p=0.3
Social status	Director 5.2, Teacher 4.5, admin. 4.6, skilled labour 5.1, unskilled labour 2.3, Farmer 5.3.		p=0.2
Exercise	1. 5.1, 2. 4.5, 3. 4.8, 4. 4.7, 5. 4.9		p=0.5

1= None, 2 = 1 hour per week, 3 = 2-4 hours per week, 4 = 5-9 hours per week, 5 = 10 + hours per week.

Figure 30

Radiology score in male vs female patients with and without iritis

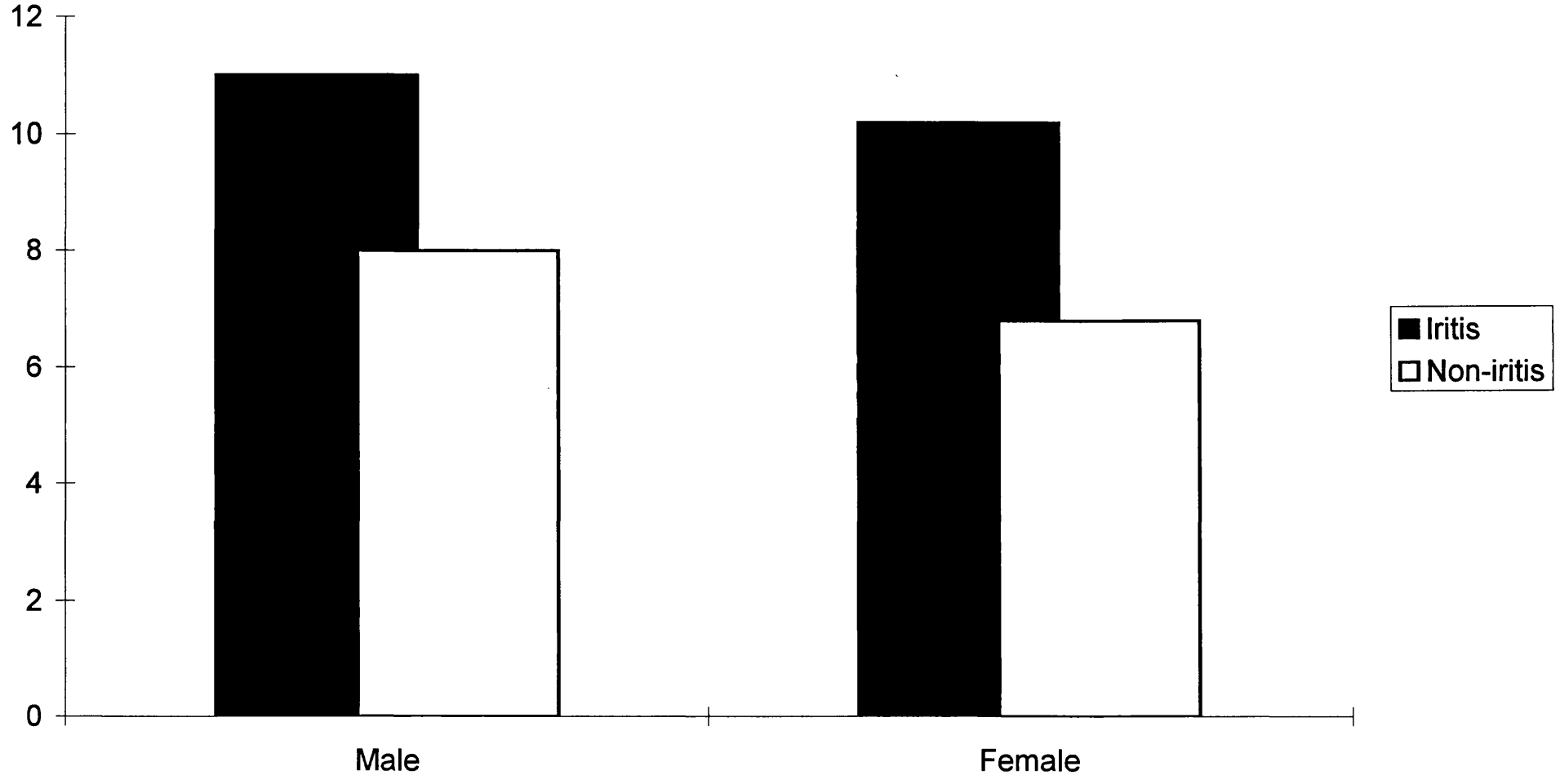


Figure 31

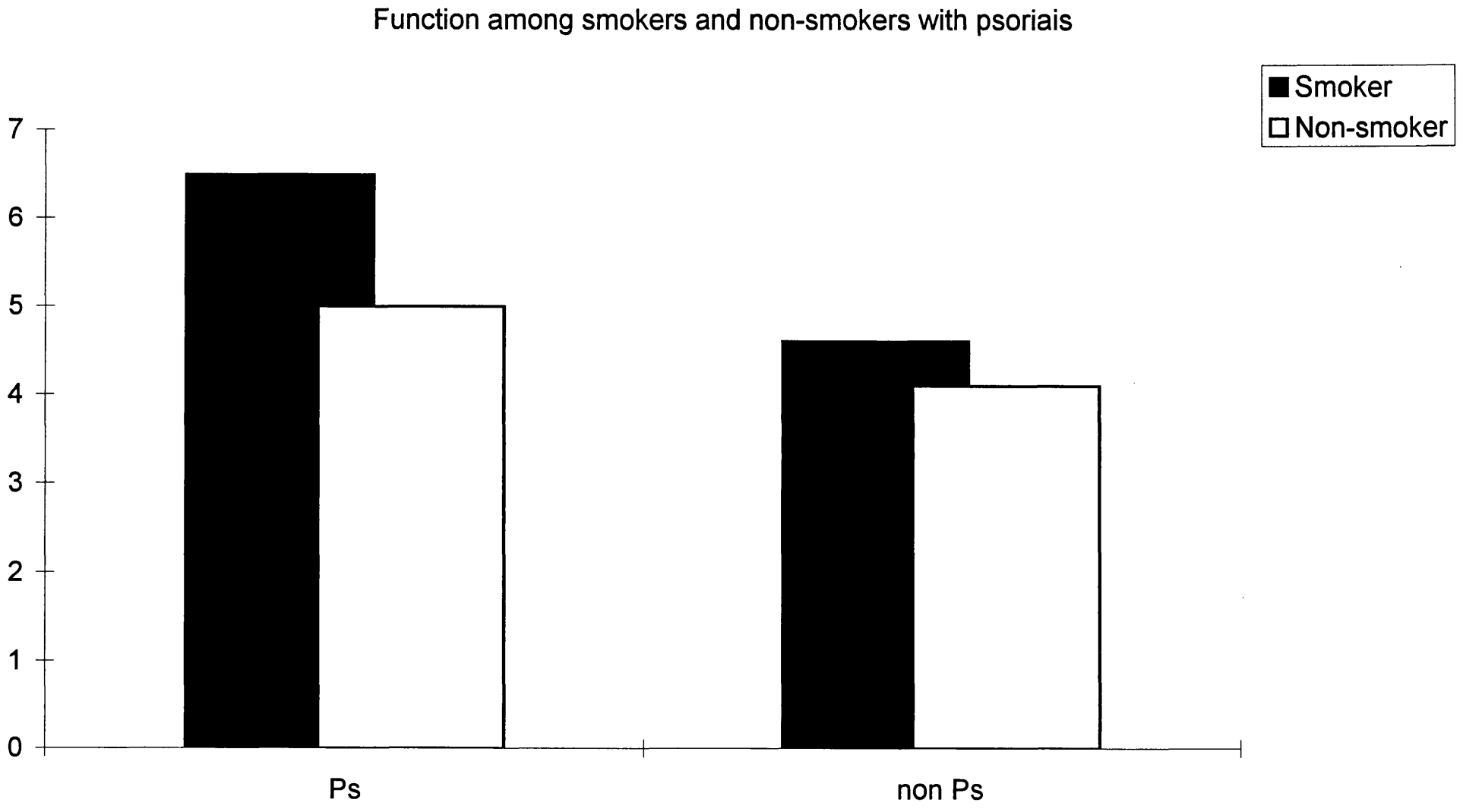
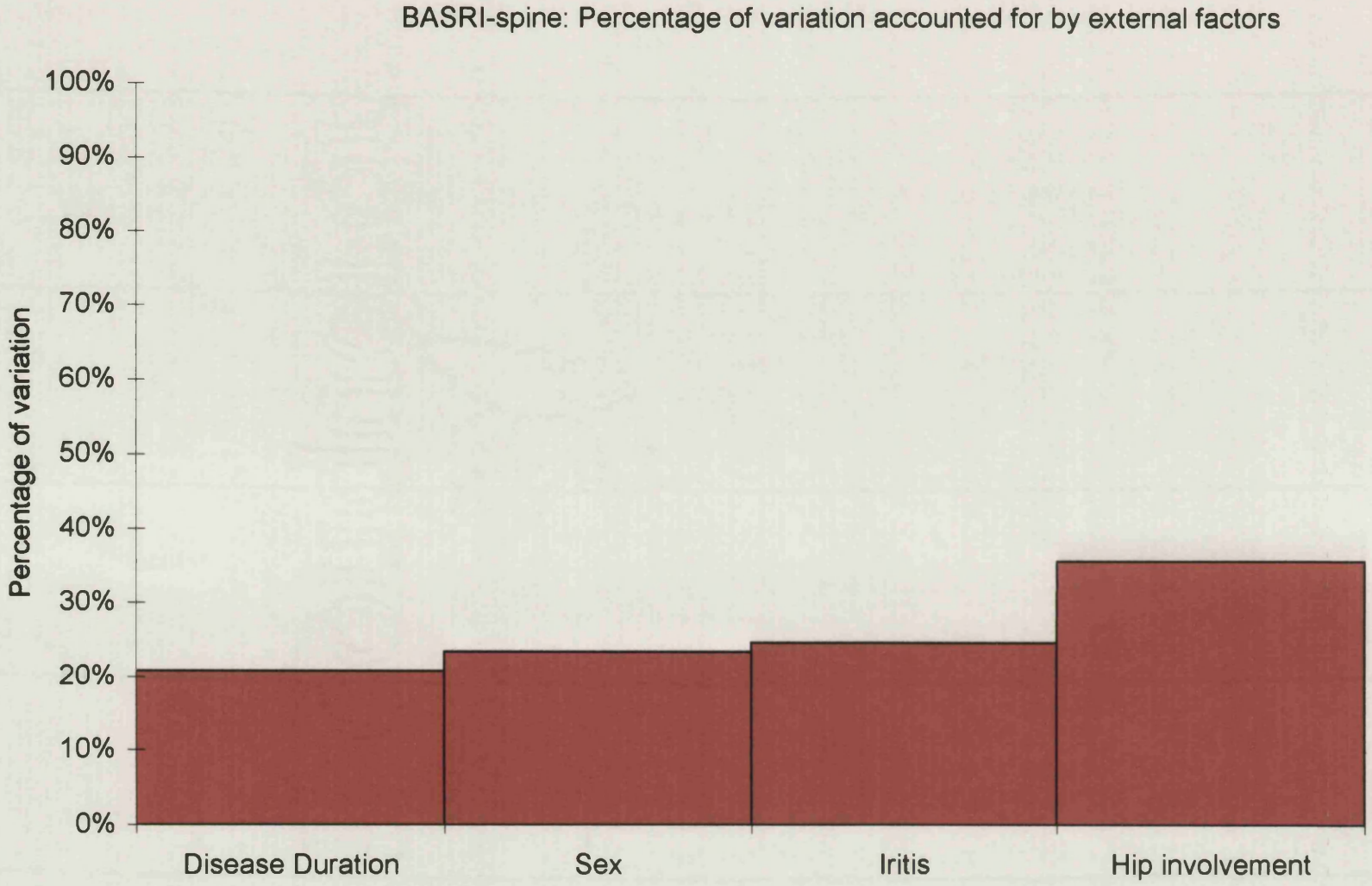

Function among smokers and non-smokers with psoriais
Smoker
Non-smoker
7
6
5
4
3
2
1
0
Ps
non Ps

Figure 32


BASRI-spine: Percentage of variation accounted for by external factors
Percentage of variation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Disease Duration
Sex
Iritis
Hip involvement

(e) Birth Order and Ankylosing spondylitis

Objective : In the HLA-B27 transgenic mouse model the first litters have been shown to have a higher percentage of diseased offspring than later litters [195]. This could be explained either by : a) the age of the mother (older maternal age decreases transmission of disease to the next generation) or b) the birth order of the offspring (ie environmental influence) has an influence on susceptibility to disease. Previous work has shown, that in humans the maternal age does not appear to have an effect on inheritance of disease [217] However, it has been shown using 162 AS patients, that the number of first-born children with AS is significantly higher than would be expected. [218]. Herein, we examine this hypothesis, that birth order of the child may effect the prevalence of disease, using 2343 patients . [Appendix 13]

Methods: The entire Bath ankylosing spondylitis database was used (n=5120). Patients of family size 2 -6 siblings were selected. When more than one sibling in the same family had AS, a random number table was used to select the child for inclusion and additional relatives were removed from the study. All adopted patients were removed from the study.
χ^2 was performed on position of birth compared to family size using SPSS. Patients were grouped as first-born versus not first-born. After stratification by family size, the number of children with AS who were expected to be first-born on the basis of an equal distribution within birth orders was computed.

Results:

Demographic date: The AS children born into family sizes of 2,3,4,5, or 6 children were comparable for sex ratio and disease duration. [Table 33]. AS patients from families of five or six children were older than those from 2, 3, or 4 child families [47 vs 45 respectively

p=0.026] and they therefore had the disease for longer. [25 vs 24 years respectively p=0.04] [Table 33].

Birth Order: Chi squared test on first born compared to later born children: When observed values of AS for the first born child were compared to those observed for later born children, the first born child is more likely to develop disease [p=0.025]. However, the first born child was only 1% more likely to develop disease. [1351 (37% of children were expected to be first born children and 1390 (38%) were born first]. [Table 34].

Chi squared test on family size units: The birth order in families of 3, 4, and 6 children deviated from the expected. Fewer of the youngest children had AS in these families (however in families of 6 children more were fourth born than expected). [Table 34 and Figure 35].

Interpretation of study: Taking the AS population as a whole there is a statistically significant effect of birth order. However, this difference is 1% and is unlikely to be clinically relevant. In addition, there was no clear biological gradient observed among families of different sizes. For example, in a family of 3 and 4 children the first born child is more likely to have AS than later born children but this is not seen among families of 5 children. In a family of 6 children there was no case of AS among the fifth or sixth born child but more than expected among the fourth born child. Factors which may be having an influence on birth order would obviously include abortion, miscarriage and stillbirth. Only live births are included in this analysis. It is possible that families of 5 or 6 children have more miscarriages and the children may be of a much later birth order (ie 7th or 8th) than recorded. There are many factors which make comparisons between families difficult. For example, we know that genetic susceptibility is required to develop AS and that women

with early onset AS are more likely to pass on the disease to their children [152]. Logically, women who suffer symptoms of AS would be less likely to have a large family. Therefore, only women with no family history of AS will be likely to have families of 5-6 children. Thus, any child born into a large family is automatically at a lower risk of AS than any child born into a small family. This means that children born 5th or 6th (higher birth order) appear to have a lower risk when compared to the population. However, when compared to first born children in a larger family they do not actually have a lower risk [Table 34].

Recent research [218] shows an increased risk of AS among first born children. In 40 families with 2 children , 26 of the AS affected children were first born, whereas the number expected was 20, a surplus of 30%. This study, does not substantiate these findings.

Birth order appears to have a statistically significant effect but the actual difference in this study between first born and later born children is only 1% and is unlikely to be clinically relevant. Thus, the effect of birth order needs to be interpreted with caution.

Table 33 : Demographic data

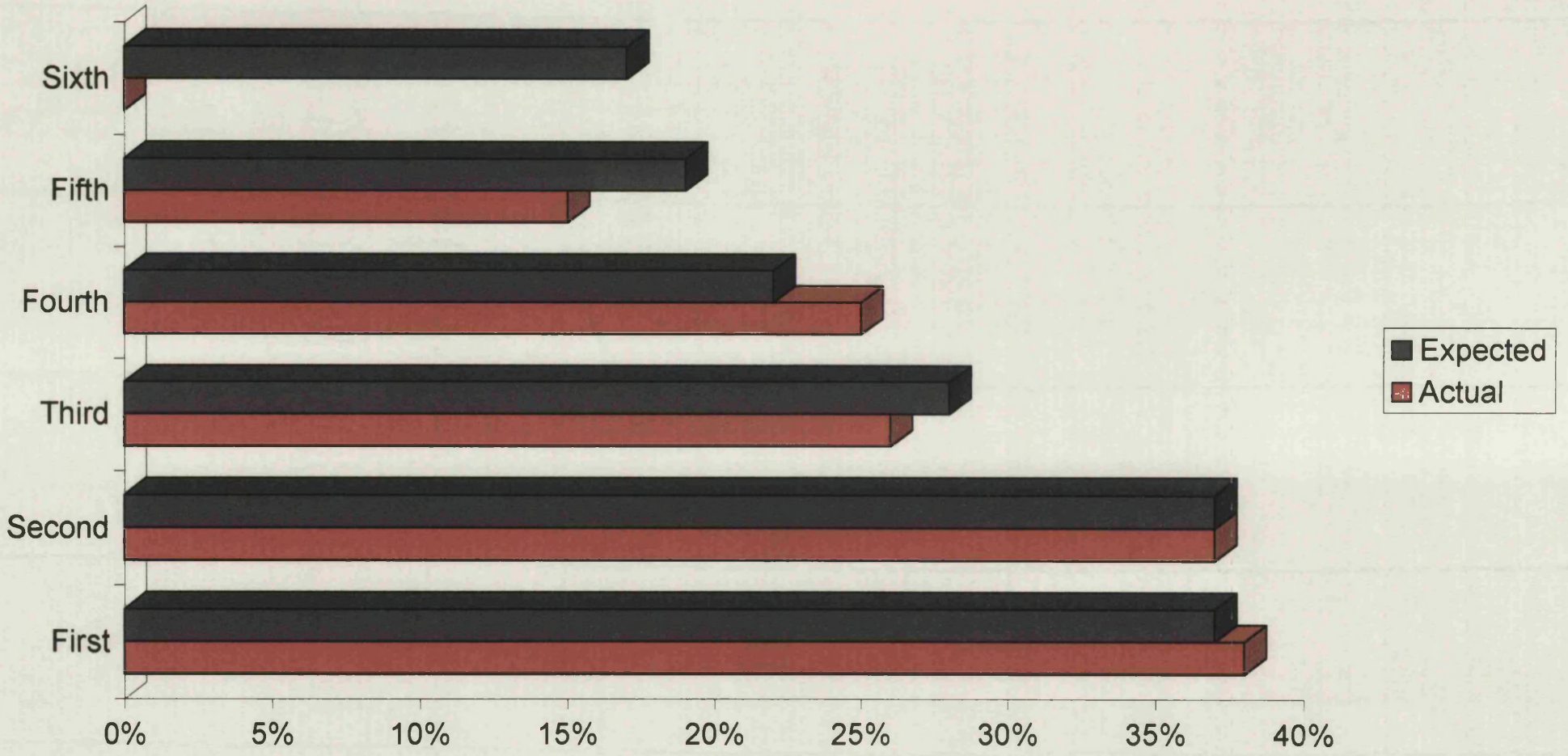
	Family size					
	Family of 2	Family of 3	Family of 4	Family of 5	Family of 6	p value
Age of AS child (sd.)	45 (13)	45 (13)	45 (13)	47 (12)	47 (11)	p=0.026
Sex ratio of child	2.3 : 1	2.3 : 1	2.3 : 1	2.3 : 1	2.3 : 1	ns
Disease duration of child (sd)	21 (14)	21 (13)	20 (13)	22 (14)	22 (13)	ns
Age at onset of child (sd.)	24 (11)	24 (11)	25 (11)	25 (13)	25 (12)	p=0.039

Table 34 : Birth order of AS individuals within the family.

	Family Size					Total	
Birth Order	2	3	4	5	6	**Observed**	**Expected**
1	741 (49.8%)	385 (35.8%)	167 (28.8%)	75 (20.6%)	22 (9.7%)	**1390 (38%)**	**1351 (37%)**
2	747 (50.2%)	375 (34.9%)	157 (27.1%)	61 (16.8%)	27 (11.3%)	**1367 (37%)**	**1351 (37%)**
3		314 (29.2%)	144 (24.9%)	70 (19.2%)	38 (21.0%)	**566 (26%)**	**607 (28%)**
4			111 (19.2%)	75 (20.6%)	102 (50.1%)	**288 (25%)**	**249 (22%)**
5				83 (22.8%)	0 (0%)	**83 (15%)**	**104 (19%)**
6					0 (0%)	**0 (0%)**	**32 (17%)**
Total	1488	1074	579	364	189	**3694**	**3694**
AS child first born	741	385	167	75	22	**1390 (38%)**	**1351 (37%)**
AS child not first born	747	689	412	289	167	**2304 (62%)**	**2343 (63%)**
						$\chi^2 = 11.16$*	
						p=0.025	

* First born child compared to later born children

Figure 35


Birth Order p<0.001
Sixth
Fifth
Fourth
Third
Second
First
0%
5%
10%
15%
20%
25%
30%
35%
40%
Expected
Actual

7 Conclusions

7.1 Summary of findings for nature of progression in AS

Radiology is fundamental to the diagnosis and evaluation of subsequent progression of AS. Apart from the New York criteria for the sacroiliac joints, no widely accepted radiological measure exist. The BASRI index (for spine, hip and combined for total) is reproducible, disease specific, sensitive to change at two years, simple to use and explain. It has now been accepted under the OMERACT [Outcome Measures in Rheumatology] criteria for Truth (ie validity , does it measure what it reports to measure), Discrimination (ie reliability and sensitivity to change), Feasibility (ie can it be applied easily). [223]

In comparison to other radiology scoring methods used for AS, the Stoke Radiology Spinal Score [224] was found to be more reliable for scoring cervical and lumber spine (intraclass correlation coefficient between 0.87 and 0.97) [225] . BASRI for cervical and lumbar spine was found to be moderately reliable when scored by independent observers [225] . However, the Larsen hip score proved unreliable when used in AS (Kappa 0.47-0.58). Therefore the BASRI hip score would constitute the only measure of change in patients with hip involvement due to AS. It is simple to use and required minimal training. The BASRI-hip was found to be sensitive to change over a one year period.

Although the Stoke index showed better reliability, it has 72 domains and takes times to complete. It is much more detailed and slower to use than BASRI and only examines the spine and neck. The BASRI global index (spine and hip score together) will examine the SI joints, neck, spine and hips within 30 seconds. Thus, is an easier system to employ when grading large numbers of radiographs for study and can be used to give a more rounded picture of the AS individual.

The disease history of AS as described by BASRI shows a progressive disorder with a great deal of variation between individuals. Those subjects with severe disease in one area were

destined to develop severe disease in other joints. Those with mild disease in one area (such as in the lumbar spine or no hip involvement) had milder disease in all other joints. As a group the rate of progression of AS is constant. However, among individuals there is a great deal of variation and definite subsets of patients exist. Some subjects do not progress at all in 10 years of disease and others show marked change in a short period. Male patients show worse axial disease than women, patients with hip disease (male or female) show more axial involvement and those subjects with iritis show more cervical involvement. Thus, males with hip disease and iritis should be seen as a high risk group compared to females without hip involvement or iritis.

The age of first radiographic change can be estimated to be before the age of 10 in those patients showing symptoms at the twenties. This extrapolation assumes that rate of change before symptoms (ie SI joints change) will be equivalent to rate of change after symptoms (ie axial change). If this assumption is true then the trigger of AS would be pulled in or before childhood.

Thus, BASRI spine and hip are reliable and valid measures. Disease duration, male sex, hip involvement and iritis are the main markers for severe radiological outcome. This is a progressive disease (with average 30% change every 10 years) which shows a great deal of variation between individuals and may begin as young as age 8.

7.2 (i) Summary of finding for influence of genetic factors on AS

The susceptibility genes for inflammatory eye, skin, bowel and joint disease appear to overlap: these conditions are prevalent more often in the same individual, in the same families and do not appear to follow a temporal pattern in onset of symptoms. Those patients with AS and inflammatory bowel disease carry the greatest genetic load. Thus, it is possible that for AS to develop there needs to be a combination of susceptibility genes. These are either genes linked to iritis and AS (therefore family member also suffer from iritis or AS but not psoriasis or inflammatory bowel disease) or genes for IBD and psoriasis (therefore family members suffer from IBD, psoriasis and/or AS). These genes may be occult or expressed. (Figure 14). Iritis predicts a poorer radiological (AS specific) outcome and psoriasis and inflammatory bowel disease are associated with more disease activity and poorer function.

In the absence of susceptibility genes linked to iritis, psoriasis and bowel disease it appears that other susceptibility factors are required for AS to develop, such as the susceptibility gene B60 and B27 (ie 77% of patients with IBD carry B27 compared to 98% of non-IBD AS patients). However, HLA B60 and B27 do not affect AS disease activity or function, while the presence of psoriasis and inflammatory bowel disease do affect these outcome measures. It is possible that patients with these secondary conditions do not carry enough AS specific susceptibility genes but do carry a higher frequency of other pathogenic factors (as well as the iritis, psoriasis and inflammatory bowel disease genes) which influence disease activity (and therefore function). AS specific susceptibility gene such as B27 can be seen to cause a younger age at onset of disease in the individual. However, this younger age at onset does not affect outcome (ie radiological change, function and disease activity). Therefore, age of first onset of symptoms is not a factor of severity of disease (ie most severe present symptoms first) but may be related to genetic susceptibility load.

However, a young onset is associated with hip involvement and hip involvement is associated with more severe AS. Yet, young age at onset without hip involvement does not have a poorer outcome and adult onset with hip involvement is associated with more severe disease. Therefore, hip involvement may represent the phenotypic expression for a severity marker.

Women appear to be protected from AS and therefore those females that do suffer from the disease carry a higher susceptibility load than both men and women without the condition. This is seen in the finding that the children and siblings of women with disease are more likely to develop the condition and women with a young age at onset are more likely transmit the condition to their children. These results have also been confirmed by findings in a French cohort [226] It has been argued [227] that the women with a negative family history are underdiagnosed previously and were therefore underrepresented in our study in comparisons to males. However, data from the RNHRD database do not support this hypothesis. From the database, of 243 women diagnosed in 1970-79 62 (26%) had a positive family history (ie father/mother, aunt/uncle, brother/sister with disease). Of patients diagnosed in 1990-99, 213 (30%) had a positive family history. In addition, familial disease is thought to be less severe [167] than sporadic illness. Thus, women with a family history may have a greater delay in diagnosis.

The are two possible explanations for higher risks associated with maternal AS: 1. women with disease may require a higher genetic load or 2. they may pass on through the uterine environment or breast milk, disease inducing factors (ie pathogenic triggers). However, a X chromosome effect is clearly ruled out by findings here and by subsequent findings from Oxford [228] . Further evidence that the effect may be genetic comes from findings that offspring of mother with disease have more previous relatives with disease (grandparents, uncle/aunt). It is possible that a triggering pathogen is found to run in families and is passed on by females (thorough breast-feeding). However, the most likely explanation is probably

the influence of genetic factors. However, disease inherited from the maternal side does not differ from that inherited from the paternal side. Susceptibility factors from the maternal side also have no impact on severity.

However, the inheritance of secondary disorders is dependent on sex of affected parent. Children of mothers with disease inherit more inflammatory bowel disease but sons inherit less psoriasis (than if the father has disease). However the genes for these conditions may be occult as only 7% of the mothers transmitting bowel disease also suffer from the disorder (93% did not have IBD). Parents who do suffer from secondary conditions do transmit them to their children. However expression of disease is not directly transmitted from diseased parent to child. Genetic information from the unaffected parent may modify the expression of disease. [As siblings have comparable disease].

There does appear to be a difference between familial and sporadic disease. Those patients with an affected parent have milder disease (and parents with AS have milder disease however we have shown that they also probably had a previous family history [20 % in females and 10% in males]). This may be a factor of selection that only those patients with milder disease have a family.

Finally, there is strong evidence that there is genetic control over the level of severity in AS. Genes contained in loci on chromosomes 18 and 20 may be involved in regulating disease activity and loci on chromosomes 2,9 and 11 may contain genes involved in regulating function. This shows that severity and susceptibility are controlled by separate determinates and that different aspects of severity (ie BASDAI and BASFI) may be regulated by different areas of the genome.

7.2 (ii) Summary of influence of environmental factors:

Moderate daily exercise is associated with lower disease activity and improved function. Those patients who followed a regular exercise regimen were in the higher education bracket and under the care of a rheumatologist. However, in any test of this type it is difficult to rule out the possibility that milder patients are more able to exercise and have less disruption to their life. Milder patients have less fatigue naturally and therefore may be able to perform exercise and continue in education. However, those who do daily activities score highly in beliefs in the benefits of exercise. Therefore they themselves feel the exercise is having an influence on their disease activity and ability to function. Using these findings suggest that an physiotherapy and education intensive program should be a highly effective treatment for those with spondylitis. It does appear that a course of this type can reduce deterioration while controls not attending a program will change by on average by 10% over 2 years. However, yet again it is difficult to rule out the possibility that this is simply regression to the mean. As patients referred to the program are by selection more severe than those not referred by their rheumatologist. However it could be argued that as these patients were more severe it would be expected that their disease would be more aggressive and more likely to deteriorate over time (than milder patients). The finding that the course may prevent advance of the disease demonstrates that this method of treatment works.

Determinants of outcome appear to be inherent to the individual. Male sex, iritis, peripheral involvement, hip disease and psoriasis all point to more severe disease. However, one environmental variable which did have a significant influence was smoking. Those patients with AS and especially those with psoriasis who do smoke have worse disease.

It is possible that the uterine environment has an influence on susceptibility to AS. In both the transgenic mouse and among the AS database there appears to be a lower birth order

among those patients with AS. However, this birth order effect might be explained by the hypothesis that women with AS are less likely to have large families. It appears that the 5th or 6th born child in a family is at a lower risk that first born children but this may just be because these children are less likely to have and AS mother.

Thus, environmental factors do appear to play a role in severity to AS. Exercise, physiotherapy, smoking and education may aid in modifying the disease expression for the individual. Alterations in the uterine enviroment may play a role in susceptibility.

7.3. What this study has shown which is new:

1. The validation of BASRI, an AS specific scoring system which meets the OMERACT criteria.

2. AS is a linearly progressing disorder which may begin as young as age 8 in patients with first symptoms age 25-28 years.

3. Inherent characteristics associated with worse disease are: hip involvement , iritis, male sex and peripheral involvement.

4. Environmental variables associated with worse disease are: smoking, poor education, and lack of regular exercise.

5. The susceptibility genes between AS, psoriasis, inflammatory bowel disease and iritis overlap.

6. Women with AS are associated with more heritable susceptibility factors.

7. Women transmit more bowel disease to children and men transmit psoriasis to sons.

8. Susceptibility does not influence severity.

9. Outcome is not comparable between affected parent and child but is comparable between siblings.

10. Loci on chromosomes 18, 20 and 2,13,11 are linked to severity of disease.

8. Discussion and future direction

8.1 Natural history of AS and factors modifying progression.

Results show that for the majority of patients, AS is a progressive disorder. The rate of progression of radiological change in an AS hospital population is 35% of available change (or 2.5 on the BASRI 2-16 scale) every 10 years. Thus, disease becomes more severe with progressing disease duration. However, although correlation with disease duration and severity of radiographs is significant it is still poor (r=0.3) and only describes 19% of the variation seen in BASRI. Thus, 80% of the variation must be accounted for by other determinants. Factors associated with severe disease (as measured either by radiographs, disease activity or function) are: hip involvement, iritis, psoriasis, inflammatory bowel disease, male sex, peripheral involvement, smoking, poor education, lack of regular exercise, genetic determinants on chromosomes 18, 20, 2 ,3 & 11, and finally siblings with severe disease.

These findings do aid understanding of AS but they may not be useful in identifying or predicting the individuals at risk of a severe outcome. For example, in terms of the environmental variables (education, exercise, smoking), it is very difficult to prove cause and effect. It is possible that the most severely affected individuals have greater disruption to their life, they may not continue their education and may not be able to do regular exercise. In addition, it is possible that those with severe disease are more likely to smoke because it psychologically helps to make them feel better. Moreover, the inherent factors (hip involvement, iritis, psoriasis, inflammatory bowel disease) associated with poor outcome may also not act as predictors to severity. Symptoms of AS occur on average at age 25 but first symptoms of hip involvement, inflammatory bowel disease or iritis do not occur until ages 30, 30 or 39 respectively. Therefore the patients with these disorders have had AS for at least 5-10 years before any development of the 'predictors' of severe disease.

In this time it is possible to see that the disease is severe without needing to observe the presence of hip disease, iritis etc. This criticism will also apply to the criteria of Amor et al [117], who found that hip arthritis, sausage-like finger or toe, oligoarthrtis and poor efficiency of nonsteriodial anti-inflammatory drugs predict a poor outcome. These factors will all take time to develop and can not be used as entry variables to predict outcome. In addition, it is perhaps unsurprising that inflammation of the eye, skin, bowel, hip and peripheral joint are associated with worse disease in the spine, as they are simply reflections of inflammation in more areas of the body. The more severe the disease, the more areas of the body become affected. Thus, worse disease is associated with worse disease. The disease spreads and more areas are inflamed in those patients with severe disease (affecting the hip, eye, peripheral joints, bowel and skin), while in those with mild disease there is little inflammation even in the areas affected with disease. These are not predictors of outcome but are themselves part of the measure of the level of inflammation in the body.

Male sex has also been used as a predictor of radiological outcome in AS. However, it could be argued that AS has long been thought of as a disease affecting men more than women. Therefore, when describing the changes seen in AS in the past and today the focus has been on the changes seen in men (3:1, male :female) and these are mostly axial spine changes. There is more change in the axial spine in men compared to women. However, if women had been used to define AS specific measures there may be a different criteria and under these new scoring assessments women may appear more severe. For example, if peripheral joint damage was included as part of an AS specific measure, women may appear worse than men. Women do have poorer function and more disease activity than men as measured both from centile reference curves and among the offspring of parents with AS. In addition, hip disease is found as often in women as in men and severity of hip involvement is comparable between the two sexes Therefore, men do have more severe axial disease than women but that may not mean that they have more severe disease generally. This may be

more a factor of how radiological outcome is defined and less that male sex really translates into more severe disease. Thus, it would appear that siblings level of disease is the only real predictor of outcome.

It is possible that in a few individuals an early prognosis of severe disease (eg as defined from figure 9), with early involvement in the neck or advanced disease in the spine could allow treatment with interventions such as anti-TNF and might prevent hip involvement occurring.

In 21% of patients with iritis [50/237], there is inflammatory eye involvement before the age of 25 [the average age of onset of symptoms of AS in these patients was 18.5 years (stdev : 5.6)]. These patients, and any subjects from a eye clinic with iritis followed by arthritis should be flagged for early aggressive intervention treatment such as physiotherapy and disease modifying agents like anti-TNF. This may prevent severe axial involvement and hip/ peripheral disease in the future.

Finally, those with a severely affected sibling should be flagged for early treatment.

Any sign of severe early disease will suggest more widespread and advanced involvement in the future. This spread of disease may be prevented if treatment is give early. In addition, all patients should be encouraged not to smoke, to be educated about their condition and to do regular exercise.

8.2 Secondary disorders and AS

There are a number of interacting findings from this study regarding the relationship between AS and the secondary disorders:

1. The secondary disorders associated with AS are found more often than expected in the same individual. Relatives of patients with AS are also more likely to suffer from the

secondary disorders than expected (in the absence of AS). Therefore, there does appear to be an overlap in the susceptibility genes of these conditions.

2. It has been shown that iritis is associated with more radiological involvement and psoriasis & bowel disease are associated with higher disease activity and poorer function.

3. In women, there appears to be more susceptibility genes than in men. Women have higher disease activity and poorer function but less radiological involvement of the axial spine.

4. The children of AS-women are more likely to develop inflammatory bowel disease and the sons of male patients more likely to develop psoriasis

Hypothesis : **Susceptibility** genes (ie inherited factors predisposing an individual to AS) do not affect the severity of inflammation. However, they may determine which regions of the body become susceptible to inflammation. Therefore, susceptibility genes for psoriasis bowel disease or iritis will make the skin, bowel or eye susceptible to inflammation. **Severity** genes determine the level of inflammation in susceptible areas.

For example, the genes linked to iritis may be present in all individuals with AS (eg the B27 genes is linked to iritis and AS). If the disease is mild (ie few severity genes) there is a low level of inflammation in the body. The patient is susceptible to iritis but does not show clinical symptoms because the inflammation level is mild. If the patient has a large number of severity genes then areas prone to disease (ie susceptible) will show clinical symptoms of inflammation. Thus, the genes appear occult among patients with mild AS and appear expressed in those with more severe AS [Figure 36].

However, in the case of inflammatory bowel disease and psoriasis genes there appears to be an effect of sex on expression of the genes. When a women carries these genes they may make the bowel susceptible to inflammation. When a man carries these genes they may make the skin (psoriasis) susceptible to inflammation. Women generally , carry more

susceptibility genes but not necessarily more severity genes. Therefore, more areas are prone to inflammation (areas susceptible) in women, but depending on the severity genes these areas may not become symptomatic. Thus, in women disease may appear to remain occult. For example only 7% of the AS mothers of children with AS-IBD also suffered from bowel disease.

Thus, the secondary disorders are controlled by related genes. These disorders control which areas of the body will become susceptible to inflammation. The sex of the individual will determine how many of these susceptibility genes are present and modify which areas are prone to inflammation. [Figure 36]

8.3 Interaction between susceptibility and severity factors.

This study has show that in addition to specific susceptibility genes such as B27 and B60, susceptibility is also associated with; sex, the secondary disorders (IBD, iritis, psoriasis), age at onset, and family history. Men show clinical symptoms of AS more often than women. Thus, women are thought to be protected in some way from developing AS. Those women who do suffer from the condition may have been exposed to higher levels of susceptibility than men. Evidence that they carry more susceptibility genes comes from the findings that they are more likely to have a previous family history of disease and more likely to pass the condition on to their children. Women also pass on bowel disease to their children even when they do not suffer from bowel disease. Thus, occult susceptibility genes exist and women may carry more of these genes.

Susceptibility is determined by genetics and bacterial triggers. Severity genes are independent from susceptibility genes and it is possible that men carry more severity genes (thus show more clinical symptoms than women). When disease is present the genes linked to IBD, psoriasis and iritis will determine which parts of the body become inflamed.

However, these genes do not determine the severity of disease. Separate severity genes determine level of inflammation. Under higher levels of inflammation, the areas susceptible (eye, skin, bowel, peripheral joint or hip) become involved. Thus, without severity genes determining severe disease, the susceptibility genes (for hip, bowel etc) remain hidden (but may be involved in determining susceptibility to AS)

Patients with a young age at onset carry more susceptibility factors. Some of these factor such as B27 cause an earlier onset of disease. Hip disease genes will be a susceptibility factor, also making the hip susceptible to disease. If genes for severe disease are present then the hip susceptibility genes (leading to earlier onset of AS) may target the inflammation to the hip. However, if AS is mild (due to mild severity genes), then the hip does not become involved even though the factors targeting the hip are present.

Thus, susceptibility genes and severity genes are separate factors. Susceptibility genes determine what areas become 'susceptible' and severity genes determine whether these areas develop disease and to what extent.

8.4 Influence of environmental factors.

Of seven environmental variables examined (smoking, activity of job, marital status, education level, social status, exercise, birth order) only three were not associated pathogenesis of AS. The others (smoking, marital status, education and exercise) were associated with variation in disease expression.

As suggested earlier, it is difficult to prove cause and effect when discussing association between traits and variables. However, the finding that the majority of environmental variables examined were associated with severity of AS suggests that to an extent the progression of AS can be modified. If it was found that smoking, exercise, and education had no effect on outcome, this result would have implied that severity is totally inherent.

Instead, these findings suggest that environmental factors may limit and moderate outcome. Patients themselves report a high belief in the effectiveness of exercise to relieve symptoms and those subject that attend the intensive education/physiotherapy course of the RNHRD report great benefits and often want to come back on repeat courses.

There were many other variables which may influence outcome but could not be examined within this study. For example, hormonal levels and bowel flora may be important determinants of severity of disease.

Thus, environmental variables do appear to play a role in pathogenesis of AS. However, much more work in this area is need to demonstrate and understand this role.

8.5 Future directions

Men are more susceptible to AS then women, and disease is transmitted more to the son than the daughter. One explanation for this has been that there may be a Y chromosome effect. In collaboration with Harvard University, where the entire Y chromosome has previously been mapped, possible susceptibility genes will be examined. Blood from 50 AS males and 50 normal males will be analysed to establish if a link does exist. To date the bloods of 36 affected AS males have been collected and analysed.

The targeting of patients with severe and active AS (i.e. those with bowel disease or hip involvement) for disease modifying drugs was attempted for this study. It was hoped that the anti-TNF drug Etanercept could be given in a pilot study. However , supplies of the drug were limited and all research was stopped by the company. However, it is planned that Infleximab may now be used on a sample of patients to establish the beneficial effects of this new treatment.

The radiographic database is currently being expanded so that concordance of disease among siblings and parent-child pairs can be compared for AS specific changes. The level of change in the axial spine is different between the sexes. Thus, concordance of disease will

highlight how much radiological change is inherited and to what degree it is determined by sex of the patient.

The increased level of susceptibility to AS among the children of women with AS could be explained by transmission of the triggering agent through breast milk. A cohort study of breast feeding levels among those AS mothers with an affected child versus those with an unaffected child will be undertaken.

The effect of the X chromosome on severity of disease will be examined using similar methods to those used in the autosmal genome screen. In addition, in collaboration with The Wellcome Trust, Oxford more work will be undertaken to further focus in on the location of the severity genes.

To examine the age of first radiological change in the sacroiliac joints a study will be undertaken (if funding permits) examining by MRI the children (age 10-16) of women with young age at onset and the children of men with a strong family history. A cohort of B27 negative children will be used as a control to examine if first changes can be observed in some of the B27 positive children of AS patients.

8.6 In Summary

Susceptibility to AS is determined by susceptibility genes and an environmental trigger. The trigger may be pulled in childhood and first radiological change may begin as young as age 8. Separate severity genes are involved in determining the rate of progression and the extent of inflammation of the disease. There are many interlinking susceptibility genes and these will determine the areas susceptible to AS. There are a number of severity genes and these will interact to determine outcome. The genetic load of susceptibility and severity genes is determined by both parents whether or not they are affected by AS. The activity level of the person will modify the activity of the disease. Smoking will make progression worse especially in those patients with psoriasis. Education may help a person deal better

with their disease and perhaps adhere to the treatments prescribed. Further work should examine what genes are involved in susceptibility and severity and what the role or function is of these genes in the body. Drug therapy may then help modify the condition or in the long term prevent the disease in those most at risk.

Figure 36: Susceptibility genes for psoriasis/ IBD and iritis in men and women with AS.

Susceptibility gene	Severity gene	Sex	Outcome	
Ps/IBD	+ mild	Female AS	Mild AS,	No secondary disorder
Ps/IBD	+++ severe	Female AS	Severe AS,	IBD
Ps/IBD	+ mild	Male AS	Mild AS ,	No secondary disorder
Ps/IBD	+++severe	Male AS	Severe AS,	Ps
Iritis	+ mild	Female AS	Mild AS,	No secondary disorder
Iritis	+++ severe	Female AS	Severe AS,	Iritis
Iritis	+ mild	Male AS	Mild AS,	No secondary disorder
Iritis	+++ severe	Male AS	Severe AS,	Iritis

9. Final Statements.

1. BASRI is a valid measure to assess radiology in AS. It gives a description of the axial spine and hips. This constitutes the only rapid measure (ie 30 seconds to complete) which can give a global score of the radiographic status of the AS individual. Thus, it may be used both in research to compare large numbers of subjects rapidly, reliably and with minimal training, as well as in the clinic for routine follow-up .

2. The radiological progression of ankylosing spondylitis as a population is linear, progressing at a rate of 35% of available change every 10 years. It may begin as young as age 8 in the sacroiliac joints and then progresses up the spine to the lumbar and then cervical spine. A quarter of patients with severe disease will develop hip involvement. Factors associated with rate of progression of disease include sex of the patient, presence of iritis or hip involvement, education level and marital status.

3. Patients with severe disease (as determined by radiographs or disease activity or function) do not just have advanced disease in the spine but also suffer from inflammation in the hips, peripheral joints, skin, eye, bowel. Thus, severe AS is a more wide spread disease than mild AS. These additional areas become involved generally after the onset of the arthritis . Thus, with increasing disease duration the disease becomes more progressive. This level of severity appears to be inherit as siblings are concomitant for disease expression.

4. Susceptibility factors and severity factors are discrete. Susceptibility is determined by genetic load (which may include those genes determining inflammation of the eye, bowel and skin) and infective triggers which may take effect even in the uterine environment. Women with AS transmit the disease more often to their children, this may because they

carry a higher susceptibility load (ie they have more diseased relatives) or there may be a uterine environmental effect. Severity genes and environmental factors may determine the level and nature of the inflammation but susceptibility factors may determine the areas 'susceptible' to disease (ie bowel, eye skin, hip , peripheral joints etc).

5. Inherent severity level of disease may be modified by factors such as exercise, education and social support.

6. Future work should examine drugs specifically targeting the underlying inflammatory process involved in AS such as the drug anti-TNF. In addition, by targeting the genes involved in severity of AS and identify their protein products, it will be possible to develop disease modifying drugs and alter prognosis of disease for the first time.

10. References

[1] Calin A & Taurog J. (Eds) 1998. The Spondylarthritides. Oxford: Oxford University Press.

[2] Massare C. Anatomo-radiologie et vérité historique a propos du bilan xéroradiographique de Ramsés II. Bruxelles-Médical 59 (1979) 163-170.

[3] La Momie de Ramsés II. Éditions Recherche sur les Civilisations. Paris 1985.

[4] Feldtkeller E. APA Bane, The Saint with the palm disease. J Rheumatol 2000, 27 (s59): P 4.8.

[5] Seronegative polyarthrtis. Wright V, Moll J. North-Holland Publishing Company 1976.

[6] Calin A Ankylosing Spondylitis sine sacroliitis. (1979) Arthritis Rheum 22: 303-304.

[7] Khan M, van der Linden S, Kushner I. Spondylitic disease without radiological evidence of sacroilitis in relatives of HLA-B27 positive ankylosing spondylitis patients. Arthritis Rheum.1985 28: 40-43

[8] Gran J, Husby G, Hordvik M. Spinal ankylosing spondylitis : a variant form of ankylosing spondylitis or a distinct disease entity ? Ann Rheum Dis 1985; 44 : 368-371.

[9] Reuven Mader. Atypical clinical presentation of ankylosing spondylitis. Semin Arthritis Rheum 1999; 29 (3): 191-196.

[10] Braun J, Sieper J, Bollow M Imaging of Sacroiliitis. Clin Rheumatol 2000; 19 :51-57.

[11] Burgos-Vargas R, Naranjo A, Castillo J. Ankylosing spondylitis in the Mexican Mestizo : Patterns of disease according to age at onset. J Rheumatol 1989; 16 (2) 186-191.

[12] Malaviy A, Mehra N : Spondyloarthropathies in India . Spine State of the Art Reviews 4 : 679- 684, 1990.

[13] Claudepierre P, Gueguen A. Ladjouze A. Features associated with juvenile onset spondyloarthropathies in North Afric . Rev Rheum Engla Ed 63 : 87 1996

[14] Burgos-Vargas R, Petty R : Juvenile ankylosing spondylitis . Rheum Dis Clin North Am 18 : 123 1992.]

[15] Martinez-Lavin M, Mansilla J, Pineda C, Pijoan C. Ankyloisng spondylitis is indigenous to Mesoamerica J Rheumatol 1995, 22 (12): 2327-30]

[16] Deesomchoik U, Tumrasvin T : Clinical comparison of patients with ankylosing spondyltis, Reiter's syndrome and psoriatic arthritis. J Med Assoc Thai 76: 61, 1993].

[17] Roussou E, Chowdhry K, Calin A. Ethnic differences in ankylosing spondylitis: disease expression in Pakistan compared to Britain. J Rheumatol 1997; 24(3): 612-613.

[18]Claudepierre P, Gueguen A, Ladjouze A, Hajjaj-Hassouni N, Sellami S, Amor B, Dougados M. Features associated with juvenile onset of spondylarthropathies in north Africa. Rev Rhum Engl Ed 1996; 63(2): 87-91]

[19] Hajjaj-Hassouni N, Maetzel A, Dougados M, Amor B. Rev Rheum Ed Fr 1993; 60(6): 420-5]

[20] Mijiyawa M, Oniankitan O, Khan M. Spondyloarthropathies in sub-Saharan Africa. Curr Opin Rheumatol. 2000, 12 : 281-286.]

[21] Sonozaki H, Seki H, Chang S, Okuyama M, Juji T. Human lymphocyte antigen , HLA-B27, in Japanese patients with ankyloisng spondylitis. Tissue Antigens 1975 ; 5 : 131-136.]

[22] Beasley R, Bennett P, Lin C. Low prevalence of rheumatoid arthrits in Chinease . Prevalence survey in a rural community. J Rheumatol Suppl 1983 : 10 ; 11-5.]

[23] Koh W, Boey M, Ann Acad Med Singapore 1998; 27 (1) :3-6]

[24] Maddison P, Isenberg D, Woo P, Glass D (Eds) 1993. Oxford Textbook of Rheumatology Vol 2. Oxford: Oxford Medical Publications.

[25] Will R, Palmer R, Bhalla A, Calin A. Osteoporosis in early ankylosing spondylitis: a primary pathological event ? Lancet 1889; 2: 1483-5.

[26] Mitra D, Ring E, Bhalla A, Collins A. Osteoporosis associated with ankylosing spondylitis. In: Ring E, ed. Current research in osteiporosis and bone mineral measurement III. London: British Institute of Radiology. 1994: 42-3

[27] Mitra D, Elvins M, Speeden D, Collins A. The prevalence of vertabral fractures in mild ankylosing spondylitis and their relationship to bone mineral density. Rheumatology 2000; 39: 85-89

[28] Maghraoui A, Borderie D, Cherruau B, Edouard R, Dougados M, Roux C. Osteoporosis, Body Composition, and Bone Turnover in Ankylosing spondylitis. J Rheumatol 1999; 26(10): 2205-2209]

[29] Mullaji A, Upadhyay S, Ho E. Bone mineral density in ankylosing spondylitis: Dexa comparison of control subjects with mild and advanced cases. J Bone Joint Surg[Br] 1994; 76-B: 660-5.

[30] Will R, Palmer A, Bhalla K, Ring F, Calin A. Bone loss as well as formation is a feature of progessive ankylosing spondylitis. Br J Rheumatol XXIX (6): 498-499

[31] Calin A. The Margaret Holroyd Essay . The individual with ankylosing spondylitis : Defining disease status and the impact of the illness. Br J Rheumatol 1995; 34 : 663-672.

[32] Ruof J, Stucki G. Validity aspects of ESR and C-reactive protein in AS. 1999 J Rheum 26:4 : 966-70.

[33] Spoorenberg A, van de Heijde D, de Klerk E, Dougados M, de Vlam K, Mielants H, van de Tempel H, van d Lindens. Relative value of ESR and C-reactive protein assessment of disease activity in AS. 1999 J Rheum 26:4 : 980-984.

[34] Will R, Magaro L, Elswood J, Calin A. Ankylosing spondylitis (AS) and acute phase reactants (APRs). The elusive relationship. Br J Rheum 1989; 26 (S2): 11

[35] Tutuncu A, Bilgie A, Kennedy G, Calin A. Interleukin-6, acute phase reactants and clinical status in ankylosing spondylitis. Ann Rheum Dis. 1994;53:425-428.

[36] Garrett S, Jenkinson T, Kennedy G, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis : The Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994; 21 (12) : 2286-2291.

[37] van der Heijde D, Bellamy N, Calin A, Dougados M, Khan M, van der Linden S. Preliminary core sets for endpoints in ankylosing spondylitis. J Rheumatol 1997;24(11):2225-2228.

[38] van der Heijde D, Calin A, Dougados M, Khan M, van der Linden S, Bellamy N. Selection of instruments in the core set for DC-ART, SMARD, Physical Therapy and Clinical Record Keeping in Ankylosing spondylitis. Progress Report of the ASAS Workign Group. J Rheumatol 1999; 26 (4) 951-954.

[39] Calin A, Garrett S, Whitelock H, Kennedy G, O'Hea J, Mallorie P, Jenkinson T. A new approach to defining functional ability in ankylosing spondylitis : The development of the Bath Ankylosing Spondylitis Functional Index. J Rheuamtol 1994; 21 (12):22812285.

[40] Dougados M, Gueguen A, Nakache J, Nguyen M, Amor B. Evaluation of a functional index and an articular index in ankylosing spondylitis. J Rheumatol 1988; 15: 302-307.

[41] Jones S, Steiner A, Garrett S, Calin A. The Bath Ankylosing Spondylitis Patient Global Score (BAS-G). Br J Rheumatol. 1996; 35: 66-71.

[42] Taylor A, Balakrishnan C, Calin A. Reference centile charts for measures of disease activity, functional impairment and metrology in ankylosing spondylitis. Arthritis Rheum 1998; 41 (6): 1119-1125.

[43] Taurog J, Maika D, Satumtira N, Dorris M, McLean I, Yanagisawa H, Sayad A, Stagg A, Fox G, Le O'Brien A, Rehman M, Zhou M, Weiner A, Splawski J, Richardson J, Hammer R. Inflammatory disease in HLA-B27 transgenic rats. Immunol Rev 1999; 169: 209-23]

[44] Taurog J, Hammer R, Ecperimental spondyloarthropathy in HLA – B27 transgenic rats. Clin Rheumatol 1996; 15 (sup 1): 22-7]

[45] Nickerson C, Harvinder S, David C. Role of enterobacteria and HLA-B27 in spondyloarthropathies : sutdues with transgenic mice. Ann Rheum Dis 1990; 49: 426-433]

[46] Brewerton D, Hart F, Nicholls A, Caffrey M, James D, Sturrock R. Ankylosing Spondylitis and HLA-27. Lancet 1973;ii: 904-907.

[47]. Woodrow J, Eastmond C. HLA B27 and the genetics of ankylosing spondylitis. Ann Rheum Dis 1978;37:504-509.

[48] Thomson G: Investigation of the mode of inheritance of the HLA assocaited diseases by the method of antigen genotype frequency among diseased individuals. Tissue Antigens 1983; 21:81-104

[49] Rosenthal A, Egbert P, Calin A, Bombardier C, Payne R. Association of HLA-B27, ankylosing spondylitis and uveitis. International Congress Series N. 450 XXIII Concilium Ophthalmologicum, Kyoto 1978 Ed K Shimizu- Co- Ed JA Oosterhuis, Excerpta Medica, Amsterdam-Oxford, Elsevier North-Holland, 940-943.

[50] Brewerton D, Caffrey M, Nicholls A, Walters D, James D. (1973) Acute anterior uveitis and HLA-A27. Lancet,2, 994.

[51] Zierhut M, Feltkamp T, Forrester J. Immunology of the eye and the joint. Immunol Today 1994 15: 49

[52] Mielants H, Veys E, Goemaere S, Goethals K, Cuvelier C and De Vos M (1991a) Gut inflammation in the spondylarthropathies: clinical, radiological, biological and genetic features in relation to the type of hisology. A prospective study. J Rheumatol; 18: 389-393.

[53]. Nuki G. Ankylosing spondylitis, HLA B27 and beyond. 1998 Lancet; 351:767-768

[54] Khan M, HLA-B27 and its subtypes in world populations. Curr Opinons in Rheumatol 1995;7:263-269

[55] Calin A, Bennett P, Jupiter J, Terasaki P. HLA B27 and sacroilitis in Pima Indians- Association in Males only . J Rheumatol 1977 (S3), 4: 44-48.

[56]. Khare S, Luthra H, David C. HLA-B27 and other predisposing factors in spondyloarthropathies. 1998 Curr Opin Rheumatol; 10: 282-289.

[57] Brown M Kennedy G, MacGregor A, Darke C, Duncan E, Shatford J Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997; 40: 1823-1858

[58] van der Linden. S, Valkenburg H, de Jongh B, Cats A. The risk of developing ankylosing spondylitis in HLA B27 positive individuals: a comparison of relatives of spondylitis patients with the general popualtion. Arthritis Rheum 1984: 27: 241-249.

[59] Calin A, Fries J The striking prevalence of ankylosing spondylitis in 'healthy' W27 positive males and females. A controled study. N Engl J Med (1975);293: 835-839.

[60]. Calin A, Marder A, Becks E, Burns T. Genetic differences between B27 postive patients with Ankylosing Spondylitis and B27 postive healthy controls. Arthritis Rheum 1983; 26: 1460-1464.

[61]Taurog J, Satumtira N, Richardson J, simmns W, Hamer W. Alleles of the dark Agouti (DA) rat strain are protective agant inflammatory disease in HLA-B27 transgenic rats. Arthritis Rheum 1996: 39:S121. 12.

[62] Brown M, Jepson A, Young A, Whittle H, Greenwood B, Wordsworth B. Ankylosing spondylitis in West Africans-evidence for a non-HLA-B27 protective effect. Ann Rheum Dis 1997; 56:68-70.

[63] Awada H, Baddoura R, Naman R, et al Weak associations between HLA-B27 and the spondylarthropathies in Lebanon. Arthritis Rheum 1997; 40 (2):388-389

[64] Chalmers I. Ankylosing spondylitis in African Blacks. Arthritis Rheum 1980;23: 1366-70

[65] Stein M, Davis P, Emmanuel J, West G. The Spondyloarthropathies in Zimbabwe: A Clinical and Immunogenetic Profile J Rheumatol 1990; 17:10 1337-1339

[66] Robinson W, van der Linden S, Khan M, Rentsch H-U, Cats A, Russell A, Thomson G. HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27+ patients. Arthritis Rheum 1989; 32 (9) 1135-1141.

[67] Rubin L, Amos C, Wade J Investigating the genetic basis for ankylosing spondylitis. Linkage studies with the major histocampatibility complex region. Arthritis Rheum 1994; 37: 1212-1220

[68]. Brown M, Pile K, Kennedy LG, Calin A, Darke C, Bell J, Wordsworth B, Cornèlis F. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 1996; 55: 268-270.

[69] Alarcon G, Garcia A, Behena S, Melin-Aldana H, andrade F, Ibañez-de-Kasep G, alcocer-Varela J, Alarcòn-Segovia D, Granados J. HLA-B alleles and complotypes in Mexican patients with seronegative spondylarthropathies. Annals of Rheumatic Diseases. 1994; 53:755-758.

[70] Derhaag P, van der Horst A, De Waal L, Feltkamp T. HLA B27(+) acute anterior uveities and other antigens of the major histocompatability complexs. Invest. Ophthalmol Vis Sci 1989; 30: 2160-2164.

[71] Reveille J, Suarez-Almazor M, Russell A, Go R, Appleyard J, Barger B, Acton R, Koopman W, McDaniel D. HLA in ankylosing spondylitis: is the HLA-B27 the only MHC gene involved in disease pathogenesis ? Semin Arthritis Rheum 1994; 23 : 295-309.

[72] Khan M, Kushner I, Braun W. Genetic heterogeneity in primary ankylosing spondylitis. J Rheumatol 1980; 7:383-386.

[73] Khan M, Kushner I , Braun W. B27-negative HLA- Bw16 in ankylosing spondylitis. Lancet 1978 1:1370

[74] Yamaguchi A, Tsuchiya N, Mitsui H. Association of HLA-B39 with HLA-B27 negivitve ankylosing spondylitis and pauciartcular juvenile rheumatoid arthritis in Japanese patients. Arthritis Rheum. 1995 38: 1672

[75]. Safwenberg J, Domeij-Nyberg B, Kjallmann M. HLA antigens in females with ankylosing spondylitis and other forms of seronegative rheumatic diseases. Scand J Rheumatol 1978; 7:177-182.

[76] Wagener P, Zeidler H, Eckert G, Deicher H. Increased frequency of HLA-Bw66 and Bw35 CREG antigens in HLA-B27 negative ankylosing spondylitis. J Rheumatol 1984; 43:253-257.

[77] Brown M, Kennedy G, Darke C, Gibson K, Pile K, Shatford J, Taylor A, Calin A, Wordsworth P. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. Arthrit Rheumat 1998; 41 (3) 460-465.

[78] Miehle W, Schattenkirchner M, Albert D, Bunge M. HLA-DR4 in ankylosing spondylitis with different patterns of joint involvement. Ann Theum Dis 1985; 44:39-44

[79] Lopez D, Barber D, Villadangos J, Lopez de Castro J. Cross-reactive T cell clones from unrelated individuals reveal similarities in peptide presentaion between HLA-B27 and HLA-DR2. J Immunol 1993; 150: 2675-2686.

[80] La N, Mathieu A, Mulargia M, Carcassi C, Vacca A , Ledda A. Association of the HLA-A2, CW2, B27, S31, DR2 haplotype with ankylosing spondylitis: a possible role for non-B27 factors in the disease. Dis Markers 1993; 11: 191-203.

[81] Monowarul Islam S, Numaga J, Fujino Y, Masuda K, Ohda H, Hirata R, Maeda H, Mitsui H. HLA-DR8 and acute anterior uveitis in ankylosing spondylitis. Arthritis Rheum 1995 ; 38 (4): 547-550.

[82] Maksymowych W. Gorodezky C, Olivo A, Alaez C, Wong C, Burgos-Vargas R, Sanchez-Corona J, Ramos-Remus C, Russell A. HLA-DRB1*08 influences the development of disease in Mexian Mestizo with Spondyloarthropathy. J Rheumatol 1997;25(5):905-907.

[83] Ploski R, Flato B, Vinje O, Maksymowych W, Førre Ø, Thorsby E. Association to HLA DRB1*08, HLA DPB*0301 and homozygosity for an HLA linked proteasome gene in juvenile ankylosing spondylitis. 1995 Hum Immunol 44: 88-96.

[84] Maksymowych W, Russel S,: Polymorphism in the LMP2 gene influences the relative risk for acute anterior uveitis in unselected patients with ankylosing spondylitis.Clin Invest Med 1995; 18:42-46

[85] Maksymowych W, Jhangri G, Gorodezky C, Luong M, Wong C, Burgos-Vargas R, Morenot M, Sanchez-Corona J, Tamos-Remus C, Russel A. The LMP2 polymorphism is associated with susceptibility to acute anterior uveitis in HLA-B27 positive juvenile and adult Mexican subjects with ankylosing spondylitis. Ann Rheumat Dis 1997;56:488-492.

[86] Ploski R, Maksymowych W, Førre. HLA-DR8 and susceptibility to acute anterior uveitis in ankylosing spondylitis: Comment. Arthritis Rheumat 1996;39 (2): 351

[87] Burney R, Pile K, Gibson K, Calin A, Kennedy G, Sinnott P, Powis S, Wordsworth P. Analysis of the MHC class II encoded components of the HLA class I antigen processing pathway in ankylosing spondylitis. Ann Rheum Dis 1994;53:58-60.

[88] Lau C, Burgos-Vargas R, Louthrenoo W, Mok M, Wordsworth P, Zeng Q. Features of spondyloarthritis around the world. Rheum Dis Clin North Am 1998; 24 (4) 753-767].

[89] Hohler T, Schaper T, Schneider P, zumBuschenfelde K, MarkerHermann E. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. Arthritis Rheumat 1998: 41 (8) : 1489-1492.

[90] Scofield RH, Harley J. Genetic analysis of HLA B in ankylosing spondylitis. J Rheumatol 1998; 25 sup 54 : 6

[91] Lòpez de Castro J. HLA-B27 polymorphism and its effect on peptide presentation and disease. J Rheumatol 1998; 25 supp 54: 5.

[92] Lopez-Larrea C, Sujirachato K, Mehra N HLA-B27 subtypes in Asian patients with ankylosing spondylitis. Tissue Antigens 1995; 45:169-76

[93] Ren E, Koh W, Sim D, Boey M, Wee G, Chan S. Possible protective role of HLA-B*2706 for ankylosing spondylitis. Tissue Antigen 1997; 49:67-9

[94] Sudarsono D, Hadi S, Mardjuadi A, Nasution A, Diekker-Saeys A, Breur-Vriesendorp B, Lardy N, Feltkamp T Evidence that HLA-B2706 is not protective against spondyloarthropathy. J Rheumatol 1999;26:7 1534-1536

[95] Brown M, Pile K, Kennedy G. Campbell D, Andrew L, Msrch R, Shatford J, Weeks D, Calin A, Wordsworth P. A Genome-wide screen for susceptibility loci in ankylosing spondylitis. Arthritis Rheumat 1998; 41 (4): 588-595

[96] Brown M, Rudwaleit M, Pile K, Kennedy L, Shatford J, Amos C, Siminovitch K, Rubin L, Calin A, Wordsworth P. The role of germline polymorphisms in the T-cell receptor in susceptibility to ankylosing spondylitis. Br J Rheumatol 1998; 37: 454-458.

[97] Moll J, Haslock I, Macrae I, Wright V. Associations between ankylosing spondyitis, psoriatic arthritis, reiter's disease, the intestinal arthropathies, and behcet's syndrome. Medicine; 53 (5): 343-363.

[98] Linssen A B27(+) disease versus B27(-) disease. Scand J Rheumatology 1990 supp 87 111-119

[99] Mielants H, Veys E, Cuvelier C, de Vos M. Subclinical involvement of the gut in undifferentiated spondyloarthropathies. Clin Exp Rheumatol 1989; 7: 499-504

[100] Leirisalo-Repo M, Turunen U, Stenman S, Helenius P, Sappala K, High frequency of silent inflammatory bowel disease in spondyarthropathy. Arthritis Rheumat; 37 (1): 23-31.

[101] Claudepierre P, Gueguen A, Ladjouze A, Hajjaj-Hassouni N, Sellami S, Amor B, Doughados M. Predictive factors of severity of spondylaorthropathy in North Africa. Br J Rheum 1995; 34: 1139-1145

[102] Vaile J, Meddings J, Yacyshyn B, Russell A, Maksymowych W. Bowel permeability and CD45RO expression on circulation CD20+ B cells in patients with ankylosing spondylitis and their relatives. J Rheumatol. 1999; 26:1 128-135

[103] Geczy A, Alexander K, Bashir H, Edmonds J, Upfold L, Sullivan J. HLA-B27, Klebsiella and ankylosing spondylitis: biological and chemical studies. Immunol Rev 1983: 70: 23-50.

[104] Ebringer R, Cawdell D, Cowling P, Ebringer A. Sequential studies in ankylosing spondylitis. Association of Klebsiella pneumoniae with active disease.Ann Rheum Dis 1978; 37: 146-151.

[105] Sahly H, Podschum R, Kekow J, Nolle B, Gross W, Ullmann U. Humoral immune response to Klebsiella capsular polysaccharides in HLA-B27-positive patients with anterior uveitis and ankylosing spondylitis. Autoimmunity 1998; 28 (4): 209-215.

[106] Makilkola O, Nissila N, Lehtienen K, Granfors K. IGA class serum antibodies against three different Klebsilla serotypes in ankylosing spondylitis. Br J Rheumatol. 1998; 37 (12): 1299-1302.

[107] Fielder M, Pirt S, Tarpe C, Wilson C, Cunningham P, Ettelaie C, Biner A, Bansal S. Ebringer A. Molecular mimicry and ankylosing spondylitis: possible role of a novel sequence in pullulanase of Klebsiella pneumoniae. FEBS Letter 1995, 369 :243-248.

[108] Sprenkels S, Kregten E, Feltkamp T. IgA Antibodies against Klebsiella and other Gram-negitive bacteria in Ankylosing spondylitis and acute anterior uveitis. Clin Rhem 1996;15 (sup1): 48-51

[109] van Kregten E, Huber-Bruning O, Vanderbroucke J, Willers J. No conclusive evidence of an epidemiological relation between Klebsiella and ankylosing spondylitis. J Rheumatol 1991; 18: 384-8

[110] Smith G. Blackwell C, Nuki G. Faecal flora in spondyloarthropathy. Br J Rheumatol. 1997;36 : 850-854.

[111] Cancion-Diaz M, Perez-Salazaar J, Dominguez-Lopez L, Antibody reponses to Klebsiella pneumoniae 60 kDa protein in familial and sporadic ankylosing spondylitis: Role of HLA B27 and protein characterization as Gro-El HSP60. J Rheumatol.

[112] Hajjaj-Hassouni N, Maetzel A, Dougados M, Amor B. Comparison of patients evaluated for spondylarthropathy in France and Morocco. Rev Rheum Ed Fr 1993; 60 (6) : 420-425.

[113] Claudepierre P, Sibilia J, RoudotThoraval F, Flipo R, Wendling D, Goupille P, Eulry F, Clerc D, Berthelot J, Vergne P, Allanore Y, LargetPiet B, Chevalier X. Factors linked to disease activity in a French cohort of patients with spondyloarthropathy. J Rheumatol. 1998; 25 (10): 1927-1931.

[114] Averns H, Oxtoby J, Taylor H, Jones P, Dziedzic K, Dawes P. Smoking and outcome in ankylosing spondylitis. Scand J Rheumatol 1996; 25: 138-42.

[115] Roussou E, Kennedy G, Garrett S, Calin A. Socio-economic status in ankylosing spondylitis: relationship between occupation and disease activity. J Rheumatol 1997; 24 (5): 908-911.

[116] Gran J, Skomsvoll. The outcome of ankylosing spondylitis: A study of 100 patients. Br J Rhematool 1997; 36: 766-771.

[117] Guillemin F, Briancon S, Pourel J, Gaucher A. Long term disability and prolonged sick leaves as outcome measures in ankylosing spondylitis. Arthritis Rheum. 1990; 33: 1001-1006.

[118] Kraag G, Stokes B, Groh J, Helewa, Goldsmith C.The effects of comprehensive home physiotherapy and spervision on patients with ankylosing spondylitis- A random controlled trial. J Rheumat 1990; 17 (2) 228-233.

[119] Band D, Jones S, Kennedy G, Garrett S, Porter J, Gray L, Richardson J, Whitelock H, Calin A. Which patients with ankylosing sponylitis derive most benifit from and inpatient management program. J Rheumat 1997; 24: 2381-2384.

[120] Hajjaj-Hassouni N, Maetzel A, Dougados M, Amor B. A comparative study of patients evaluated for spondylarthropathy in France and Morocco.Rev Rheum (Engl edn) 1993; 60: 341-346.

[121] Amore B, Santos R, Nahal R, Listrat V, Dougados M. Predictive factors for the long-term outcome of spondyloarthropathies. J Rheumato 1994; 21 (10) 1883-1887.

[122] Jacoby R, Newell R, Hicking P. Ankylosing spondylitis and trauma: the medicolegal implications. A comparative study of patients with non-specific back pain. Ann Rheum Dis 1985;44: 307-311.

[123] Kennedy G, Will R, Calin A. Sex ratio in the spondylarthripathies and its relationship to phenotypic expression, mode of inheritance and age at onset.

[124] W James. Sex ratio and hormone in HLA related rheumatic diseases Ann R Dis 1991; 50: 401-404

[125] Hart F. Bechterew's syndrome in women: is it different from that in men ? Scand J Rheumatol 1980; 32:38-40

[126] Gran J, Husby G, Hordivik M, Stormer J, Romberg-Andersen O. Radiological changes in men and women with ankylosing spondylitis. Ann Rheum Dis 1984; 43:570-5

[127] Ostensen M, Ostensen H. Ankylosing spondylitis-the female aspect. J Rheumatol 1998;25 (1):120-124]

[128] Spector T, Ollier W, Perry L . Free and serum testosterone levels in 276 males: A comparative study of rheumatoid arthritis, ankylosing spondylitis and healthy controls. Clin Rheumatol 1989; 8: 37-41

[129] Masi A, Do sex hormones play a role in ankylosing spondyltis ? Rheum Dis Clin North Am 1992; 18:153-76

[130] Tapia-Serrano R, Jemenez-Balderas F, Murrieta S, Bravo-Gatica C, Guerra R, Mintz G. Testicular function in active ankylosing spondyltis.Therapeutic response to human chorionic gonadotrophin. J Rheumatol 1991; 18: 841-8

[131] Ivanyi P, Hampl R, Starka L , Mickova M. Genetic association between H-2 gene and testosterone metabolism in mice. Nature New Biology 1972;238:280-281

[132] Giltay E, Popp-Snijders C, van Schaardenburg D, Dekker-Saeys B, Gooren L, Dijkmans B. Serum Testosterone Levels are not elevated in patients with ankylosing spondylitis.J Rheum 1998 ; 25:12]

[133] Giltay E, VanSchaardenburg D, Gooren L, PoppSnijders C, Dijkmans B. Androgens and Ankylosing Spondylitis: A role in the pathogenesis ? Ann of New York Academy of Sciences 1999;846: 340-365]

[134] Testosterone and testosterone free index in mild ankylosign spondylitis: relationship with bone mineral density and vertebral fractures. Mitra D, Elvins D, Collins A. J Rheumatol 1999; 26:2414-2417

[135] Averns H, Oxtoby J, Taylor H, Jones P, Dziedzic K, Dawes P. Radiological outcome in ankylosing spondylitis: use of the stoke ankylosing spondylitis spine score (SASSS) Br J Rheumat. 1996;35:373-6,

[136] Ralston S, Urquhart G, Brzeski M, Sturrock R. A new method for the radiological assessment of vertebral squaring in ankylosing spondylitis. Ann Rheum Dis 1992; 51:330-3.

[137] MacKay K, Mack C, Pande I, Roussou E, Calin A. The Bath Ankylosing Spondylitis Radiology Index: definition and correlation with functional, disease activity and metrology indices [abstract]. Arthritis Rheum. 1996; 39 Suppl 9:S206.

[138] K Mackay, C Mack, S Brophy, A Calin. The Bath Ankylosing spondylitis radiology index (BASRI): A new, validated approach to disease assessment. Arthritis Rheum. 1998;41 (12): 2263-2270.

[139] Boers M, Brooks P, Strand C, Tugwell P. The OMERACT filter for outcome measures in rheumatology. J Rheumatol 1998 ; 25: 1-2.

[140] Larsen A, Dale K, Morten E. Radiographic evaluation of Rheumatoid Arthritis and related conditions by standard reference films. Acta Radiologica 1977; 18: 481-491

[141] Mackay K, Brophy S, Mack C, Doran M, Calin A. The development and validation of radiograpic grading system for the hip in ankylosing spondylitis: the Bath Ankylosing Spondylitis Radiology Hip Index (BASRI-H). 2000 J Rheumatol. 27 (12): 2866-2872.

[142] Kirkwood B. Essentials of Medical Statistics. Blackwell Science 1988

[143] Altman D, Chitty L. Charts of fetal size. 1. Methodology. Br J Obstet Gynaecol 1994; 101: 29-34.

[144] Graudal N, Grethe Jurik A, de Carvalho A, Graudal H. Radiograpic progression in rheumatoid arthritis: A long term prospective study of 109 patients. Arthritis Rhuem. 1998; 41 (8): 1470-1480.

[145] Calin A. Ankylosing spondylitis sine sacroliitis. Arthritis Rheum 1979; 22 : 303-304.

[146] Khan M, van der Linden S, Kushner I. Spondylitic disease without radiological evidence of sacroilitis in relatives of HLA-B27 positive ankylosing spondylitis patients. Arthritis Rheum. 1985; 28: 40-43.

[147]. Wolfe F, Sharp J. Radiographic outcome of recent-onset rheumatoid arthritis: A 19 year study of radiographic progression. Arthtitis Rheum. 1998; 41 (9): 1571-1582.

[148]. Kennedy G, Edmunds L, Calin A. The natural history of ankylosing spondylitis : Does it burn out ? J Rheumat 1993; 20 (4): 688-692

[149]. Oostveen J, Prevo R, den Boer J, van de Laar M. Early detection of sacroiliitis on magnetic resonance imaging and subsequent development of sacroiliitis on plain radiography. A prospective, longitudinal study. J Rheumatol 1999; 26(9): 1953-8.

[150] Baggle S, Lyons J, Angell E, Barkhuizen A, Han Y, Planck S. HLA-B27 transgenic Lewis rats and iritis after gram-negative infection. Investigative Ophthalmology and Visual Science 1996;37 (3):172

[151]. Taurog J, Richardson J, Croft, J. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. Journal of experimental Medicine (1994) 180, 2359-2364.

[152] Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. 1999 The Lancet; 354: 1687-1690.

[153] Moll J, Haslock I, Macrae I, Wright V. Association between ankylosing spondylitis, psoriatic arthritis, reiter's disease, the intestinal arthropathies and Behcet's syndrome. Medicine (1974); 53(5): 343-64

[154] Lee F.I, Bellary S.V, Francis C. Increased occurrence of psoriasis in patients with Crohn's disease and their relatives. *Am. J. Gastroenterol.* 1990; 85: 962-923

[155] Yates VM, Watkinson G, Kelman A. Further evidence for an association between psoriasis, Crohn's disease and ulcerative colitis. *Br. J. Dermatol.* 1982;106:323-30

[156] Hammer B, Ashurst P, Naish J. Diseases associated with ulcerative colitis and Crohn's disease. Gut 1968; 9:17-21.

[157]. Catsarou-Casari A, Katsambas A, Theodoropoulos T, Stratigos J. Ophthalmlogical manifestations in patients with psoriasis. Acta Derm Venereol (Stockn); 64 :557-558

[158]. Tromp G Kuivaniemi H, Rapael S, Alakokko L, Christiano A, Considine E Genetic linkage of familial granulomatous inflammatory arthritis, skin rash and uveitis to chromosome 16. *Am. J. Hum.Genet.* 1996;59 5:1097-1107

[159]. Nair R, Henseler T, Jenisch S, Stuart P, Bichakjian C, Lenk W, Westphal E, Guo Sun-Wei, Christophers E, Voorhees J, Elder J. Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Human Molecular Genetics* 1997; 6: 1349-1356

[160]. Curran M, Lau K, Hampe J, Schreiber S, Bridger S, Macpherson A, Cardon L et al and Lennard-Jones J. Genetic Analysis of inflammatory bowel disease in large european cohort supports linkage to Chromosome 12 and 16. *Gastroenterology* 1998; 115: 1066-1071.

[161]. Tiilikainer A, Lassus A, Karvonen J. Psoriasis and HLA-Cw6. *Br. J. Dermatol.* 1980; 102: 179-84.

[162]. Mielants H, Veys E, Joos R, Cuvelier C, De Vos M Repeat ileocolonoscopy in reactive arthritis *J Rheumatol* (1986b) ; 14: 456- 8

[163] Brewerton D, Caffrey M, Hart F, James D, Sturrock R. Ankylosing spondylitis and HLA B27. *Lancet* 1973;1:904-906

[164]. Careless D, Inman R. Acute anterior uveitis-clinical and experimental aspects. *Semin Arthritis Rheum* 1995; 24, 6 : 432-441

[165]. Mielants H, Veys E, Cuvelier C, de Vos M. Subclinical involvement of the gut in undifferentiated spondyloarthropathies. Clin Exp Rheumatol 1989; 7: 499-504

[166]. Maksymowych W, Chou C, Russell A. Matching prevalence of peripheral arthritis and acute anterior uveitis in individuals with ankylosing spondylitis. *Ann Rheum Dis* 1995; 54: 128-130.

[167] Calin A, Kennedy G, Edmunds L, Wills R. Familial versus sporadic ankylosing spondylitis: Two different diseases ? Arthritis and Rheumatism. 1993 ; 36 (5) : 676

[168] Woodrow J, Eastmond C. HLA B27 and the genetics of anklosing spondylitis. Annals of the Rheumatic Diseaease. 1978; 37: 504-509.

[169] Brewerton D, Caffrey M, Nicholls A, Walters D, James D. HL-A 27 and arthropathies associated with ulcerative colitis and psoriasis. Lancet 1 956-958.

[170] Calin A, Elswood J. The natural history of juvenile-onset ankylosing spondylitis: a 24 year retrospective case control study. Br. J Rheumatol. 1988;27: 101-103

[171] Calin A, Elswood J. The relationship between pelvic, spinal and hip involvement in ankylosing spondylitis- one disease process or several ? Br. J Rheumatol. 1988;27 393-395.

[172] Calin A, MacKay K, Santos H, Brophy S. A New Dimension to Outcome: Application of the Bath Ankylosing Spondylitis Radiology index. J Rheumatol. 1999;26 (4): 988-992.

[173] Caplanne D et al. Late onset spondylarthropathy: clinical and biological comparison with early onset patients. Ann Rheum Dis 1997;56 (3): 176-179

[174] Calin A, et al . Late onset ankylosing spondylitis: A distinct disorder ? Br J Rheumatol 1991;30: 69-70.

[175] Uksila J, Toivanen P, Granfors K. Enteric infections and arthritis: bacteriological aspects. In: Calin A, Taurog T. Editors. The Spondylarthrides Oxford University Press 1998: 167-177.

[176]. Calin A: Ankylosing spondylitis Clin Rheum Dis 1985 **11**:41-60

[177]. Mackay K, Brophy S, Mack C, Calin A. Patterns of radiological axial involvement in 470 Ankylosing Spondylitis (AS) patients. Arthritis Rheum 1997; **40**(9): 561

[178] Calin A. Ankylosing spondylitis in Maddison P, Isenberg D, Woo P, Glass D. Oxford Textbook of Rheumatology Vol 2. Oxford Medical Publications 1993

[179] Armstrong B.D, MacFarlane D, Panayi G. Ankylosing spondylitis and Klinefelter's syndrome: Does the X chromosome modify disease expression ? Br J Rheumatiol. (1985);**24** : 277-281.

[180] van der Linden S, Valkenburg H, de Jongh B, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. Arthritis Rhuem (1984) ; **27**, 3 : 241-249

[181] Mφller P, Vinje O, Dale K, Berg K, Kåss E. Family studies in Bechterew's Syndrome (ankylosing spondylitis). Scand J Rheumatology (1984)**13**: 1-10

[182] Calin A, Marder A, Becks E, Burns T. Genetic differences between B27 positive patients with ankylosing spondylitis and B27 positive healthy controls. Arthritis Rhuem 1983; **26** ; 12 1460-1464

[183] Thomson G. HLA disease associations: models for the study of complex human genetic disorders. Clin Lab Sci 1995; 32: 183-219.

[184] Lynn Ah. Kwoh CK, Venglish CM, Aston CE, Chakravarti A. Genetic epidemiology of rheumatoid arthritis. Am J Hum Genet (1995);**57** (1) 150-159

[185] Simmons W, Summerfield S, Roopenian D, Slaughter C, Zuberi A, Gaskell S, Bordoli R, Hoyes J, Moomaw C, Colbert R, Leong L, Butcher G, Hammer R, Taurog J: Novel HY Peptide Antigens Presented by HLA-B27. J Immunol 1997;**159**:2750-2759

[186] Brown M, Kennedy G, MacGregor A, Darke C, Duncan E, Shatford J, Taylor A, Calin A, Wordsworth P. Susceptibility to ankylosing spondylitis in twins. Arthritis Rhuem; 40 (10): 1823-1828.

[187] Taylor A, Balakrishnan C, Calin A. Reference centile charts for measures of disease activity, functional impairment, and metorlogy in ankylosing spondylitis. Arthritis Rheum 1998; 41 (6): 1119-1125.

[188] Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. Lancet 1999; 354: 1687-90.

[189] Krug H, Taurog J. HLA-B27 has no effect on the phenotypic expression of progressive ankylosis in ank/ank mice. J Rheumatol 2000 May, 27(5): 1257-9

[190] A. Awada H, Abi-Karam G, Baddoura R, Okais J, Attoui S. Clinical, radiological and laboratory findings in Lebanese spondylarthropathy patients according to HLA-B27 status. Joint Bone Spine 2000; 67(3):194-8.

[191] Burden A, Javed S, Bailey M, Hodgins M, Connor M, Tillman D. Genetics of psoriasis: paternal inheritance and a locus on Chromosome 6p. J Invest Dermatol 1998 Jun; 110(6): 958-60.

[192]. Rahman P, Gladman D, Schentag C, Petronis A. Excessive paternal transmission in psoriatic arthritis. Arthritis Rheum 1999 Jun; 42(6): 1228-31.

[193] Wright V, Moll J. Seronegative polyarthritis. Elsevier/North-Holland Publishing 1976.

[194]. Mattey D, Hassell A, Dawes P, Jones P, Yengi L, Alldersea J, Strange R, Fryer A. Influence of polymorphism in the managanese superoxide dismutase locus on disease outcome in rheumatoid arthritis: evidence for interaction with glutathione S-transferase genes. Arthritis Rheum 2000 Apr; 43(4): 859-64.

[195] Weinreich S, Hoebe, Ivanyi P. Maternal age influences risk for HLA-B27 associated ankylosing enthesopathy in transgenic mice. Ann Rheum Dis 995; 54: 754-6.

[196] Calin A. Ankylosing spondylitis and its management: Rheumatology in Practice 1994;Winter 14-6.

[197] Gall V: Exercise in the Spondyloarthropathies: Arthritis Care Res 1994;7:215-20.

[198] Hidding A, Van der Linden S, De Witte L: Therapeutic effects of individual physical therapy in ankylosing spondylitis related to duration of disease. Clin Rheumatol 1993;12:334-340.

[199] Kraag G, Stokes B, Groh J, Helewa A, Goldsmith C: The effect of comprehensive home physiotherapy and supervision on patients with ankylosing spondylitis - A randomized controlled trial. J Rheumatol 1990;17:228-33.

[200] Helliwell PS, Abbott CA, Chamberlain MA: A randomised trial of three different physiotherapy regimes in ankylosing spondylitis. Physiotherapy 1996;82:85-90.

[201] Russell P, Unsworth A, Haslock I: The effect of exercise on ankylosing spondylitis - A preliminary study. Br J Rheumatol 1993;32:498-506.

[202] Viitanen JV, Lehtinen K, Suni J, Kautiainen H: Fifteen months' follow-up of intensive inpatient physiotherapy and exercise in Ankylosing Spondylitis. Clin Rheumatol 1995;14:413-9.

[203] Kraag G, Stokes B, Groh J, Helewa A, Goldsmith CH: The effect of comprehensive home physiotherapy and supervision on patients with ankylosing spondylitis - A 8-month followup. J Rheumatol 1994;21:261-3.

[204] Pearcy MJ, Wordsworth BP, Portek I, Mowat AG: Spinal movements in ankylosing spondylitis and the effect of treatment. Spine 1985;10:472-4.

[205] Tomlinson MJ, Barefoot J, Dixon AJ: Intensive In-patient physiotherapy courses improve movement and posture in ankylosing spondylitis. Physiotherapy 1986;75: 6-9.

[206] O'Driscoll SL, Jayson MIV, Baddeley H: Neck movements in ankylosing spondylitis and their responses to physiotherapy. Ann Rheum Dis 1978;37:64-6.

[207] Viitanen JV, Suni J, Kautiainen H, Liimatainen M, Takala H: Effect of physiotherapy on spinal mobility in ankylosing spondylitis. Scand J Rheumatol 1992;21:38-41.

[208] Roethlisberger FJ, Dickson WJ: Management and the worker. Cambridge, MA: Harvard University Press, 1939.

[209] Hidding A, Van der Linden S, Gielen X, de Wite L, Dijkmans B, Moolenburgh D: Continuation of group therapy is necessary in ankylosing spondylitis. Arthritis Care Res 1994;7:90-6.

[210] Stenström CH: Therapeutic exercise in rheumatoid arthritis. Arthritis Care Res 1994;7:190-7.

[211]Stenström CH, Arge B, Sundbom A: Home exercise and compliance in inflammatory rheumatic diseases - A prospective clinical trial. J Rheumatol 1997;24:470-6.

[212] Roussou E, Kennedy LG, Garrett S, Calin A: Socioeconomic status in ankylosing spondylitis: Relationship between occupation and disease activity. J Rheumatol 1997;24:908-11.

[213] FitzGerald JD, Grossman JM. Validity and reliability of retrospective assessment of disease activity and flare in observational cohorts of lupus patients. Lupus 1999;8: 638-644.

[214] Caldwell J, Furst D, Smith A, Clark J, Bonebrake R, Gruhn W, McIlwain H, Logue C. Flare during drug withdrawal as a method to support efficacy in

Rheumatoid Arthritis: Amiprilose hydrochloride as an example in a double blind, randomised study. J Rheumatol 1998;25:1; 30-35

[215] Wolfe F, Johnston C, Yee B. Preliminary criteria for flare in rheumatoid arthritis. Arthritis Rheumat. 1997; 40(9): S1688

[216] Will R, Magaro L, Elswood J, Calin A. Ankylosing spondylitis (AS) and acute phase reactants (APRs). The elusive relationship. Br J Rheum 1989; 26 (S2): 11

[217] Raza K, Kennedy G, Calin A. Maternal age and the risk of developing ankyloing spondylitis. An Rheum Dis 1997; 56: 209-210.

[218] Paudoin P, van der Horst-Bruinsma E, Dekker-Saeys A, Weinreich S, Bezemer P, Dijkmans B. Increased risk of developing ankylosing spondylitis among first-born children. Arthritis Rheum 2000; 43 (12) : 2818-2822.

[219]. Goodman K, Correa P. Transmission of Helicobacter pylori among siblings. Lancet 2000 ; 355 (9201): 358-62.

[220] . Nielsen H, Dorup J, Herlin T, Larsen K, Nielsen S, Pedersen F. Epidmeiology of juvenile chronic arthritis: risk dependent on sibship, parental income, and housing. J Rheumatol 1999; 26 (7): 1600-5

[221]. Bingley P, Douek I, Rogers C, Gale E. Influence of maternal age at delivery and birth order on risk of type 1 diabetes in childhood: prospective population based family study. BNJ 2000 ; 321(7258):420-4

[222]. Dahlquiest G, Ivarsson S, Lindberg B, Forsgren M. Maternal enteroviral infection during pregnancy is a risk determinant for childhood IDDM. Diabetes. 1995; 44: 408-13.

[223] van der Heijde D, van der Linden S, Dougados M, Bellamy N, Russell A, Edmonds J. Ankylosing spondylitis : Plenary discussion and results of voting on selection of domains and some specific instruments. J Rheumatol 1999; 26 (4): 1003-1005.

[224] Dawes P. Stoke Ankylosing Spondylitis Spine Score J Rheumatol. 1999 Apr;26(4):993-6.)

[225] Radiological scoring methods in ankylosing spondylitis: reliability and sensitivity to change over one year. Spoorenberg A, de Vlam K, van der Heijde D, de Klerk E, Dougados M, Mielants H, van der Tempel H, Boers M, van der Linden S. J Rheumatol 1999; 26 (4): 997-1002]

[226] Miceli- Richard C, Said-Nahal R, Breban M. Impact of sex on inheritance of ankylosing spondylitis. Lancet 2000: 355(9209): 1097-8; discussion 1098.

[227] Feldtkeller E, Braun J, Impact of sex on inheritance of ankylosing spondylitis. Lancet 2000; 355 (9209): 1097-7, Discussion 1098.

[228] Hoyle E, Laval S, Calin A, Wordsworth B, Brown M. The X-chromosome and suscetibility to ankylosing spondylitis. Arthritis Rheum 2000; 43(6): 1353-5.

Appendix 1 : The BAS indices

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

Please place a mark on each line below to indicate your answer to each question, relating to the past week.

1. How would you describe the overall level of fatigue/tiredness you have experienced?

NONE ——————————————— VERY SEVERE

2. How would you describe the overall level of AS neck, back or hip pain you have had?

NONE ——————————————— VERY SEVERE

3. How would you describe the overall level of pain/swelling in joints other than neck, back or hips you have had?

NONE ——————————————— VERY SEVERE

4. How would you describe the overall level of discomfort you have had from any areas tender to touch or pressure?

NONE ——————————————— VERY SEVERE

5. How would you describe the overall level of morning stiffness you have had from the time you wake up?

NONE ——————————————— VERY SEVERE

6. How long does your morning stiffness last from the time you wake up?

0 hrs 1/2hr 1 hr 11/2hrs 2 or more hrs

[The VAS are scored in cm and the BASDAI score is calculated as :
the sum of question 1,2,3,4 + the average of question 5 and 6, all divided by 5.]

Bath Ankylosing Spondylitis Functional Index (BASFI)

Please draw a mark on each line below to indicate your level of ability with each of the following activities, during the last week.

Note: An aid is a piece of equipment which helps you to perform an action or movement.

1. Putting on your socks or tights without help or aids (eg sock aids):

EASY _____ IMPOSSIBLE

2. Bending forward from the waist to pick up a pen from the floor without an aid:

EASY _____ IMPOSSIBLE

3. Reaching up to a high shelf without help or aids (eg helping hand):

EASY _____ IMPOSSIBLE

4. Getting up out of an armless dining room chair without using your hands or any other help:

EASY _____ IMPOSSIBLE

5. Getting up off the floor without help from lying on your back:

EASY _____ IMPOSSIBLE

6. Standing unsupported for 10 minutes without discomfort:

EASY _____ IMPOSSIBLE

7. Climbing 12-15 steps without using a handrail or walking aid (one foot each step):

EASY _____ IMPOSSIBLE

8. Looking over your shoulder without turning your body:

EASY _____ IMPOSSIBLE

9. Doing physically demanding activities (eg, physiotherapy exercises, gardening, or sports):

EASY _____ IMPOSSIBLE

10. Doing a full days activities whether it be at home or at work:

EASY _____ IMPOSSIBLE

[The VAS are scored in cm and BASFI is calculated by taking the average of the 10 questions.]

Bath Ankylosing Spondylitis Patient Global Score (BAS-G)

Please place a vertical mark on the scale below to indicate the effect your disease has had on your well-being over the last week.

NONE — WORST POSSIBLE

[BAS-G is calcuated by measuring the VAS scale in cm]

ARTHRITIS & RHEUMATISM
Vol. 41, No. 12, December 1998, pp 2263–2270
© 1998, American College of Rheumatology

THE BATH ANKYLOSING SPONDYLITIS RADIOLOGY INDEX (BASRI)

A New, Validated Approach to Disease Assessment

KIRSTEN MACKAY, CHRISTOPHER MACK, SINEAD BROPHY, and ANDREI CALIN

Objective. To develop a reproducible and simple radiologic scoring system for the spine in patients with ankylosing spondylitis (AS): the Bath Ankylosing Spondylitis Radiology Index for the spine (BASRI-s).

Methods. Radiographs of 470 patients with AS were scored using the New York criteria for the sacroiliac joints and, similarly, grading the lumbar and cervical spine on a scale of 0–4 (for normal, suspicious, mild, moderate, and severe). These 3 scores were added together to produce the BASRI-s score (scored 2–12). Radiographs of 188 patients were used to test reproducibility. Blinded radiographs of 89 non-AS patients were included, randomly, to assess disease specificity. Sensitivity to change was assessed using 177 radiographs from 58 AS patients.

Results. Intra- and interobserver variation showed 75–86% and 73–79% complete agreement at all sites, respectively. Specificities of 0.83–0.89 suggested that the lumbar and cervical spine BASRI scores were disease specific. Sensitivity to change became apparent at 2 years ($P < 0.001$). Using a lateral view *and* an anteroposterior view of the lumbar spine was more sensitive than using a lateral view alone. Grading a set of radiographs (sacroiliac joints, lumbar spine, and cervical spine) took 30 seconds.

Conclusion. BASRI is a reliable method for grading radiographic changes in patients with AS. It is disease specific, sensitive to change, valid, simple, and rapid to perform.

Supported by a grant from the Coates Charitable Trust, the Col. W. W. Pilkington Trust, the Harris Charitable Trust, and the National Ankylosing Spondylitis Society.

Kirsten R. MacKay, MRCP, Christopher Mack, FRACP, Sinead Brophy, BSc, Andrei Calin MD, FRCP: Royal National Hospital for Rheumatic Diseases, Bath, UK.

Address reprint requests to Andrei Calin, MD, FRCP, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL, UK.

Submitted for publication February 23, 1998; accepted in revised form June 23, 1998.

Ankylosing spondylitis (AS) is a chronic, progressive condition with fluctuating disease activity. A number of measures are used simultaneously to monitor outcome (1–10), and these are fundamental in assessing the natural history of AS and the effectiveness of specific management strategies in terms of outcomes research (11). Characteristic radiologic change is essential for the diagnosis of AS and is considered the "gold standard" for disease status, but little work has been done to assess disease progression in radiologic terms. Radiographs have the advantage over other measures of being objective and uncomplicated by diurnal variation (12).

No classification defining global radiologic change in AS exists. The New York (NY) criteria for AS include the only widely accepted radiology measure specific for the disease, but this refers purely to the sacroiliac (SI) joints (13–15). The Rome criteria use the presence of radiologic sacroiliitis to diagnosis AS but ignore the rest of the spine (16). End-stage cervical and lumbar spine disease may be readily recognizable in terms of a "bamboo spine," but less severe changes have not been adequately described. A number of systems for scoring some part of the spine in AS have been published (12,17–20), but these are not used widely. None of them score the whole spine. Some have poor reproducibility, are insensitive to disease progression, and are slow to use (21). All ignore the posterior structures of the spine, classifying those who have only posterior element fusion as normal or as having "mild" radiographic changes when in fact the spine may be completely fused (see Figures 1A and B and Figure 2).

The Bath Ankylosing Spondylitis Radiology Index for the spine (BASRI-s), a new radiologic scoring system, was designed to address these problems. This report describes the BASRI-s, its inception, and its uses. Radiologic changes in the hip have specifically been excluded from this study, since those AS patients who develop hip disease represent a small, distinct subset of

Table 1. Bath Ankylosing Spondylitis Radiology Index (BASRI) for the spine*

Score	Grade	System applies to both the lumbar and the cervical spine (grade each as 0–4)
0	Normal	No change
1	Suspicious	No definite change
2	Mild	Any number of erosions, squaring, or sclerosis, with or without syndesmophytes, on ≤2 vertebrae
3	Moderate	Syndesmophytes on ≥3 vertebrae, with or without fusion involving 2 vertebrae
4	Severe	Fusion involving ≥3 vertebrae

* For the lumbar spine, examine both the anteroposterior and lateral radiographs together. The score for the lumbar spine is a composite of the 2 views. If, as in Figures 1A and B, one view shows syndesmophytes and fusion but the other view shows lesser changes, the overall score will relate to the view showing the more significant change. This system applies equally to the cervical spine, but only to the lateral radiograph. See Figures 1 and 2.

patients whose disease occurs at a younger age (22–26). An index documenting radiologic hip change is being developed.

PATIENTS AND METHODS

A consensus approach (27) was used to determine a suitable scoring system for radiographs of patients with AS. Radiographs of 470 patients who had been diagnosed according to the NY criteria were scored openly and assigned to 1 of 5 severity grades based on the NY grading of SI joint disease (0 = no disease, 1 = suspicious for disease, 2 = minimal disease, 3 = moderate disease, and 4 = severe disease). The radiographs used were an anteroposterior (AP) pelvis film, AP and lateral lumbar spine films, and a lateral cervical spine film. The SI joints, lumbar spine, and cervical spine were each assessed separately. The lumbar spine was defined as extending from the lower border of T12 to the upper border of S1, and the cervical spine as extending from the lower border of C1 to the upper border of C7.

The discriminating features of each radiologic severity group were defined and used as the basis to describe a method of assessing the severity of radiologic change in AS. The system was then repeatedly tested in a blinded manner by 3 experienced readers (KM, CM, and AC) and modified openly on several occasions before the final format was determined. Rules for scoring the lumbar and cervical spine are shown in Table 1. The NY criteria were used to score the SI joints (15).

To assess whether an AP or lateral radiographic view was more appropriate for scoring the lumbar spine, 58 sets of lumbar spine radiographs with both AP and lateral views were scored using 1) the AP view alone, 2) the lateral view alone, and 3) the AP and lateral views combined. Sensitivity and specificity for each view was determined and compared with the score obtained with both radiographic views. A similar process was used for assessment of cervical spine radiographs.

Following definition of the scoring system, radiographs of 188 consecutive patients with AS and 89 without AS were scored randomly and blindly by the 3 readers to validate the BASRI. The mean (±SD) age of this AS population was 44.5 ± 10.9 years, and the sex ratio was 3:1 (males:females). Two hundred sixty-three SI joint, 160 lumbar spine, and 145 cervical spine radiographs from the AS cohort were scored, assessing intra- and interobserver variation. Because these data were nonparametrically distributed, tending, with increasing disease duration, to cluster toward a higher score (worse disease), a kappa statistic was used to determine the significance of the intra- and interobserver variability.

Sensitivity to change over time was determined by scoring serial radiographs of 58 patients, assessing 177 time intervals of 1, 2, 3, and 4 years. Radiographs were obtained on 2 occasions 12, 24, 36, and 48 months apart, and were available in 20, 31, 28, and 23 cases, respectively. The mean time from diagnosis of these 58 patients was 18 years (range 0–44 years). All radiographs were blinded as to the name and date of the radiograph, and a Wilcoxon signed rank test for nonparametric data was used to determine the earliest point at which sensitivity to change became apparent.

The specificity and positive predictive value against other rheumatic conditions was assessed using 305 radiographs from the AS cohort and 78 radiographs from the 89 non-AS patients. Radiographs of non-AS patients were interspersed with the AS patients' films, such that readers were unaware of the diagnosis. A cutoff of grade 2 (definite) disease was used, and all radiographs were then classified into 1 of 2 groups: those with and those without AS changes. The SI joints were not viewed at this point.

The non-AS cohort we studied consisted of consecutive outpatients who were attending the Royal National Hospital for Rheumatic Diseases, a tertiary referral center, and who had had cervical or lumbar spine radiographs obtained for evaluation of symptoms. Their mean (±SD) age was 57.9 ± 16.8 years, and the sex ratio was 1:3 (males:females). This cohort included 41 patients with rheumatoid arthritis, 21 with mechanical back pain, 10 with fibromyalgia, 10 with osteoporosis, and 7 with psoriatic arthritis.

RESULTS

Lumbar and cervical spine: which film should be used? Using 58 sets of AP and lateral lumbar spine radiographs, 3 scores were derived as described above. This third, or combination, score differed from the AP or lateral scores if syndesmophytes or fusion were seen at *different* levels on each projection. This occurred in 3 of the 58 patients. The "combination" score differed from the AP score alone in 9 of the 58 patients (15.5%) and from the lateral score alone in 21 (36%). Overall, the use of 2 projections changed the score 46% of the time. Assuming that the combination view gives the truest assessment, the sensitivity for the AP view alone is 0.83 and that for the lateral view alone is 0.73 (see Figures 1A and B).

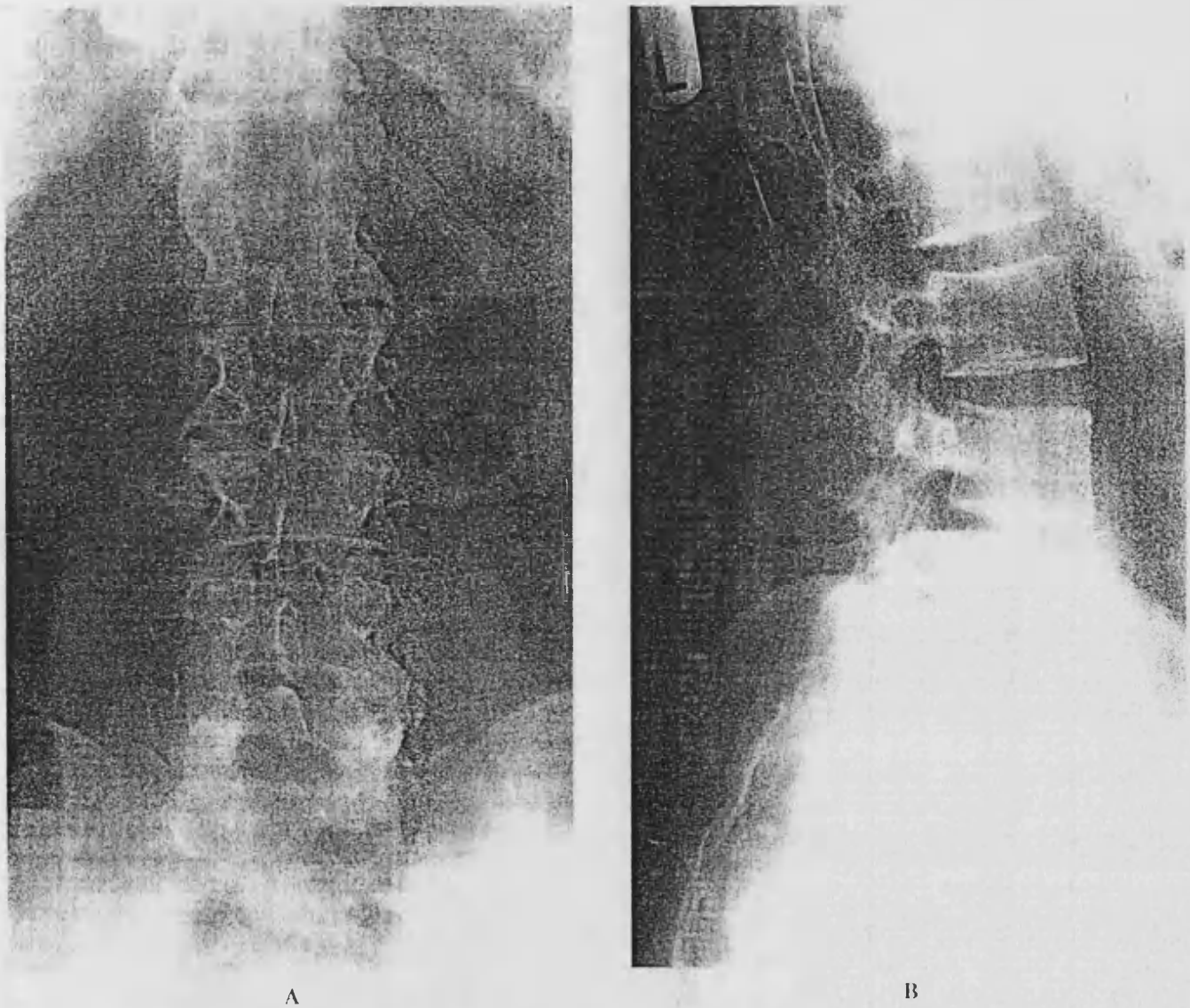


Figure 1. A, Anteroposterior (AP) and B, lateral radiographic views of the lumbar spine of an ankylosing spondylitis patient, obtained on the same day. Syndesmophytes and fusion can be seen in the AP view but not in the lateral view. In a scoring system that uses only the lateral radiograph, the findings on the film in B could be classified as normal.

In the cervical spine, this situation was not evident (see Figure 2). Only 20 cases were available in the population studied, since AP views of the cervical spine were rarely taken. The AP view influenced the overall score only once.

Validation of the BASRI. *Intraobserver variation.* Two hundred blinded SI joint radiographs were assessed twice by a single observer, with 86% complete agreement, giving a kappa score of 0.69. Results for the lumbar and cervical spine were similar, with kappa scores of 0.65 (75% complete agreement) and 0.73 (81% complete agreement), respectively (see Tables 2 and 3). The main errors were reading the SI joints as grade 3 rather than grade 4 in 12 of 200 cases (6%), scoring the lumbar spine as grade 4 rather than grade 3 in 7 of 97 cases (7%), and in distinguishing suspicious (grade 1) from mild (grade 2) disease in the cervical spine in 5 cases. Variation of >1 grade occurred only twice when scoring the lumbar spine and never when scoring the SI joints or the cervical spine.

Interobserver variation. Reproducibility between 2 readers was assessed using 263 SI joint radiographs

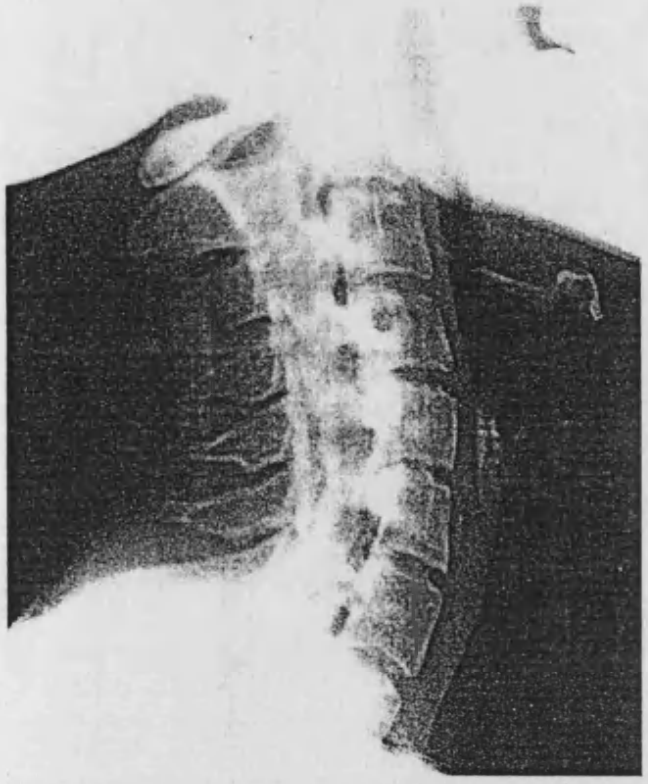


Figure 2. Cervical spine radiograph, showing fusion of the posterior elements but no anterior fusion. Any scoring system that ignored the posterior elements would classify the findings on this film as normal.

scored by each assessor on separate occasions. There was 78% complete agreement between observers, with a kappa score of 0.55. The 160 sets of lumbar spine radiographs used reached 73% complete agreement (κ = 0.64), and the 145 cervical spine radiographs reached 79% complete agreement (κ = 0.69) (see Tables 2 and 3). Both the lumbar and cervical spine scores outperformed the established NY criteria for SI joint assessment. Considerable difficulty in separating grades 3 and 4 existed in SI joint assessment (8 occasions), while

Table 2. Summary of intra- and interobserver variations at the various skeletal sites scored as part of the Bath Ankylosing Spondylitis Radiology Index

	Intraobserver variation		Interobserver variation	
Skeletal site	Complete agreement (%)	Kappa statistic	Complete agreement (%)	Kappa statistic
Sacroiliac joints	86	0.69	78	0.55
Lumbar spine	75	0.65	73	0.64
Cervical spine	81	0.73	79	0.69

Table 3. Kappa statistics and strengths of agreement

Kappa statistic	Strength of agreement
<0.2	Poor agreement
0.2–0.4	Fair agreement
0.4–0.6	Moderate agreement
0.6–0.8	Good agreement
0.8–1.0	Very good agreement

distinguishing mild from suspicious disease was the main problem for the lumbar and cervical spine scoring (5 occasions). Discrepancies of >1 grade occurred while scoring the SI joints in 3 of 263 films, the lumbar spine in 5 of 160 films, and the cervical spine in 10 of 145 radiographs.

Disease specificity. Specificity for the lumbar spine was 0.89, and that for the cervical spine was 0.83. The positive predictive value for the lumbar spine was 0.97, and that for the cervical spine was 0.95.

Sensitivity to change. Using Wilcoxon's signed rank test for nonparametric data, the BASRI-s demonstrated a significant change in radiologic score ($P <$ 0.001) at 24 months for the SI joints, the lumbar spine, and the cervical spine. Where the time interval between radiographs was 12 months, 30% of cases showed changes of at least 1 grade within this period, but this was not statistically significant ($P < 0.07$).

Scoring speed. The mean time taken to score 1 set of films (SI joints, lumbar spine, and cervical spine) was less than 30 seconds.

DISCUSSION

Any measure that documents disease status must be reproducible and sensitive to change. It should be disease specific and have both face validity and predictive validity (11). To be clinically useful, it needs to be easy and quick to use, with few training requirements. The use of standard measures is essential to allow comparison of results between clinical studies (28). This is illustrated well in the rheumatoid arthritis literature by the frequent use of radiologic grading systems, such as the Larsen and Sharp scores for the hand and the ACR (American College of Rheumatology) response criteria or EULAR (European League Against Rheumatism) Core Data Set (29–32). Using these criteria in epidemiologic studies can improve knowledge of disease and set a standard against which new treatments may be assessed. Knowledge of the natural history of a disease such as AS, which has an unknown etiology, can provide

insights into causal factors and provide patients with prognostic information and expectations (33). Distinguishing between markers of disease activity and those that attempt to measure damage and function is important (11). The BASRI was designed to fill a perceived gap in the range of outcome measures for AS.

Given that the NY criteria for the SI joints are well established, they were incorporated into the BASRI without change. To gain credibility, any newly developed score needs to perform as well as these criteria, and we have used the performance data for the NY criteria as the "gold standard." The BASRI was reproducible, with intra- and interobserver variations equivalent to or better than those of the NY criteria. The main problem for grading the lumbar and cervical spine was distinguishing suspicious disease from mild disease on 5 occasions because of difficulty in determining whether squaring was present. Difficulty in distinguishing between grades 1 and 2 was not seen with the SI joints because bilateral grade 2 or unilateral grade 3 sacroiliitis was part of the entry criteria for the study. A potential method of overcoming this problem for the spine would be to measure each vertebra individually to determine the presence or absence of squaring compared with the population mean, as done in the method described by Ralston and colleagues (18). However, this would considerably increase the time taken for scoring, thereby reducing the clinical usefulness of the BASRI.

The lumbar and cervical spine were shown to be disease specific, but the SI joints were excluded from this part of the study because the presence of sacroiliitis (as part of the NY criteria) was one of the entry criteria for the AS cohort. Since only 7 of the 89 non-AS cohort had psoriatic arthritis, further work is under way to determine whether the BASRI would be able to differentiate AS patients from those with Reiter's disease or psoriatic arthritis.

Little information has been available regarding radiographic sensitivity to disease progression and the frequency with which repeat radiographs should be performed. The BASRI was found to be sensitive to change over a 2-year period, which suggests that radiographs at intervals of <2 years, for either routine or study purposes, are not warranted. The radiographs in the study were blinded for the date, confirming that the BASRI could determine "forward progression" (i.e., could identify the earlier of 2 radiographs performed on the same individual).

Other radiologic scoring systems for the lumbar spine have used a single lateral radiograph to grade radiologic change (12,19). Although classic radiologic features can be seen on this view, some changes, such as syndesmophyte formation and fusion between vertebrae, can be missed. The score generated using the AP view alone differed from the combined score (using both the AP and lateral views) 15% of the time but less frequently than with the lateral view alone, which differed 36% of the time. The best screening view for assessing the lumbar spine appears not to be the traditional lateral projection, but rather, the AP projection. If grade 4 change is seen on this view, no further radiographs are required, but if lesser disease is evident, then both the AP and lateral views are essential to define fully the severity of change. This did not apply to the cervical spine. The combination of views therefore used to determine the BASRI for the spine was: a lateral cervical spine, an AP and lateral lumbar spine, and an AP pelvis.

The use of the AP lumbar spine radiograph does increase the radiation dose slightly. A lateral lumbar spine radiograph incurs a dose of 19.0 milliSieverts (mSv), the addition of an AP lumbar spine radiograph increases the dose by 7.0 mSv (34), which is equivalent to the radiation exposure incurred during a transatlantic flight. The total radiation exposure for AP pelvis and AP and lateral lumbar spine radiographs is 31.3 mSv (34). No data are available for the cervical spine, but the nearest comparable examination would be a lateral chest radiograph, incurring a dose of 0.66 mSv (34). The risk of death from a fatal cancer following a lumbar spine radiograph is ~1:10,000 (35). The risk of dying in a road traffic accident in 1 year is 1:8,000 (35).

The thoracic spine has not been included in the BASRI-s because of technical difficulties and excessive radiation exposure. The scapulae and ribs tend to overlie the vertebrae, making a good view impossible. So, it is difficult to produce a well-penetrated radiograph. Lung disease will also add to any difficulties because the lungs overlie the thoracic spine in the lateral position. Attempts to improve the penetrance will increase the radiation exposure by an amount that is dependent upon the individual (e.g., muscle thickness affects penetration). Standard radiographs (as used by Larsen for other conditions) (29) have not been used since there are no available "gold standard" radiographs in AS. The BASRI was compared with the only other available index (21) and was found to be more reproducible, equally sensitive to change, and easier to use. Larsen scored only the hips in AS but found less reproducibility than in other diseases, perhaps because new bone formation occurs in spondylarthropathies. He suggests

that his index should be used with caution in these conditions (29).

The BASRI was easy to use and to explain. The mean time taken to score a set of radiographs for 1 subject was <30 seconds, and the required training was minimal. Cross-sectional data generated using the original cohort of 470 patients suggest that the disease ascends with time (36), which is consistent with many earlier studies and supports the instrument's face validity. Additionally, these data suggest that radiology may help in defining subgroups of patients, especially if used in conjunction with a variety of other indices. Studies using longitudinal data to confirm the validity of the BASRI as a prognostic marker are in progress. A pilot study investigating the relationship between metrology and an earlier version of the BASRI revealed a good correlation between the 2 scores (37). Following from this, the relationships between radiology, metrology, function, and disease activity (3–5) are currently being investigated.

The BASRI, as described above, is a modified version of studies previously published in abstract form (38), involving the entire axial skeleton, including the hips. Further work has since been undertaken delineating the spinal score, or BASRI-s, allowing for a more precise interpretation of the grading system, and this system has been compared with other available radiologic scoring systems (20,21). The present study uses a separate cohort of patients from those used in the previous studies and was undertaken to validate the BASRI-s in a new AS population.

Hip changes have not been included in the BASRI-s because patients who develop hip disease appear to represent a distinct subgroup. Hip arthritis is associated with the age at disease onset, occurring at a younger age. Ascending spinal disease seems to be a product of time—the longer the disease, the greater the spinal change (22). The majority of patients with hip disease have developed some radiographic change by the age of 23 (23). In a prospective study begun in 1947, 150 war veterans with AS were followed up for 33 years. Peripheral joint disease occurred early, and those whose hips were normal after 10 years of AS did not subsequently develop hip arthritis (24). Other evidence has shown that hip disease is associated with a more severe outcome. Amor and colleagues (25) include hip arthritis as 1 of 7 entry variables correlating with disease severity (odds ratio 22.85; 95% confidence interval 4.43–118) (25). Because hip disease affects only 18–37% of the AS population (22), the use of a global score (BASRI-g) for every AS patient, with a maximum score of 16 rather than 12, may inappropriately dilute the score of the majority of AS patients. Those with severe, or grade 4, spinal disease without hip arthritis would rate only 12 on a 16-point global scale despite having a bamboo spine, poor metrology, and poor function. It may be better to grade these populations separately, using the BASRI-s for one and the BASRI-g for the other.

To maintain simplicity, the BASRI does not pick up minor radiologic change. The score does not change with each additional erosion. Evidence of squaring or sclerosis will always remain grade 2, or *mild* disease, until fusion between 2 vertebrae or the presence of ≥3 syndesmophytes is identified. This is because spinal restriction in patients without bridging syndesmophytes may be related to soft tissue inflammation and potentially reversible factors, and therefore should be classed as mild disease (12,22). As well as scoring the type of damage that occurs in AS (erosions, squaring, syndesmophytes), the BASRI also incorporates the extent of the involvement (i.e., number of vertebral levels with syndesmophytes or fusion). However, it does not differentiate between those with a complete "bamboo spine" and those who have ≥3 fused vertebrae. Both score a grade 4, or *severe* disease. This definition of severe disease has been used because fusion of ≥3 vertebrae involves at least 60% of the lumbar spine, which limits spinal movement significantly. Our preliminary work correlating radiology with metrology supports this (37), but it does mean that the BASRI suffers from a ceiling effect. However, the same plateau effect and broad grading system are seen in the NY criteria, which have been widely accepted for scoring the SI joints and were the blueprint for the BASRI.

The limited scale of 1–4, necessitating fairly broad categories of radiologic grade, potentially explain why sensitivity to change for the BASRI is seen at 2 years. However, it might be that relevant change is truly slow and can only be seen at 2 years whatever the design of the index. Thus, the 72-point scale of the Stoke Ankylosing Spondylitis Spine Score (SASSS) (12) was no more sensitive to change (21). Unlike rheumatoid arthritis, where erosions tend to occur within the first few years, progression in AS appears to proceed quite slowly. Only 35% of a 470-patient cohort (mean disease duration 21 years, range 2–50 years) developed grade 4 changes in the lumbar spine (36). It is likely that the majority are diagnosed with grade 4 SI changes because of the lateness of their presentation, not the speed of the radiologic change. This lack of radiologic progression to severe spinal disease in the majority suggests that the potential ceiling effect is of little consequence.

Various other radiologic scores for AS have been published in the past 10 years (12,17–19). These have not attempted to define progression in the entire axial skeleton. The SASSS, as mentioned above, grades the lumbar spine alone, using a scale of 0–72. It scores the edges of each vertebral body from the lower border of T12 to the upper border of S1, using a 0–3 scale (1 for erosions, squaring, or sclerosis; 2 for syndesmophytes; and 3 for a total bony bridge). The maximum possible score is therefore 72, which represents a completely ankylosed spine. The SASSS does not take into account the posterior elements. This means that a subject with only posterior fusion would have a low score, and the low SASSS would be inconsistent with the clinical picture (see Figures 1A and B and Figure 2). Using the SASSS is slow, taking more than twice the time of the BASRI to grade just the lumbar spine (21). It does not use the AP lumbar spine film, which we have shown to be necessary (see Figures 1A and B). It is less reliable than the BASRI and is not sensitive to progression (21).

The Glasgow Radiological Index (GRI) (18–19) is a composite score. The SI joints are scored using the NY criteria, but a maximum score of 8 can be attained (unlike the NY criteria, the mean of the SI joints is not taken). The lumbar spine score includes the Vertebral Concavity Index, which measures the concavity of an individual vertebra, comparing it with a normal reference range. If the vertebra is squared, it is scored 1. The maximum concavity score is 5. In addition, each syndesmophyte identified is scored 1, allowing for a maximum possible score of 12. All 3 scores are added for a potential total of 25. The GRI applies only to the lumbar spine and SI joints. It is slow to perform, taking $\geq$3 minutes (18). It scores syndesmophytes but not fusion. It ignores erosions, the posterior elements, and uses only a lateral radiograph. The followup study (19) evaluated 41 patients at 5-year intervals. The abstract states that progressive change was detected. There was no correlation between the GRI and other clinical and laboratory parameters, namely, chest expansion, spondylometry, the erythrocyte sedimentation rate, and IgG levels.

In conclusion, radiology is fundamental to the diagnosis and tracking the subsequent progression of AS. Apart from the New York criteria for the SI joints, no widely accepted radiologic criteria exist. The BASRI, as a radiologic classification system, aims to satisfy the OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials) filter goal: specifically, one aspires toward truth, discrimination, and feasibility (39). The BASRI fulfills these criteria because it is reproducible, specific, sensitive to change at 2 years, simple and quick to use, and easy to explain. It is a global index, scoring the SI joints and the lumbar and cervical spine.

ACKNOWLEDGMENTS

The authors thank Ms P. Porch, Senior Radiographer, for her help with labeling of radiographs, and the various Charitable Trusts who have provided funding for this research: the Coates Charitable Trust, the Col. W. W. Pilkington Trust, the Harris Charitable Trust, and the National Ankylosing Spondylitis Society.

REFERENCES

1. Dougados M, Gueguen A, Nakache JP, Nguyen M, Mery C, Amor B. Evaluation of a functional index and an articular index in ankylosing spondylitis. J Rheumatol 1988;15:302–7.
2. Dougados M, Gueguen A, Nakache JP, Nguyen M, Amor B. Evaluation of a functional index for patients with ankylosing spondylitis [letter]. J Rheumatol 1990;17:1254–6.
3. Calin A, Garrett S, Whitelock H, Kennedy LG, O'Hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281–5.
4. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286–91.
5. Jenkinson TR, Mallorie PA, Whitelock HC, Kennedy LG, Garrett SL, Calin A. Defining spinal mobility in ankylosing spondylitis (AS): the Bath AS Metrology Index. J Rheumatol 1994;21:1694–8.
6. Jones SD, Steiner A, Garrett SL, Calin A. The Bath Ankylosing Spondylitis Patient Global Score [abstract]. Br J Rheumatol 1995;34 Suppl 1:114.
7. Creemers MCW, van 't Hof MA, Franssen MJAM, van de Putte LBA, Gribnau FWJ, van Riel PLCM. Disease activity in ankylosing spondylitis: selection of a core set of variables and a first step in the development of a disease activity score. Br J Rheumatol 1996;35:867–73.
8. Daltroy L, Larson M, Roberts WN, Liang M. A modification of the Health Assessment Questionnaire for the spondyloarthropathies. J Rheumatol 1990;17:946–50.
9. Mander M, Simpson JM, McLellan A, Walker D, Goodacre JA. Carson Dick W. Studies with an enthesis index as a method of clinical assessment in ankylosing spondylitis. Ann Rheum Dis 1987;46:197–202.
10. Laurent MR, Buchanan WW, Bellamy N. Methods of assessment used in ankylosing spondylitis clinical trials: a review. Br J Rheumatol 1991;30:326–9.
11. Rigby AS, Silman AJ. Outcome assessment in clinical trials of ankylosing spondylitis. Br J Rheumatol 1991;30:321–5.
12. Averns HL, Oxtoby J, Taylor HG, Jones PW, Dziedzic K, Dawes PT. Radiological outcome in ankylosing spondylitis: use of the Stoke Ankylosing Spondylitis Spine Score (SASSS). Br J Rheumatol 1996;35:373–6.
13. Bennett PH, Burch TA. Population studies of the rheumatic diseases. Amsterdam: Excerpta Medica Foundation; 1968.
14. 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.
15. Moll JMH, Wright V. New York clinical criteria for ankylosing spondylitis. Ann Rheum Dis 1973;32:354–63.
16. Kellgren JH, Jeffrey MR, Ball J. The epidemiology of chronic

rheumatism. Vol. 1. Oxford: Blackwell Scientific Publications; 1963.
17. Taylor HG, Wardle T, Beswick EJ, Dawes PT. The relationship of clinical and laboratory measures to radiological change in ankylosing spondylitis. Br J Rheumatol 1991;30:330–5.
18. Ralston SH, Urquhart GDK, Brzeski M, Sturrock RD. A new method for the radiological assessment of vertebral squaring in ankylosing spondylitis. Ann Rheum Dis 1992;51:330–3.
19. Chaudhuri K, Armstrong S, Murphy E, Zoma A, York J, Sturrock RD. Evaluation of a radiological index of spinal disease in ankylosing spondylitis—10 year follow-up study [abstract]. Br J Rheumatol 1997;36 Suppl 1:128.
20. MacKay K, Mack CS, Pande I, Roussou E, Calin A. The Bath Ankylosing Spondylitis Radiology Index: definition and correlation with functional, disease activity and metrology indices [abstract]. Arthritis Rheum 1996;39 Suppl 9:S206.
21. Mack CS, MacKay KR, Calin A. Radiology in ankylosing spondylitis: a comparison of two scoring systems. Br J Rheumatol 1996;35 Suppl 1:23.
22. Khan MA. Ankylosing spondylitis: clinical features. In: Klippel J, Dieppe P, editors. Rheumatology. Mosby-Year Book Europe Limited; 1994. p. 3:25.1–25.10
23. Calin A, Elswood J. The relationship between pelvic, spinal and hip involvement in ankylosing spondylitis—one disease process or several? Br J Rheumatol 1988;27:393–5.
24. Carette S, Graham D, Little H, Rubenstein J, Rosen P. The natural disease course of ankylosing spondylitis. Arthritis Rheum 1983;26:186–90.
25. Amor B, Santos RS, Nahal R, Listrat V, Dougados M. Predictive factors for the long term outcome of spondyloarthropathies. J Rheumatol 1994;21:1883–7.
26. Riley MJ, Ansell BM, Bywaters EGL. Radiological manifestations of ankylosing spondylitis according to age at onset. Ann Rheum Dis 1971;30:138–48.
27. Bellamy N, Buchanan WW. Outcome measurement in osteoarthritis clinical trials: the case for standardisation. Clin Rheumatol 1984;3:293–303.
28. Felson DT. Choosing a core set of disease activity measures for rheumatoid arthritis clinical trials. J Rheumatol 1993;20:531–4.
29. Larsen A, Dale K, Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol 1977;18:481–91.
30. Sharp JT, Lidsky MD, Collins LC, Moreland J. Methods of scoring the progression of radiologic changes in rheumatoid arthritis: correlation of radiologic, clinical and laboratory abnormalities. Arthritis Rheum 1971;14:706–20.
31. Tugwell P, Boers M. OMERACT Conference on Outcome Measures in Rheumatoid Arthritis Clinical Trials: conclusion. J Rheumatol 1993;20:590.
32. Scott DL. A simple index to assess disease activity in rheumatoid arthritis. J Rheumatol 1993;20:582–4.
33. Little H. The natural history of ankylosing spondylitis. J Rheumatol 1988;15:1179–80.
34. Hart D, Hillier MC, Wall BF, Shrimpton PC, Bungay D. Doses to patients from medical x-ray examinations in the UK: a review of information held in the National Patient Dose Database. National Radiological Protection Board. Report 289. London (UK): Her Majesty's Stationers Office; 1995.
35. Annals of the ICRP series. Recommendations of the International Commission for Radiological protection. Vol. 21. London (UK) Pergamon; 1991. pp. 1–3.
36. MacKay K, Brophy S, Mack C, Calin A. Patterns of radiological involvement in 470 ankylosing spondylitis patients [abstract]. Arthritis Rheum 1997;40 Suppl 9:S61.
37. Kennedy LG, Jenkinson TR, Mallorie PA, Whitelock HC, Garrett SL, Calin A. Ankylosing spondylitis (AS): the correlation between a new metrology score and radiology. Br J Rheumatol 1995;34: 767–70.
38. Pande I, MacKay KR, Chatfield C, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI): a new validated approach to disease assessment. Br J Rheumatol 1995;34 Suppl 3:1203.
39. Boers M, Brooks P, Strand C, Tugwell P. The OMERACT filter for outcome measures in rheumatology. J Rheumatol 1998;25:1–2.

Erratum

In the article entitled "Outcome of Renal Transplantation in Ninety-Seven Cyclosporine-Era Patients with Systemic Lupus Erythematosus and Matched Controls" published in the August 1998 issue of *Arthritis & Rheumatism* (Stone et al, pp 1438–1445), the name of the third author of reference 4 was listed incorrectly. The correct reference is Reveille JD, Bartolucci A, Alarcón GS. Prognosis in systemic lupus erythematosus: negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. Arthritis Rheum 1990;33:37–48. We regret the error.

Appendix 3

The Development and Validation of a Radiographic Grading System for the Hip in Ankylosing Spondylitis: the Bath Ankylosing Spondylitis Radiology Hip Index

KIRSTEN MacKAY, SINEAD BROPHY, CHRISTOPHER MACK, MICHELE DORAN, and ANDREI CALIN

ABSTRACT. ***Objective.*** To develop a simple but reproducible radiological scoring system for the hip in ankylosing spondylitis (AS).

Methods. A consensus approach was used to develop a grading system whereby hip radiographs of 470 patients with AS were scored from 0 to 4 (normal, suspicious, mild, moderate, and severe), producing the Bath Ankylosing Spondylitis Radiology Hip Index (BASRI-hip or BASRI-h). The system was designed to be specific for AS radiographic change at the hip and includes circumferential joint space narrowing, osteophytes, erosions, and protrusio acetabulae. This score can then be added to the BASRI-spine, forming the grading system BASRI-total, scored 2–16. Radiographs of 134 patients were used to test reproducibility. Blinded radiographs of 100 non-AS patients were included randomly to assess disease specificity. Sensitivity to change was determined using 438 radiographs from 122 patients, over 219 time intervals.

Results. Inter and intraobserver variation for the right and left hip showed 83-84% and 94–96% complete agreement, respectively. Disease specificity was 0.76. If non-AS patients with a total hip replacement were excluded, disease specificity was 0.83. Sensitivity to change became apparent at one year ($p < 0.001$). Grading the hips using a single radiograph takes seconds.

Conclusion. BASRI-h is a reliable method for grading hip radiographic change in AS. It is disease-specific, sensitive to change, simple to use, and rapid to perform. (J Rheumatol 2000;27:2866–72)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS RADIOGRAPHIC INDEX RADIOLOGY
BATH ANKYLOSING SPONDYLITIS RADIOLOGY INDEX HIP

Ankylosing spondylitis (AS) is a chronic, progressive condition with fluctuating disease activity. A number of measures[1-10] can be used simultaneously to monitor outcome and these are fundamental in assessing the natural history of AS[11]. Characteristic radiological appearances at the sacroiliac (SI) joints are essential for the diagnosis of AS[12,13], but no classification completely defining axial radiological change in AS exists. We developed the Bath Ankylosing Spondylitis Radiology Index (BASRI-spine or BASRI-s), which describes a system for scoring radiological change in the AS spine[14]. The hips were excluded from this score as those developing hip disease represent a small, distinct subset of patients, developing more severe disease, at a younger age[15-19]. Hip disease in AS has not been satisfactorily defined. It is difficult to distinguish between AS hip changes and those of osteoarthritis (OA) in older patients with AS. The Larsen score [originally designed for grading radiological change in rheumatoid arthritis (RA)] has occasionally been applied to the hip radiographs of patients with AS, but is less reproducible if used to score conditions where there is increased bony deposition, as occurs in AS, with the development of syndesmophytes[20].

The Bath Ankylosing Spondylitis Radiology Hip Index (BASRI-hip or BASRI-h) is a new radiological scoring system designed to address these problems. We describe the BASRI-hip and its inception and uses. It can be used independently or in conjunction with the BASRI-spine[14] to produce the BASRI-total (BASRI-t) score for the axial skeleton. No additional radiographs will be required to score the BASRI-hip if an anteroposterior (AP) pelvic view has already been taken to review the SI joints.

MATERIALS AND METHODS

A consensus approach[21] was used to determine a suitable scoring system for hip radiographs in patients with AS. Four hundred seventy AP pelvic radiographs of AS patients, diagnosed using the New York criteria[12], were scored openly by 3 experienced readers and placed in one of 5 severity

From the Royal National Hospital for Rheumatic Diseases (RNHRD) Bath, United Kingdom.

Supported by a grant from the Coates Charitable Trust, Col WW Pilkington Trust, and the National Ankylosing Spondylitis Society.

K.R. MacKay, MRCP, Registrar in Rheumatology, RNHRD, and Arthritis Research Campaign (ARC) Clinical Training Fellow, Oxford University; S. Brophy, BSc, Research Assistant; M. Doran, MRCPI, Registrar in Rheumatology, RNHRD, now Research Fellow, Department of Clinical Epidemiology, Mayo Clinic, Rochester, MN, USA; C.S. Mack, FRACP, Registrar in Rheumatology, RNHRD; A. Calin, MD, FRCP, Consultant Rheumatologist, RNHRD.

Address reprint requests to Dr. A. Calin, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath, BA1 1RL, United Kingdom.

Submitted July 21, 1999 revision accepted June 19, 2000.

grades: 0 = no disease, 1 = suspicious disease, 2 = minimal disease, 3 = moderate disease, and 4 = severe disease. These were based on the New York grading of SI joint disease, which was used as a "blueprint" in view of its widespread acceptance. The discriminating features determining the assignment of radiographs into different categories were used as the basis to describe the radiographic changes. The system was repeatedly tested in a blinded fashion by 3 experienced readers (KM, CM, and AC) and modified openly on several occasions before a final format was agreed on. Circumferential joint space narrowing appeared to be the most important discriminating feature and as such, differing degrees of narrowing were used to distinguish between radiological grades. However, erosions, osteophytes, and protrusio acetabulae were important in aiding the differentiation between grades 1, 2, and 3. Rules for scoring the hip radiographs are shown in Table 1 and illustrated in Figures 1 and 2.

Following definition of the scoring system, radiographs of 134 consecutive patients with AS and 100 without AS were chosen to assess the reliability of the scoring system, predict sensitivity to change, and determine disease specificity. The mean age of the AS population was 45.9 ± 10.6 years and the sex ratio was 4.8:1 (male:female). The non-AS cohort were consecutive outpatients attending the Royal National Hospital for Rheumatic Diseases (RNHRD), a tertiary referral center. Pelvic radiographs, in this group had been taken for the evaluation of symptoms. Their mean age was 54.5 ± 17.5 years and the sex ratio was 1:2 (male:female). The cohort included 48 with RA, 21 with OA or mechanical back pain, 7 with fibromyalgia, 3 with osteoporosis, 12 with psoriatic arthritis, 4 with polymyalgia rheumatica, 3 with inflammatory bowel disease, and 2 with trochanteric bursitis.

Table 1. BASRI-hip scoring system. Increase BASRI-h grades 1 and 2 (suspicious and minimal) by one grade if 2 of the following bony changes are present: erosions, osteophytes, or protrusio.

Grade		Description
0	Normal	No change
1	Suspicious	Possible focal joint space narrowing
2	Minimal	Definite narrowing, leaving a circumferential joint space > 2 mm
3	Moderate	Narrowing but with circumferential joint space ≤ 2 mm or bone-on-bone apposition of < 2 cm
4	Severe	Bone deformity or bone-on-bone apposition > 2 cm or total hip replacement

Reliability. Two independent observers scored the 234 radiographs in a blinded fashion, on separate occasions, to assess interobserver variation. All SI joints had been obscured and radiographs from the non-AS cohort interspersed such that the readers were unaware of the diagnosis. One hundred of the AS radiographs were then randomly chosen, by a nonreader, and scored by a single observer on 2 consecutive days, assessing intraobserver variation. A kappa statistic was used to determine the significance of inter and intraobserver variability.

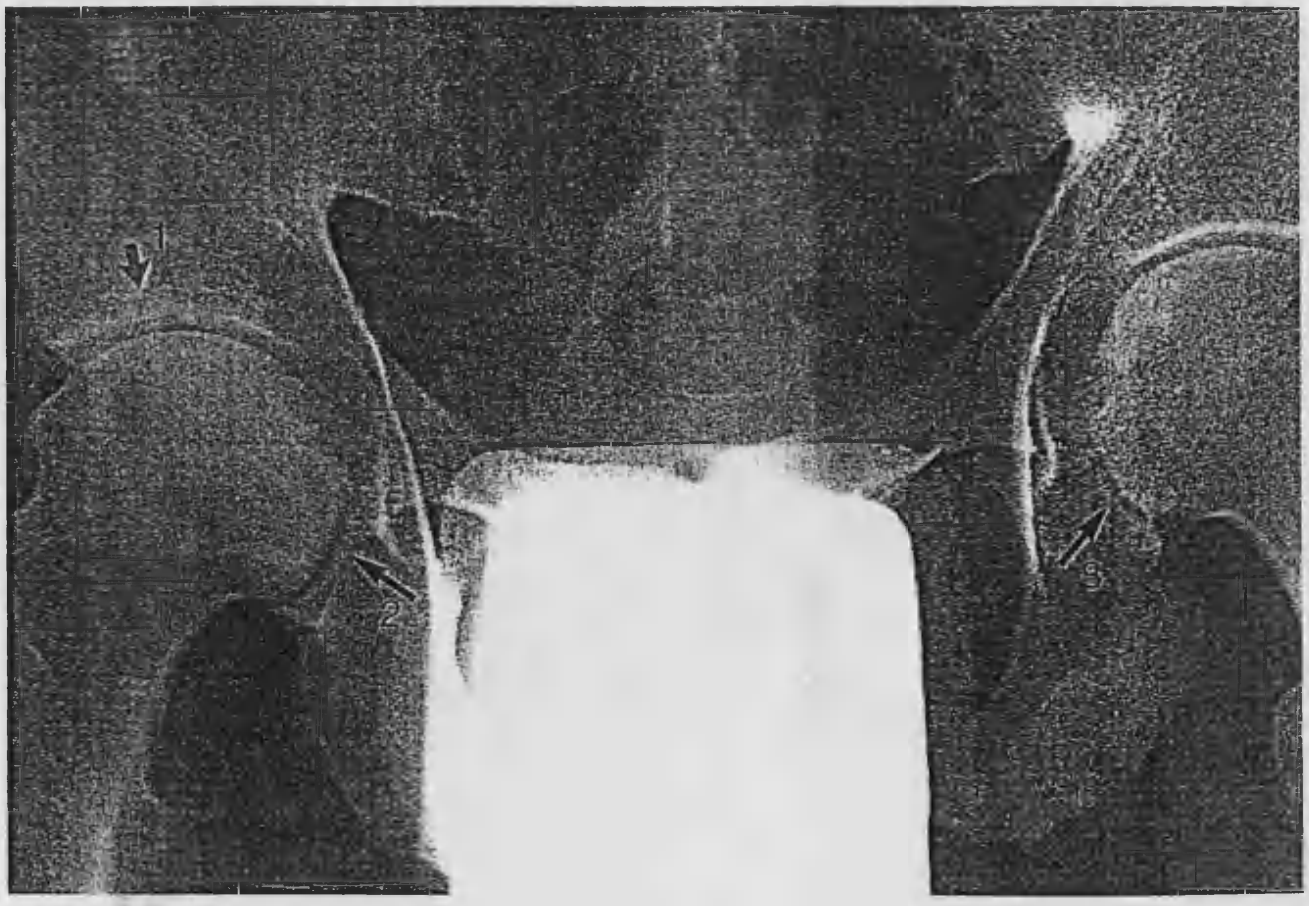


Figure 1. These pelvic radiographs are of the same AS patient. The first was taken when he was age 25 and had had disease for 5 years. Shown here: a BASRI h score of grade 2 (mild disease) for the right hip with erosions (arrow 1), osteophytes and focal joint space narrowing (arrow 2). BASRI-hip score for the left hip is grade 1 (suspicious disease) with focal joint space narrowing only (arrow 3).

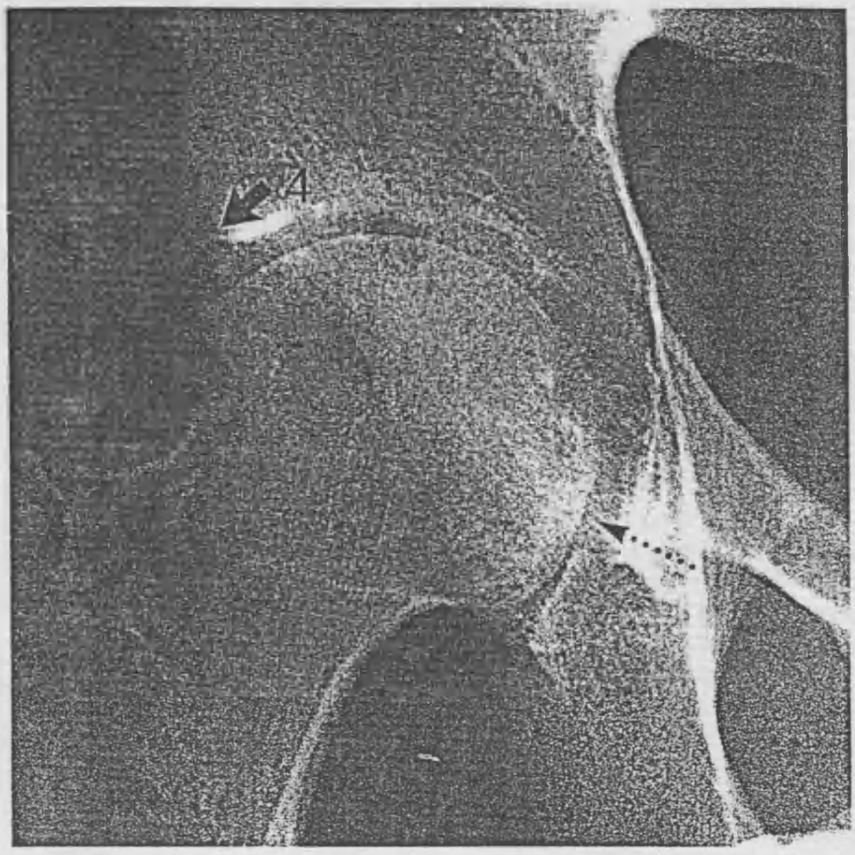


Figure 2. Same patient as Figure 1. Close up view of the right hip taken 18 months later. The BASRI-h grade is now moderate (grade 3) because of bone-on-bone apposition of < 2 cm (broken arrow). An osteophyte can be seen at the acetabular margin (arrow 4).

Disease specificity. The specificity of the BASRI-h against other rheumatic conditions was assessed using all 234 radiographs. A cutoff of grade 2 (definite) disease was agreed upon and all radiographs were then classified into one of 2 groups: those with and those without AS changes. Measurement of disease specificity was undertaken to determine whether the BASRI-h measures AS-specific change.

Sensitivity to change. Sensitivity to change over time was determined by scoring 438 serial radiographs of 122 patients, assessing 219 time intervals of 1, 2, 3, or 4 years. Patients were included if pelvic radiographs were available on 2 occasions 12, 24, 36, or 48 months apart. Pairs of radiographs were available in 60, 65, 55, and 55 cases, respectively. The mean time from diagnosis of these 122 patients was 18 years (range 0–44). All radiographs were blinded for name and date of the radiograph and a Wilcoxon signed-rank test for nonparametric data was used to determine the earliest point at which sensitivity to change became apparent.

RESULTS

Intraobserver variation. One hundred pelvic (AP) radiographs were assessed, on 2 occasions, by a single observer, with 94% complete agreement for the right hip and 96% complete agreement for the left hip. The kappa scores showed good and very good agreement at 0.86 and 0.91, respectively, for the right and left hips (Table 2). The main

Table 2. Intraobserver variation.

	Right hip, κ 0.86 (n = 100)					Left hip, κ 0.91 (n = 100)				
	1st Reader					1st Reader				
	0	1	2	3	4	0	1	2	3	4
2nd reader										
0	72	2	2			72	2			
1	1	1				1		1		
2			8	1				8		
3				1					4	
4					12					12

errors were distinguishing between suspicious (grade 1) and minimal (grade 2) disease in 4% of cases.

Interobserver variation. Reproducibility between 2 readers was assessed using 134 pelvic (AP) radiographs scored by each observer on separate occasions, revealing 83% complete agreement between readers for the right hip and 84% complete agreement for the left hip. Kappa scores showed good agreement at 0.75 and 0.74, respectively (Table 3). Distinguishing minimal from suspicious disease was again the main problem, in 6% of cases.

Sensitivity to change. Using a Wilcoxon signed-rank test for nonparametric data, the BASRI-hip showed a significant change in radiological score at one year ($p < 0.001$; Table 4).

Disease specificity. A BASRI-hip score of grade 2 was used as the cutoff to determine AS radiological change. Disease specificity is defined as "the proportion of true positives that are correctly identified as such and is 1 minus the false negative rate".[22] Hence, the disease specificity of the BASRI-hip in this study was $1 - 0.24 = 0.76$ (76%). If non-AS patients with a total hip replacement (THR) are excluded (because they automatically score grade 4, whatever the reason for their THR), disease specificity becomes 0.83. Hence, 24 of the 200 hips studied in the non-AS cohort scored ≥ 2.

Scoring speed. The mean time taken to score a pair of hips on a single AP pelvic radiograph is 10 s.

DISCUSSION

The use of standard measures documenting disease status is essential to allow comparison of results between clinical studies, to improve knowledge of disease natural history, and to set a standard against which new treatments may be assessed[23]. Any such measure must be reproducible, responsive (sensitive to change), disease-specific, and valid (i.e., measures what it claims to measure)[11,24,25]. To be useful it must be clinically feasible, being simple to use with few training requirements[24,25]. The RA literature demonstrates this well by the frequent use of the American College of Rheumatology Response Criteria, European League Against Rheumatism Core Data Set, and radiological scoring systems such as the Larsen and Sharp scores[20,26-28]. The only radiological scoring system currently in use for the AS hip has been the Larsen score, which was designed primarily to describe the hip in RA[20]. Larsen states that the score is not as reliable when used to grade conditions, such as psoriatic arthritis or AS, where new bone formation is a prominent feature[20]. The BASRI-hip, in conjunction with the BASRI-spine[14], was designed to fill a perceived gap in the range of outcome measures for AS.

As the New York criteria[12] for the SI joints are well established they were used as the basis for the BASRI scores. The BASRI-hip was developed in an identical way to the BASRI-spine score[14] and was equally reproducible, with kappa scores showing good agreement. In addition, it performed as well as the New York criteria for the SI joints when tested in the same patient cohort on a separate occasion[14].

Although the BASRI-hip was shown to be disease-specific it did not perform as well as the BASRI-spine[14]. This may have been caused by 2 principal factors. All patients in the non-AS group had pelvic radiographs taken because of hip pain, whereas those in the AS cohort had radiographs taken to visualize the SI joints and did not necessarily have hip pain. Additionally, a hip replacement always scores grade 4 whatever its cause. Of the 100 patients included in the non-AS cohort, 24 were graded as if they had AS (i.e., they had scores ≥ 2), but 7 of the 24 scored grade 4 because of a hip replacement. If these 7 were excluded, only 17/100 scored ≥ 2 (minimal disease), giving a specificity of 83%. Thirteen of those 17 had severe RA. It is likely that the BASRI-hip is describing aspects of inflammatory arthritis, e.g., circumferential joint space narrowing, that are central to both RA and AS. During the validation of BASRI-hip, the SI joints were covered prior to scoring because sacroiliitis (as part of the New York criteria[12]) was a necessary entry requirement for any patient with AS in the study.

Little information has been available regarding radiographic sensitivity to disease progression and at what frequency repeat radiographs should be performed. The BASRI-hip was sensitive to change over a one year period. Repeating radiographs at intervals of less than one year, for either routine or study purposes, is therefore not warranted. By definition, the patients used to study sensitivity to

Table 3. Interobserver variation.

	Right hip, κ 0.75 (n = 134)					Left hip, κ 0.74 (n = 134)				
	1st Reader					1st Reader				
	0	1	2	3	4	0	1	2	3	4
2nd reader										
0	72	3	4			71	2	3		
1	2	9	3			4	10	3		
2		2	8	2	1	3			3	
3			1	4	2		1		5	3
4				1	20				1	17

Table 4. 1. Sensitivity to change: one year

	Right hip, p < 0.001 (n = 60) 12 Months					Left hip, p = 0.014 (n = 60) 12 Months				
	0	1	2	3	4	0	1	2	3	4
Baseline										
0	29	4	1			34	4			
1		7	4				3			
2			3	1				5	2	
3				2	1					
4					8					12

2. Sensitivity to change: 2 years

	Right hip, p < 0.0001 (n = 65) 24 Months					Left hip, p = 0.006 (n = 65) 24 Months				
	0	1	2	3	4	0	1	2	3	4
Baseline										
0	29	3	2			26	2		1	
1		9	4				1	2		
							3			
2			2	2				6	1	2
3				1	3					1
4					10					11

3. Sensitivity to change: 3 years

	Right hip, p < 0.001 (n = 60) 36 Months					Left hip, p = 0.0001 (n = 60) 36 Months				
	0	1	2	3	4	0	1	2	3	4
Baseline										
0	33	1	5			27	3	2	1	
1	1	5	1	1			3	2	2	1
2			3	4				1	5	1
3					3				1	4
4					3					7

4. Sensitivity to change: 4 years

	Right hip, p < 0.0001 (n = 56) 48 Months					Left hip, p < 0.0001 (n = 56) 48 Months				
	0	1	2	3	4	0	1	2	3	4
Baseline										
0	26	1	4			23	3	2	1	
1		1	1	1	2		3	2	2	1
2			8		1			1	5	1
3				3	3				1	4
4					5					7

change each had more than one pelvic radiograph available. It is possible, therefore, they had more severe hip disease than other patients with only a single pelvic radiograph. This could mean that sensitivity to change becomes apparent at one year only in those with severe hip disease. However, to reduce radiation dosage, it is our policy to perform a single AP pelvic radiograph to assess both the SI joints and hips together. Hence, the additional pelvic radiograph may have been taken to assess the SI joints rather than the hips, and this was the case in at least half of the patients included. The radiographs in the study were blinded for date and the BASRI-hip was able to identify the earlier of 2 radiographs performed on the same individual, determining "forward progression."

It would be quite feasible to use the index in routine medical practice, since the BASRI-hip was simple to use and explain, requiring minimal training. Moreover, scoring a pair of hips takes only seconds. The face and construct

lidity[25] of the index is supported by cross sectional data nerated using the cohort of 470 patients[29]. These data ncur with earlier studies suggesting that hip disease velops in those whose AS begins at a younger age and ıo tend to have more severe disease[15-19]. Work with longi-dinal data is under way to confirm the validity of using ıth the BASRI-hip and the BASRI-spine as prognostic arkers. To date, only limited data are available deter-ining the relationship between radiology, metrology, func-ın, and disease activity, and we now plan to expand this to .plore the interrelationship between these phenomena[3-5].

The BASRI-hip, as described above, is a modified :rsion of work previously published in abstract form, volving the whole axial skeleton[30]. Further work has since :en undertaken delineating the hip score and allowing a ore precise interpretation of the grading system. The ırrent study uses a separate cohort of patients to the evious work and was undertaken to validate the BASRI-p in a new AS population. Hip changes have been :scribed separately from the BASRI-spine, as those devel-ɔing hip disease appear to represent a distinct cohort of .sease affecting between 18 and 37% of the AS popula-on[15] and 22% of the 470 patients with AS studied[29]. Hip thritis is associated with the age of disease onset, occur-ng by the age of 23 in the majority[16]. This is unlike ɔcending spinal disease, which seems to be a product of me[15,17]. Hip disease is also associated with a worse utcome and has been included as one of 7 entry variables orrelating with disease severity (odds ratio 22.85, .43–118)[18]. To avoid inappropriately diluting the overall ɔore of patients with AS it is important to be able to sepa-ate the hip and spine scores as necessary. Additional radi-graphs are not necessary to score the BASRI-hip if the BASRI-spine has already been undertaken, since the AP iew of the pelvis should include the hips when the SI joints re scored.

Radiology is fundamental to the diagnosis and subse-uent progression of AS. Apart from the New York criteria or the SI joints, no widely accepted radiological criteria xist. The BASRI-hip is reproducible, disease-specific, ensitive to change at one year, and simple to use and :xplain. It can be used in conjunction with the BASRI-pine[14] to produce a global radiological index scoring the :hole axial skeleton, which will be a valuable addition to ıe range of outcome measures in AS.

ACKNOWLEDGMENT

The authors thank P. Porch, Senior Radiographer, for her help.

REFERENCES

1. Dougados M, Gueguen A, Nakache JP, Nguyen M, Mery C, Amor B. Evaluation of a functional index and an articular index in ankylosing spondylitis. J Rheumatol 1988;15:302-7.
2. Dougados M, Gueguen A, Nakache JP, Nguyen M, Amor B. Evaluation of a functional index for patients with ankylosing spondylitis [letter]. J Rheumatol 1990;17:1254-6.
3. Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: The development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281-5.
4. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gainsford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: The Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286-91.
5. Jenkinson T, Mallory P, Whitelock H, Kennedy LG, Garrett S, Calin A. Defining spinal mobility in ankylosing spondylitis. The Bath AS Metrology Index. Rheumatol 1994;21:1694-8.
6. Jones S, Steiner A, Garrett SL, Calin A. Bath Ankylosing Spondylitis Patient Global Score (BAS-G). Br J Rheumatol 1996;35:66-71.
7. Creemers MCW, Van't Hof MA, Franssen MJAM, Van de Putte LBA, Gribnau FWJ, Van Riel PLCM. Disease activity in ankylosing spondylitis: Selection of a core set of variables and a first set in the development of a disease activity score. Br J Rheumatol 1996;35:867-73.
8. Daltroy L, Larson M, Roberts WN, Liang M. A modification of the Health Assessment Questionnaire for the spondyloarthropathies. J Rheumatol 1990;17:946-50.
9. Mander M, Simpson JM, McLellan A, Walker D, Goodacre JA, Carson Dick W. Studies with an enthesis index as a method of clinical assessment in ankylosing spondylitis. Ann Rheum Dis 1987;46:197-202.
10. Laurent MR, Buchanan WW, Bellamy N. Methods of assessment used in ankylosing spondylitis clinic trials: A review. Br J Rheumatol 1992;30:326-9.
11. Rigby AS, Silman AJ. Outcome assessment in clinical trials of ankylosing spondylitis [editorial]. Br J Rheumatol 1991;30:321-5.
12. Moll JMH, Wright V. New York Clinical Criteria for Ankylosing Spondylitis. Ann Rheum Dis 1973;32:354-63.
13. Kellgren JH, Jeffrey MR, Ball J. The epidemiology of chronic rheumatism. In:Kellgren JH, Jeffrey MR, Ball J, editors. The epidemiology of chronic rheumatism. Vol 1. Oxford: Blackwell Scientific; 1963:326-7.
14. Mackay KR, Mack CS, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI): A new validated approach to disease assessment. Arthritis Rheum 1998;41:2263-70.
15. Calin A. Terminology, introductions, diagnostic criteria and overview. In: Calin A, Taurog JD. The spondyloarthritides. Oxford: Oxford University Press; 1998:1-15.
16. Calin A, Elswood J. The relationship between pelvic, spinal and hip involvement in ankylosing spondylitis — one disease process or several? Br J Rheumatol 1988;27:393-5.
17. Carette S, Graham D, Little H, Rubenstein J, Rosen P. The natural disease course of ankylosing spondylitis. Arthritis Rheum 1983;26:186-90.
18. Amor B, Santos RS, Nahal R, Listrat V, Dougados M. Predictive factors for the long term outcome of spondyloarthropathies. J Rheumatol 1994;21:1883-7.
19. Riley MJ, Ansell BM, Bywaters EGL. Radiological manifestations of ankylosing spondylitis according to age at onset. Ann Rheum Dis 1971;30:138-48.
20. Larsen A, Dale K, Morten E. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiologica 1977;18:481-91.
21. Bellamy N, Buchanan WW. Outcome measurement in osteoarthritis clinical trials: The case for standardisation. Clin Rheumatol 1984;3:293-303.
22. Kirkwood B. Sources of error. In: Kirkwood B. Essentials of medical statistics. Oxford: Blackwell Scientific; 1988:163.
23. Felson DT. Choosing a core set of disease activity measures for

Appendix 4 : Submitted to Journal of Rheumatology.

The Natural History of Ankylosing Spondylitis as Defined by Radiological Progression

SINEAD BROPHY[1], KIRSTEN MACKAY[1&2], AHMED AL-SAIDI[1], GORDON TAYLOR[3] and ANDREI CALIN[1].

ABSTRACT. Objective. Radiological status is an important objective endpoint in the assessment of ankylosing spondylitis (AS). Herein we investigate the disease development of AS using radiological change.

Methods. The existing radiographs (n=2284) of 571 AS patients attending the Royal National Hospital for Rheumatic Diseases were scored retrospectively using the Bath Ankylosing Spondylitis Radiology Index. [1] Progression of disease was initially examined cross-sectionally. Univariant analysis was used to examine factors associated with joint involvement. [2]. Progression of disease was then examined longitudinally for those patients with films at time of symptom onset. [3] Rate of progression of radiological change was calculated using longitudinal data of two sets of radiographs taken 10 years apart [patient number = 54]. The results from this were used to extrapolate backwards to age at first radiological change.

Results. [1] Progression to cervical spine disease is a function of : disease duration, severity of hip and lumbar involvement and a history of iritis. [$p<0.001$]. Lumbar involvement was associated with disease duration, age now, and severity of cervical and hip involvement [$p<0.001$]. Hip involvement was a marker for cervical disease and associated with disease duration [$p<0.001$][2] Longitudinal analysis reveals marked variation among patients with a slow general rate of progression. [3] The progression of AS over any ten year period is linear [First 10 years = 30% (sd: 0.3) of potential change, 10-20 yrs = 40% (sd: 0.3) change, 20-30 yrs = 35% (sd: 0.4) change

(p=0.5)]. Backward extrapolation suggests that the approximate time of first radiological change is at the age of 8 years.

Conclusion. 1 AS is a linearly progressive disease with approximately 35% change every 10 years. Spinal involvement is largely an expression of disease duration while the hips become involved in about 25% of individuals and may predict a more severe outcome for the cervical spine. 2. Backward extrapolation shows that the disease process may start as young as 8 years of age. However, the time interval between the disease trigger and radiological change remains unknown.

Key words: ankylosing spondylitis, radiology, disease history ,BASRI

Appendix 5

A New Dimension to Outcome: Application of The Bath Ankylosing Spondylitis Radiology Index

ANDREI CALIN, KIRSTEN MACKAY, HELENA SANTOS, and SINEAD BROPHY

ABSTRACT. Our aim was to develop a reproducible and simple radiological scoring system for ankylosing spondylitis (AS) to use in cross sectional and prospective studies. Regarding validation of the BASRI (Bath Ankylosing Spondylitis Radiology Index), radiographs of 470 patients with AS were scored using the New York criteria for the sacroiliac joints. The lumbar and cervical spine, and hips were similarly graded 0–4. These scores were added together to give BASRI-t (total) and if the hips are excluded to give BASRI-s (spine). Radiographs of 188 patients were used to test reproducibility. Blinded radiographs of 89 non-AS patients were included randomly to assess disease specificity. Sensitivity to change was assessed using 177 radiographs from 40 patients. Regarding the cross sectional study, 2200 radiographs of 550 (104 F:446 M) patients were randomly selected and scored using BASRI. The frequency distribution of BASRI-t and BASRI-s were plotted using a probit plot. Inter and intraobservation showed between 73 and 82% and 73 and 88% complete agreement, with specificity of 0.78–0.89, suggesting scores are disease-specific. Sensitivity to change became apparent at 2 years ($p < 0.05$). Scoring required 30 seconds to complete. BASRI-t was found to be normally distributed using a probit plot. The mean BASRI scores (total, spinal, hip) increased with disease duration. The correlation, however, was poor ($r = 0.293, 0.347, 0.263$, respectively). Those with hip involvement had more severe spinal disease ($p < 0.0001$). Men had more severe spinal disease than women ($p < 0.0001$). We conclude BASRI is a reliable and rapid method to grade radiographic changes in AS. Using this scoring system it can be seen that AS is a slowly progressive disease with much individual variation. Hip patients have more severe spinal disease than those without hip involvement and men have more severe spinal disease than women. (J Rheumatol 1999;26:988–92)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS RADIOLOGY BASRI OUTCOME

Ankylosing spondylitis (AS) is a chronic inflammatory progressive disorder mainly affecting the axial skeleton and the peripheral joints. The disease is a result of interaction between genetic and environmental triggers. A number of measures can be used simultaneously to monitor outcome and these are fundamental in assessing the natural history of AS[1-6]. Characteristic radiological appearances at the sacroiliac (SI) joints are essential for diagnosis of AS[7], but no classification completely defining axial and hip radiological change exists in AS. The Bath Ankylosing Spondylitis Radiology Index-total (BASRI-t) is a new system for scoring radiological change for the spine and hip in AS[8]. Results can be divided into Bath Ankylosing Spondylitis Radiology Index-spine (BASRI-s), which combines the scores of the SI joints, lumbar spine, and the cervical spine, and the BASRI-hip (BASRI-h). We describe BASRI-t, BASRI-s, and BASRI-h and demonstrate their value in a cross sectional study.

From the Epidemiology Department, Royal National Hospital for Rheumatic Diseases, Bath, UK.

Supported by the National Ankylosing Spondylitis Society, John Coates Charitable Trust, and Col. W.W. Pilkington Charitable Trust.

A. Calin, MD, FRCP, Consultant Rheumatologist; K. Mackay, MRCP, Specialist Registrar; H. Santos, MD, Visiting Research Fellow; S. Brophy, BSc, Research Assistant.

Address reprint requests to Dr. A. Calin, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath, BA1 1RL, UK; E-mail: mpssb@bath.ac.uk

MATERIALS AND METHODS

Validation of BASRI. Existing Radiographs of 470 patients, diagnosed using the New York criteria for AS, were used to develop the method. They were scored openly and placed in one of 5 severity grades based on the NY scale for SI joint disease. The radiographs were an anteroposterior (AP) pelvis, an AP and lateral lumbar spine, and a lateral cervical spine. The lumbar spine was defined as extending from the lower border of T12 to the upper border of S1, and the cervical spine from the lower border of C1 to the upper border of C7. The discriminating features of each group were defined and used as the basis to describe a method of assessing severity of radiological change in AS. The system was then repeatedly tested in a blinded fashion by 3 experienced readers and modified openly on several occasions before a final format was agreed on. Rules for scoring the lumbar, cervical and hip radiographs are shown in Table 1.

To assess whether AP or lateral radiograph was more appropriate, 58 sets of lumbar and cervical spine radiographs with both AP and lateral views were scored using (1) AP alone, (2) the lateral alone, and (3) both views' contribution (as a composite score). Sensitivity and specificity for each view was determined and compared to Score 3.

After definition of the scoring system, radiographs of 188 consecutive patients with AS and 89 without AS were scored randomly and blindly by the 3 readers to validate BASRI. The mean age of the population was 44.5 ± 10.9 years and the sex ratio 3:1 (M:F), disease duration = 23 years. Assessment included inter and intraobserver variation, sensitivity to change over a yearly period (1, 2, 3 yrs, etc.), and specificity (cutoff of grade 2 — definite disease). The non-AS cohort studies were consecutive outpatients attending the Royal National Hospital for Rheumatic Diseases and had

Table 1A. BASRI-spine.

Score	Grade	System Applies to Both Lumbar and Cervical Spine
0	Normal	No change
1	Suspicious	No definite change
2	Mild	Any number of erosions, squaring, sclerosis ± syndesmophytes on ≤ 2 vertebrae
3	Moderate	Syndesmophytes on ≥ 3 vertebrae ± fusion involving 2 vertebrae
4	Severe	Fusion involving ≥ 3 vertebrae

Table 1B. BASRI-hip.

Score	Grade	Description
0	Normal	No change
1	Suspicious	Focal joint space narrowing
2	Mild	Circumferential joint space narrowing > 2 mm
3	Moderate	Circumferential joint space narrowing ≤ 2 mm or bone-on-bone apposition of <1 cm
4	Severe	Bone deformity or bone-on-bone apposition ≥1 cm

NB: Increase the grade by 1 if 2 of 3 of the following bony changes are present: erosions, osteophytes, protrusion.

pelvic radiographs taken. Their mean age was 57.9 ± 16.8 years, sex ratio 1:3 (M:F). The cohort included 41 rheumatoid arthritis, 21 mechanical back pain, 10 fibromyalgia, 10 osteoporosis, 7 psoriatic arthritis.

Cross sectional study. We randomly selected and scored 2200 sets of films of 550 (4:1, M:F) patients with AS using BASRI-t. The frequency distributions of BASRI-t and BASRI-s were plotted using a probit plot to establish parametric distribution. The probit plot is a scatter diagram that is linear if data are normally distributed and curved if they are not. Of the 550 patients, there were 423 with known disease duration.

Statistical methods. For validation of BASRI: The data were nonparametrically distributed; therefore an unweighted kappa statistic was used to determine the significance of inter and intraobserver variability. The Wilcoxon signed rank test was used to assess significance of change over time. For the implementation of BASRI: correlations were established using Pearson's correlation coefficient for normally distributed variables or Spearman's rank order for non-normally distributed variables. Independent t tests or Wilcoxon rank sum test and chi-squared were used to evaluate mean scores. The SPSS software program was used for all analyses.

RESULTS

Lumbar spine: which film should be used. Using 58 sets of AP and lateral lumbar spine radiographs, 3 scores were derived as described above. The combination score differed from both the AP and lateral scores if syndesmophytes or fusion was seen at different levels on each projection and occurred in 3/58 cases. The combination score differed from the AP alone in 9/58 (16%) cases and the lateral alone in 21/58 (36%) cases. Over all, the use of 2 projections changed the score 46% of the time.

Intraobserver variation. The complete agreement and kappa scores were (Table 2): 86% agreement (kappa 0.69) for the SI joints, 75% agreement (kappa 0.65) for the lumbar spine, 81% agreement (kappa 0.73) score for the cervical spine, and 87 and 88% agreement (kappa 0.70 and 0.75) for the right and left hip.

Interobserver variation. Reproducibility between readers revealed 78% agreement (kappa 0.55) for the SI joints, 73% agreement (kappa 0.64) for the lumbar spine, 79% agreement (kappa 0.69) for the cervical spine, and 82 and 78% agreement (kappa 0.64 and 0.57) for the right and left hip.

Disease specificity. To validate lumbar and cervical spine, radiographs of 89 non-AS outpatients were scored. For the hip component 51 non-AS outpatients were scored. Grade 2 was taken as the cutoff. A sample of 188 patients with AS was used (disease duration = 23 yrs; age 44.5 ± 10.9). Specificity for the lumbar spine was 0.89, for the cervical spine it was 0.83, and for the hips 0.78.

Table 2. Summary of inter and intraobserver variation in BASRI-total.

Skeletal Site	Intraobserver Variation		Interobserver Variation	
	Complete Agreement, %	Kappa	Complete Agreement, %	Kappa
SI joints	86	0.69	78	0.55
Lumbar spine	75	0.65	73	0.64
Cervical spine	81	0.73	79	0.69
Hips	87	0.70	78	0.57

Sensitivity to change. Serial radiographs of 40 patients were scored over 177 time intervals (4.4 intervals/patient). All radiographs were blinded for the name and date of radiograph. Scoring was performed by a single observer. The patients were assessed over a time period of one year [years between radiographs: 1 yr (n = 24), 2 yrs (n = 31), 3 yrs (n = 30), 4 yrs (n = 26)]. A significant change in radiological score ($p < 0.05$) at 2 years was observed for the SI joints, lumbar and cervical spine, and the hips. The magnitude of change for the BASRI-spine was from 7.0 to 7.9 in a 2 year period. Forty-two percent of patients had a change in BASRI-s score in a 2 year period. The smallest detectable difference between scores over a 2 year period is a change in BASRI-s of 0.5, i.e., 0.5 change in SI joints score, and a change in score of 1 for the lumbar spine and cervical spine.

Frequency distribution of BASRI-t and BASRI-s. BASRI-t was normally distributed using a probit plot. BASRI-s was not normally distributed.

Cross sectional study. The mean BASRI-t and BASRI-s scores over 5 year intervals were plotted against disease duration (Figure 1). Correlation was poor: $r = 0.293$, $p < 0.01$ and $r = 0.347$, $p < 0.01$, respectively.

BASRI-s for those patients with hip disease (n = 101) and those without (n = 322) was plotted against disease duration (Figure 2). The BASRI-s was higher for those with hip disease ($p < 0.0001$). There was no difference in disease duration between the 2 groups (20 and 21 yrs, respectively; $p < 0.2$).

The mean BASRI-t was higher for men (n = 351) than for women (n = 72) (8.9 vs 7.2, respectively; $p < 0.0001$). Disease duration was comparable (20 vs 21 yrs; $p < 0.27$) (Figure 3). More men than women had severe disease in the SI joints (odds ratio, OR = 1.74; 95% confidence interval, CI, 1.1–2.7; $p < 0.016$). More men than women had severe lumbar spine disease (OR = 2.6, 95% CI 1.4–4.6; $p < 0.001$). More men than women had severe cervical spine disease (OR = 2.3, 95% CI 1.3–3.9; $p < 0.002$). The numbers of men and women with severe hip disease were comparable at all stages of disease.

DISCUSSION

The BASRI is reproducible, with inter and intraobserver variation equivalent to or better than the NY criteria. The BASRI was found to be sensitive to change over a 2 year period, which suggests radiographs at intervals of less than 2 years are not warranted. The BASRI was easy to use; the mean time taken to score a set of radiographs was less than 30 seconds. To maintain simplicity, BASRI does not pick up minor radiological change. The score does not change with each additional erosion or sclerosis, and will always remain grade 2 or mild disease until there is fusion between 2 vertebrae or ≥ 3 syndesmophytes are identified.

The changes in BASRI-t and BASRI-s over time may

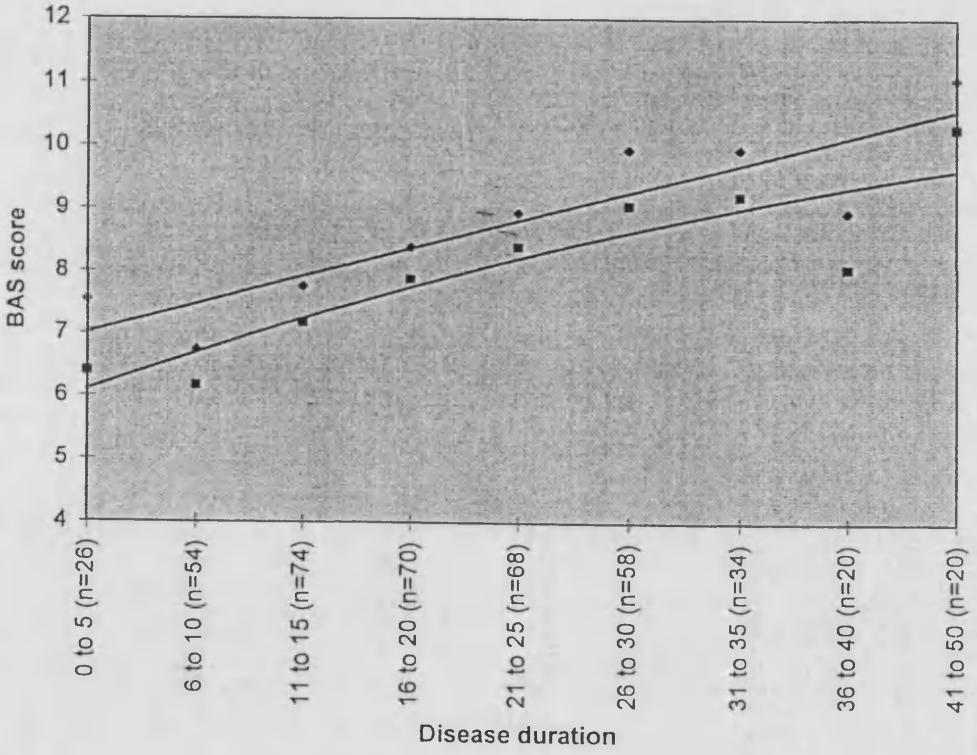


Figure 1. BASRI-total and BASRI-spine versus disease duration (n = 423). ◆ BASRI-t, r = 0.293. ■ BASRI-s, r = 0.347.

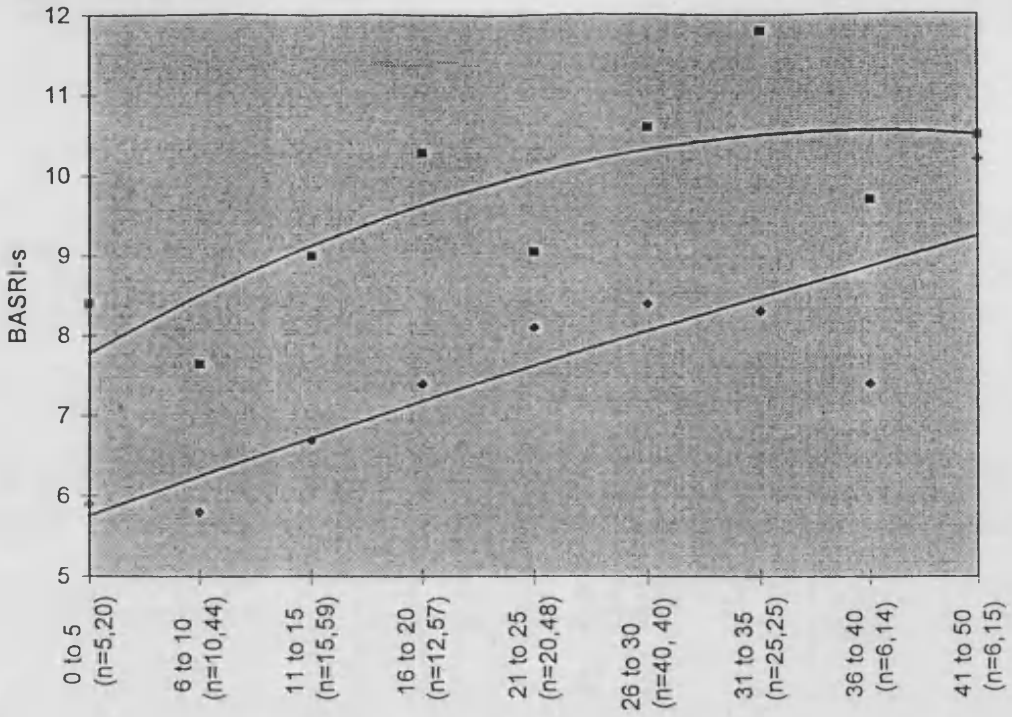


Figure 2. BASRI-spine — patients with hip disease (n = 101) versus no hip disease (n = 322). ■ BASRI-s (H). ◆ BASRI-s (NH). $p < 0.001$.

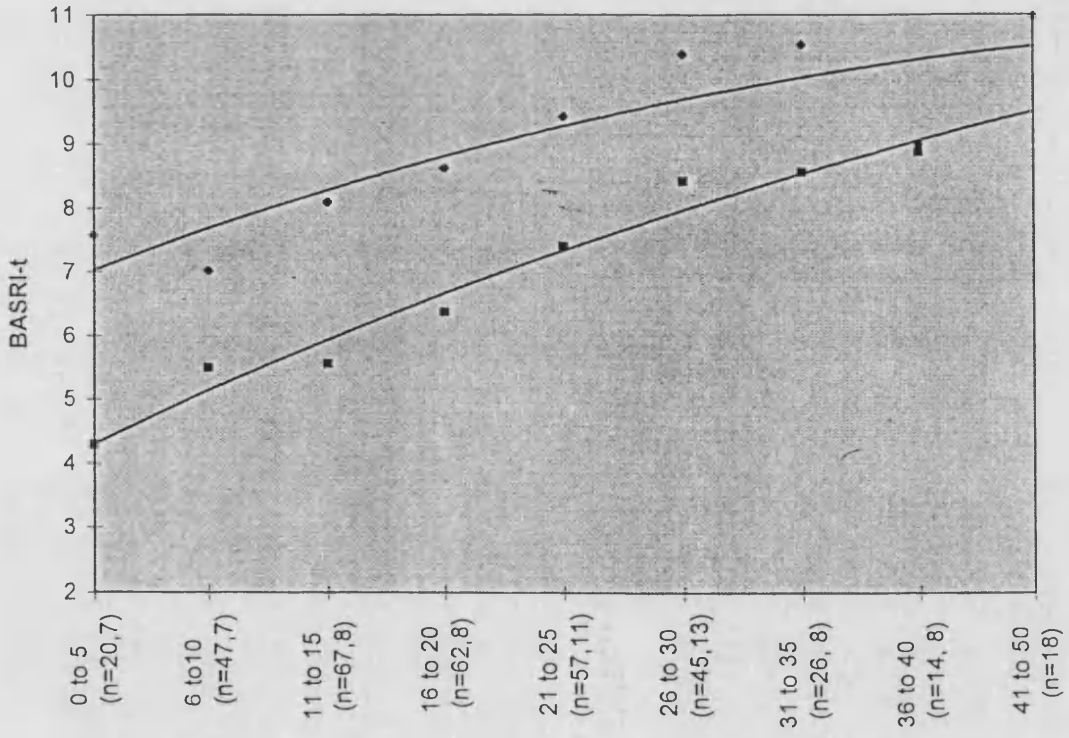


Figure 3. BASRI-total — radiology of men (n = 354) versus women (n = 72). ◆ Male. ■ Female. $p < 0.0001$.

indicate that AS is a slow progressive disease, or of course, that BASRI is too insensitive to pick up clinically relevant change. However, lack of correlation between BASRI scores and disease duration shows that outcome is extremely variable for different individuals. The change in BASRI-s in patients with and without hip involvement shows that patients with hip disease have more severe axial disease. This supports other studies that suggest hip involvement is predictive of more severe disease[9]. Comparison of male versus female has shown that men have more severe disease in the SI joints, lumbar spine, and the cervical spine, but not in the hips. Clearly, clinical significance does not equate with statistical significance. Further studies will be required to define the value of these findings.

The correlation of severity with disease duration is poor if taken individually. In the scoring of the lumbar spine, only half the patients develop severe disease after 45 years. For the cervical spine, 25% never develop any cervical involvement.

In conclusion, radiology is fundamental to diagnosis and progression of AS. Apart from the New York criteria for the SI joints, no widely accepted radiological criteria exist. BASRI as a radiological classification system is a valuable tool that is reproducible, specific, sensitive to change at 2 years, simple, and fast to use. Using the BASRI in cross sectional study of AS shows that this is a slowly progressive disease with much individual variation. Some of this variation can be accounted for: patients with hip involvement have more severe spinal disease and men have more spinal involvement than women. In conclusion, BASRI is an important outcome measure joining the metrology index (BASMI)[1], functional index (BASFI)[3], disease activity index (BASDAI)[5], and global score (BAS-G)[6] in the assessment of our patients.

REFERENCES

1. Jenkinson T, Mollorie P, Whitelock H, Kennedy G, Garrett S, Calin A. Defining spinal mobility in ankylosing spondylitis. The Bath Metrology Index. J Rheumatol 1994;21:1694–8.
2. Dougados M, Gueguen A, Nakache J, Nguyen M, Mery C, Amor B. Evaluation of a functional index and an articular index in ankylosing spondylitis. J Rheumatol 1988;15:302–7.
3. Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281–5.
4. Mander M, Simpson JM, McLellan A, Walker D, Goodacre JA, Carson Dick W. Studies with an enthesis index as a method of clinical assessment in ankylosing spondylitis. Ann Rheum Dis 1987;46:197–202.
5. Garrett S, Jenkinson T, Kennedy G, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis. The Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286–91.
6. Jones S, Steiner A, Garrett S, Calin A. Bath Ankylosing Spondylitis Patient Global Score. Br J Rheumatol 1995;34:114.
7. Rigby S, Silman A. Outcome assessment in clinical trials of ankylosing spondylitis [editorial]. Br J Rheumatol 1991;30:321–5.
8. Mackay K, Mack C, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index: a new validated approach to disease assessment. Arthritis Rheum 1998;41:2263–70.
9. Amor B, Santos R, Nahal R, Listrat V, Dougados M. Predictive factors for the long term outcome of spondyloarthropathies. J Rheumatol 1994;21:1883–7.

Appendix 6 : In Press. Journal of Rheumatology.

Inflammatory eye, skin and bowel disease in spondylarthritis : Genetic, phenotypic and environmental factors.

SINEAD BROPHY[1], STEPHAN PAVY[1], PETER LEWIS[2], GORDON TAYLOR[2], LINDA BRADBURY[3], DUNCAN ROBERTSON[4], CHRIS LOVELL[5] and ANDREI CALIN[1]

ABSTRACT. ***Objective:*** To explore the nature of the inter-relationship between inflammatory disease of the spine/ joints, skin, eye and bowel [i.e. ankylosing spondylitis (AS), psoriasis (Ps), iritis (I), inflammatory bowel disease (IBD)].

Methods: The study used four approaches: 1. Analysis of the prevalence of secondary disorders within the AS individual (χ^2 and matched pair analysis). 2. A study of the temporal relationship between the onset of the different conditions. 3. An evaluation of the prevalence of disease among first degree relatives and 4. The influence of secondary disorders on outcome of AS.

Results : 1. Among 3287 patients with AS, more than expected had either spondylitis associated with multiple co-disorders or pure AS (with no co-diseases) while fewer had AS plus one single co-disease than expected. (χ^2=32.2 p<0.001). In a matched pair analysis, patients with AS and a secondary disorder were more likely to have yet another additional concomitant disease. eg IBD-AS (n=335) patients had a higher prevalence of iritis [45.4% vs 36.7% O.R: 1.4 (1.1-2.0)] or psoriasis [23.9% vs 14.3% O.R: 1.9 (1.3-2.8)] than controls. 2. Among our database subjects, the symptomatic onset of the spinal disease precedes or is contemporaneous with gut, skin and eye involvement (Matched pair T-test , p<0.001). 3. Patients with multiple disorders predict the highest prevalence of co-diseases (ie Ps, IBD, I or AS) within family members, followed by those AS patients with only IBD, Ps or iritis in descending order. 4. Both Ps and IBD increase severity in terms of function and disease activity of AS in the patient. Radiological change is greatest for those AS subjects with iritis.

Conclusion: There is a striking overlap within patients and family members of rheumatological, dermatological and gastroenterological diseases. The susceptibility

genes of these co-disorders appear to overlap with each other and with AS : 1. A patient with two inflammatory conditions is at an increased risk of developing an additional related inflammatory disorder. 2. Those with enteropathic spondylarthritis would appear to carry the greatest genetic load in terms of first degree relatives developing inflammatory conditions (including Ps and I which are not seen in the index IBD-AS patient) 3. The secondary disorders do not precede AS (arguing against Ps and IBD allowing for an environmental conduit to pathogenic triggers in AS).
The susceptibility factors for these inflammatory conditions may be additive or have a synergistic effect on each other. There is evidence for a shared gene hypothesis.

Key words: Spondylarthritis, iritis, inflammatory bowel disease, psoriasis.

INTRODUCTION

The spondylarthritides are a group of disorders characterised by involvement of the sacroiliac joints and peripheral inflammatory arthropathy together with target organs including the eye, bowel and skin[1]. Patients with ankylosing spondylitis (AS) can develop psoriasis (Ps) or inflammatory bowel disease (IBD), whereas those with psoriasis or enteropathy may develop joint involvement. The link between these diseases might be genetic, with shared susceptibility genes. This hypothesis is supported by the finding that B27 negative spondylitis patients are more likely to have secondary forms of disease linked to psoriasis or IBD[2]. Thus, in the absence of the B27 susceptibility effect, genes such as those for the other inflammatory conditions may be important in predisposing the individual to ankylosing spondylitis. Alternatively, these conditions may be linked by environmental factors, the inflamed gut and skin allowing the conduit of pathogenic triggers which induce AS. The HLA-B27 transgenic rat develops clinical features including: inflammatory gastrointestinal disease, skin lesions, spinal lesions and peripheral arthritis. These rats generally develop bowel inflammation which is then followed by arthritis. However, if the HLA B27 transgenic rat is maintained in a sterile environment neither the inflammatory gastrointestinal disease, nor the arthritis develop[4]

Thus there is evidence that the link between these inflammatory conditions could be genetic or environmental. How these diseases are linked will have an important impact on our understanding and treatment of AS and the associated risks to relatives of affected individuals[5] . This study explores the nature of the inter-relationship between inflammatory disease of the spine / joints, skin, eye and bowel [i.e ankylosing spondylitis (AS), psoriasis (Ps), iritis (I), inflammatory bowel disease (IBD)].

METHODS

The database

The Bath RNHRD Ankylosing Spondylitis Database consists of 4953 patients. These subjects are defined as those with symptomatic disease and sacroiliitis diagnosed by x-ray, fulfilling the New York criteria for AS (ie all patients had at least sacroiliitis stage II bilateral or stage III unilateral disease) and meeting the Amor (1991) and the European Spondylarthropathy Study Group diagnostic criteria for the Spondylarthropathies. These patients represent a sub-group of spondyarthritis as they only include patients diagnosed with Ankylosing Spondylitis. Subjects are either those referred to the Royal National Hospital for Rheumatic Diseases or are members of the National Ankylosing Spondylitis Society (NASS). Among the patients, 1915 (39%) have iritis, 811 (16%) psoriasis and 404 (8%) inflammatory bowel disease (IBD:158 Crohn's disease and 246 ulcerative colitis). The diagnoses for iritis and psoriasis were ascertained by the GP, rheumatologist, ophthalmologist or dermatologist. A gastroenterologist was required for the diagnosis of inflammatory bowel disease. In each case the diagnosis was recorded as representing one point in time. Two studies have been performed to validate the diagnosis of ankylosing spondylitis in those patients recruited through NASS. 146 subjects were assessed by a rheumatologist and 100% were confirmed as having AS according to the New York Criteria (personal communication, M. Brown, Oxford) The general practitioners of a further 240 NASS members were contacted to determine whether their patient's AS had been confirmed radiologically. In 229 (95.4%) cases, AS with radiological evidence of sacroliitis was confirmed . We contacted the GP's of 120 Ps-AS patients and 139 IBD-AS patients. Of these, 77 (64%) and 112 (81 %) replied confirming Ps in 65 (84%) of cases and

IBD in 108 (96%) of cases. Iritis was diagnosed by an eye specialist in 1551 (81%) cases of 1915 reported with iritis (GPs or rheumatologist diagnosed 19%)

Data processing and statistical methods

1. Of the 4953 subjects on the database, 3287 had a complete data set with full family and personal data (the disease duration and sex ratio of those analysed compared to those without complete records was comparable : 21.3 yrs , 2.3:1 M:F and 20.0 yrs , 2.1:1 M:F respectively). The prevalence of the three individual secondary disorders (psoriasis, IBD and iritis) was determined. From these data the expected numbers of patients with none, one, two or three of these conditions was established assuming independence between the diseases. The expected number was compared with the observed number using the χ^2 test. Patients with a secondary disorder were matched with an available and appropriate control (without the specific condition) for disease duration, age and sex (ie psoriasis patients were matched with those without psoriasis [600 pairs of 811 Ps patients], IBD patients with those without IBD [335 pairs of 404 IBD patients], and iritis patients matched with those without iritis [735 pairs]. All pairs were matched for exactly the same number of years of disease duration and age. These pairs were compared using McNemar's χ^2 for prevalence of secondary disorders as outlined above.

2. Patients with a confirmed diagnosis for all reported secondary conditions and arthritis were asked to report when symptoms of each condition first began. Patient recollection was verified by GP reports.

3. Patients with a confirmed diagnosis of disease were asked 'to your knowledge do any members of your family have a diagnosis of psoriasis, inflammatory bowel disease, iritis or ankylosing spondylitis'.

4. Patients with multiple disease completed disease activity (Bath Ankylosing Spondylitis Disease Activity Index [BASDAI][6]) and function (Bath Ankylosing Spondylitis Functional Index [BASFI][7]) assessments (three assessments were made on each patient. The results of the last assessment were used as these were completed in the same month by all subjects) . The radiographs of RNHRD patients (n= 660) were blindly scored by two independent observers using the Bath Ankylosing Spondylitis Radiology Index [BASRI[8]] and then sub-grouped according to presence of secondary disorders. Matched pair T-test were used to compare groups.

RESULTS

1. Analysis of the prevalence of secondary disorders within the individual: There were more people with spondylitis and multiple co-diseases (ie more than one secondary disorder) than expected and more with pure ankylosing spondylitis (ie no secondary diseases) but there were fewer with spondylitis and only one single co-disease than expected. The observed /expected numbers for patients with no co-diseases were 2069 /1973, with one co-disease 1722 /1872, with two co-diseases 454 /417 and with three co-diseases 42 /25. (χ^2 =32.2 $p<0.001$).

Psoriatic patients had a higher prevalence of iritis. 48% vs 40% [Odds Ratio : 1.4 95% confid. (1.1-1.7)] and more IBD than controls [O.R: 1.3 (1.1-2.0)] 12.5% vs 9.4%. (Demographic data : Ps & controls (number, sex ratio, disease duration (sd), age (sd)) n =600, M:F 3.2:1, dis dur = 22 (10) ys, age = 47 (11))

Iritis patients had a higher prevalence of psoriasis [OR: 1.4 (1.1-1.8)] 18.2 % vs 14% and IBD [OR: 1.5 (1.1-2.2)] 10.6% vs 7.3% than controls (Demographic data : Iritis & controls (number, sex ratio, disease duration (sd), age (sd)) n=735, M:F=2.3:1, dis dur = 21 (10) yrs, age = 45 (11) yrs).

IBD patients had a higher prevalence of iritis [O.R: 1.4 (1.1-2.0)] 45.4% vs 36.7% and psoriasis [O.R: 1.9 (1.3-2.8)] 23.9% vs 14.3% than controls (Demographic data : IBD & controls (number, sex ratio, disease duration (sd), age (sd)) n=335, M:F=2.3:1, disease duration =22 (10) yrs, age = 47 (11) yrs).

2. Temporal relationship between onset of the different conditions: Patient recollection of age of onset of symptoms and diagnosis was confirmed using GP reports. The GP and patients recollection was comparable for age/year in 95% of cases of ankylosing spondylitis, inflammatory bowel disease and psoriasis and 100% of those with iritis. [There were 47 patients with psoriasis were the GP had records of when the first symptoms were presented. Of these 45 (ie 95%) reported the same year of presentation as the patient recalled. There were 110 patients with IBD were the GPs had records and verified the patient recollection as correct in 105 (95%) of these cases. In the sample with iritis only 58 GPs had records (as patients may generally present directly to casualty or the eye department and not to the GP), of these all 58 were in the same year as given by the patient. However, many of the GPs for the non-included samples reported that results had been computerised and the data was not available or the patient was new and there were no backdated records]. Onset of AS symptoms occurs significantly before advent of iritis ($p<0.001$) and IBD ($p<0.001$) symptoms. Onset of Ps and AS symptoms occurs at comparable ages. Gut, skin and eye symptoms do not precede those of AS. [Table 1]

3. The prevalence of disease among first degree relatives: Relatives of patient with IBD-AS are at an increased risk of developing psoriasis and iritis. (Tables 2 and 3) Those with multiple disease predict the highest prevalence of co-diseases (ie Ps, IBD, I or AS) within family members, followed by those with IBD, psoriasis and lastly iritis.

4. The influence of secondary disorders on outcome of AS: Disease activity and function are worse for patients with psoriasis and/or IBD. However, those with iritis are comparable to those with primary disease. [Table 4] Radiological change was worse for those with iritis than for patients with pure AS [Table 5].

DISCUSSION

Our data explore the nature of the inter-relationship between the spinal disease, AS, and the extra-spinal co-disorders. We are particularly intrigued by the relative role of environment and genetics in the inter-relationship of these inflammatory disorders. We have shown that: (1) in an individual with AS, the presence of one concomitant disorder enhances the probability of there being a second or third co-disease. (2) the symptoms of AS precede or are contemporaneous with the concomitant disorders (3) family members are at increased risk of secondary disorders even in the absence of their expression in the index case and finally (4) the expression of severity (ie BASRI, BASDAI, BASFI) in AS is influenced by the presence of the secondary conditions. Thus, it can be concluded that the susceptibility genes of these co-disorders overlap with each other and with AS and impact on disease severity.

This study is exploratory and there is no guarantee that clinical subsets will necessarily simplify the disease from a genetic perspective. In addition, in terms of the increased prevalence of secondary disorders within the individual, it is recognised that patients seen at a tertiary referral clinic may have more co-diseases than those seen in the general population. Similarly, patients who join a self help group (NASS) may be more likely to have multiple disorders. This suggests that a bias towards multiple disease (Berkson's Bias) may be observed among the total sample. However, more patients than expected were found without a concomitant disease (pure AS) and fewer were

found with AS and a single secondary disorder. This is not a finding predicted by the bias. In addition, the matched data used control and sample patients from the same population which were equally likely to have multiple disease as there were no selection differences between the subgroups (ie both entered the hospital/self help group system).

A link between the secondary disorders in the absence of AS has been previously described. Psoriatic patients suffer from IBD more than controls[9], the prevalence of psoriasis is increased in Crohn's disease[10,11,12] and there is a higher incidence of iritis than expected among psoriasis patients[13]. Genes on chromosome 16 are associated with iritis[14], psoriasis[15] and Crohn's disease[16] . However, no gene has been identified from the region. These inflammatory disorders are linked to chromosome 6, perhaps through HLA-Cw6, in the case of psoriasis[17], B62 in IBD[18] and B27 for AS [19]and iritis[20]. Thus, there is evidence to support the finding in this study that the genetic susceptibility to inflammatory bowel, skin, eye and joint disease overlaps and may be additive.

The suggestion that the triggering agent for AS may enter the body through the inflamed gut or skin (as proposed in the HLA transgenic rat) is not supported by our human data. There was little overt (symptomatic) inflammation due to IBD prior to the onset of symptomatic AS. However, we do appreciate that occult bowel involvement may occur early in AS[21] and that the clinical expression of psoriasis varies, very mild disease (ie minor scalp involvement) could be overlooked by the patient and GP. In fact, it is not know when the actual onset of disease begins in any of these conditions. It is possible that the trigger is pulled in the womb and disease begins in the infant with age of symptom onset simply a reflection of severity of disease. However, this study has not found evidence that overt and symptomatic IBD or Ps are involved in

triggering AS (ie allowing the conduit of environmental pathogens to induce AS). Conceivably, the results may be different in a population chosen from a gastroenterological or dermatological clinic[22]. For example, 7 of 19 patients with non AS spondylarthritis followed long term went on to develop AS, all had initially presented inflammatory gut lesions. Thus, evolution of non-AS-SpA to full blown AS was associated with gut inflammation at disease onset[23]. However, the finding that onset of bowel disease and the other inflammatory conditions do not follow a temporal pattern but may occur at any time (before or after the onset of arthritis), supports the hypothesis that susceptibility genes of inflammatory skin, eye and bowel disease may overlap with those for inflammatory joint disease.

The prevalence of disease among family members of the index case represents an estimate of the occurrence of disease within this population. The observation of familial aggregation has been previously identified. [9] Herein, we reinforce the previous observations and suggest that the strongest genetic load can be seen among the IBD-AS sufferers with the least observed among the iritis-AS patients (Table 3). The data from the relatives of the pure AS subjects suggest that occult genes associated with IBD & Ps and/or the presence of iritis genes may be required for the development of ankylosing spondylitis (see figure 1)

Patients with psoriasis and / or IBD had poorer function and greater disease activity. Psoriasis/IBD genes may have an additive effect on susceptibility to and severity of AS. Alternatively, an inflammatory/immunological response to a sizeable area of body (skin surface or bowel) may have an effect of potentiating inflammation elsewhere. Indeed, it is recognised that active inflammatory bowel disease can coincide with flares in peripheral joint disease[23]. Disease activity and function can be modified and therefore are measures of inflammation and soft tissue involvement. However,

radiological assessment is measured in terms of disease specific change and is not directly related to the level of pain/fatigue (ie disease activity) nor poor function (as this can be due to pain or soft tissue damage). Ps /IBD does not appear to increase these disease specific radiological changes. Conversely, iritis appears to be a strong phenotypic marker for more severe radiological disease. Moreover, peripheral arthritis in a patient with ankylosing spondylitis enhances the likelihood that iritis will develop[24]. The secondary diseases do appear to have a genetic overlap with AS in terms of susceptibility genes. The susceptibility factors for these conditions may be additive or have a synergistic effect on each other. The presence of these conditions has a pronounced effect on the phenotypic expression. The patient is more likely to have multiple disorders, develop these disorders after the onset of AS and have a poorer outcome in terms of the spondylitis. These findings point to the striking overlap within the patient and their family of rheumatological, dermatological and gasteroentrological processes. The impact of a relevant family history is clearly demonstrated and the data enhance our understanding of how the shared gene hypothesis can have an impact on disease expression.

REFERENCES:

1. V.Wright, J. Moll. Seronegative Polyarthritis. Elsevier/North-Holland Biomedical Press. 1976

2. Linssen A. B27(+) disease versus B27(-) disease. Scand J Rheumatology 1990; Supp 87 : 111-119.

3. Baggle S, Lyons J, Angell E, Barkhuizen A, Han Y, Planck S. HLA-B27 transgenic Lewis rats and iritis after gram-negative infection. Investigative Ophthalmology and Visual Science 1996; 37 (3):172

4. Taurog J, Richardson J, Croft, J. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. Journal of experimental Medicine 1994; 180 : 2359-2364.

5. Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. 1999 The Lancet; 354: 1687-1690.

6. Garrett S, Jenkinson T, Kennedy L, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis : the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol. 1994; 21 : 2286-91.

7. Calin A, Garrett S, Whitelock H , Kennedy L, O'Hea J, Mallorie P. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994; 21: 2281-5.

8. McKay K, Mack C, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI): A new validated approach to disease assessment. Arthritis Rheum 1998 ; 41 (12) : 2263-2270

9. Moll J, Haslock I, Macrae I, Wright V. Association between ankylosing spondylitis, psoriatic arthritis, reiter's disease, the intestinal arthropathies and Behcet's syndrome. Medicine 1974 ; 53(5): 343-64

10. Lee F.I, Bellary S.V, Francis C. Increased occurrence of psoriasis in patients with Crohn's disease and their relatives. Am. J. Gastroenterol. 1990; 85: 962-923

11. Yates VM, Watkinson G, Kelman A. Further evidence for an association between psoriasis, Crohn's disease and ulcerative colitis. Br. J. Dermatol. 1982;106:323-30

12. Hammer B, Ashurst P, Naish J. Diseases associated with ulcerative colitis and Crohn's disease. Gut 1968; 9:17-21.

13. Catsarou-Casari A, Katsambas A, Theodoropoulos T, Stratigos J. Ophthalmlogical manifestations in patients with psoriasis. Acta Derm Venereol (Stockn) 1984; 64 (6) :557-559

14. Tromp G Kuivaniemi H, Rapael S, Alakokko L, Christiano A, Considine E. Genetic linkage of familial granulomatous inflammatory arthritis, skin rash and uveitis to chromosome 16. Am. J. Hum.Genet. 1996;59 5:1097-1107

15. Nair R, Henseler T, Jenisch S, Stuart P, Bichakjian C, Lenk W, Westphal E, Guo Sun-Wei, Christophers E, Voorhees J, Elder J. Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. Human Molecular Genetics 1997; 6: 1349-1356

16. Curran M, Lau K, Hampe J, Schreiber S, Bridger S, Macpherson A, Cardon L et al and Lennard-Jones J. Genetic Analysis of inflammatory bowel disease in large european cohort supports linkage to Chromosome 12 and 16. Gastroenterology 1998; 115: 1066-1071.

17. Tiilikainer A, Lassus A, Karvonen J. Psoriasis and HLA-Cw6. Br. J. Dermatol. 1980; 102: 179-84.

18. Mielants H, Veys E, Joos R, Cuvelier C, De Vos M Repeat ileocolonoscopy in reactive arthritis J Rheumatol 1986 ; 14: 456- 8

19 Brewerton D, Caffrey M, Hart F, James D, Sturrock R. Ankylosing spondylitis and HLA B27. Lancet 1973;1:904-906

20. Careless D, Inman R. Acute anterior uveitis-clinical and experimental aspects. Semin Arthritis Rheum 1995; 24, 6 : 432-441

21. de Vlam K, Mielants H, Cuvelier C, De Keyser F, Veys E, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease : prevalence and HLA association. J Rheumatol 2000; 27 (12): 2860-2865.

22. Mielants H, Veys E, Cuvelier C, De Vos M, Goemaere S, De Clercq L, Schatteman L, Gyselbrecht L , Elewaut D. The evolution of spondyloarthripathies in relation to gut histology. III. Relation between gut and joint. J Rheumatol 1995; 22 (12): 2279-84.

23. Mielants H, Veys E, Cuvelier C, de Vos M. Subclinical involvement of the gut in undifferentiated spondyloarthropathies. Clin Exp Rheumatol 1989; 7: 499-504

24. Maksymowych W, Chou C, Russell A. Matching prevalence of peripheral arthritis and acute anterior uveitis in individuals with ankylosing spondylitis. Ann Rheum Dis 1995; 54: 128-130.

Table 1: Mean age of onset of the inflammatory disorders [Iritis (I), psoriasis (Ps), inflammatory bowel (IBD)] compared to onset of AS [all cohorts are independent and do not include subsets of the same individuals].

	AS (yrs)	I (yrs)	Ps (yrs)	IBD (yrs)	Mean difference (Confidence Intervals)
Pure AS (n=2221)*	25				
AS I (n=151)	23	33			AS vs I : 10 (7.4-10.8)
AS Ps (n=40)	27		26		AS vs Ps : none
AS IBD (n=74)	24			30	AS vs IBD : 6 (3.4-9.7)
AS I Ps (n = 30)	28	39	32		AS vs I : 11 (6.6-15.2), AS vs Ps : 4 (0.5-8.8)
AS I IBD (n=66)	22	34		31	AS vs I : 12 (9.6-15.8), AS vs IBD: 9 (5.1-12.2)
AS Ps IBD (n=18)	23		26	31	AS vs Ps : none , AS vs IBD : 6.6 (1.7-11.6)
AS I Ps IBD (n=26)	23	32	33	33	AS vs I : 9 (4.5-14.8), AS vs Ps : 10 (2.5-16.9), AS vs IBD : 10 (3.6-16.1)

* Includes original 2069 and additional recruited subjects.

Table 2 First degree relatives

	Ps	IBD	Iritis	AS	Order
1. AS pure (n = 138)	10%	7%	6%	25%	1
2. AS + iritis (n=142)	5%	3%	13%	29%	2
3. AS + Ps (n=42)	33%	12%	0%	14%	3
4. AS + iritis + Ps (n= 26)	19%	8%	15%	19%	4
5. AS + IBD + Ps (n=17)	12%	24%	6%	24%	5
6. AS + iritis + IBD (n=63)	14%	14%	16%	29%	6
7. AS + IBD (n=76)	17%	24%	8%	24%	6
8. AS +IBD + Ps + Iritis (n=23)	17%	17%	26%	30%	8
Population Rate*.	1-2%	0.07-0.1%	0.2%	0.25 %	

- The Spondylarthritides Ed A. Calin, J Taurog. 1998 Oxford University Press and Linssen A, Rothova A, Valkenburg H, Dekker-Saeys A, Luyendiijk L, Kiilstra A, Feltkamp T. The Lifetime cumulative incidence of acute anterior uveitis in a normal population and its relation to ankylosing spondylitis and histocompatibility antigen HLA-B27. Investigative Opthalmology & Visual Science, 1991 ; 32 (9): 2568-2578.

Based on patient recollection only.

Table 3 : Relatives of AS patients affected with inflammatory diseases not identified in the index case.

	+ IBD				- IBD		
	Relatives with:				Relatives with:		
	Ps	Iritis	Summary		Ps	Iritis	Summary
AS + IBD	13/76	6/76	16/76	AS pure	14/138	8/138	21/138
AS + iritis + IBD	9/63		9/63	AS + iritis	7/142		7/142
AS +Ps + IBD		1/17	1/17	AS + Ps		0/42	0/42
			26/156				28/322
			(17%)				(9%)

p=0.01

	+ Iritis				- Iritis		
	Relatives with:				Relatives with:		
	IBD	Ps	Summary		IBD	Ps	Summary
AS + iritis	4/142	7/142	11/142	AS pure	10/138	14/138	23/138
AS + iritis + IBD		9/63	9/63	AS + IBD		13/76	13/76
AS + iritis + Ps	2/26		2/26	AS + Ps	5/42		5/42
			22/231				41/256
			(10%)				(16%)

p=0.033

Table 4: Phenotypic expression of secondary disease

	AS duration	BASDAI	BASFI	Mean Difference between 1^0 AS vs 2^0 AS [BASDAI] (confidence interval of mean)	Mean Difference between 1^0 AS vs 2^0 AS [BASFI] (confidence interval of mean)
Pure AS (n=2221)	19.0 yrs	4.1	3.9		
AS I (n=1342)	23.7 yrs	3.9	4.0	ns	ns
AS Ps (n=389)	19.3 yrs	4.7	4.6	0.5 (0.3-0.7)	0.4 (0.2-0.7)
AS IBD (n=341)	25.7 yrs	4.5	4.8	0.4 (0.05-0.7)	0.8 (0.6-1.1)
AS I Ps (n=323)	25.6 yrs	4.6	4.8	0.4 (0.2-0.6)	0.8 (0.4-1.0)
AS I IBD (n=150)	25.3 yrs	4.3	4.6	ns	0.6 (0.2-1.1)
AS Ps IBD (n=44)	20.5 yrs	5.9	5.4	1.7 (1.2-2.4)	1.6 (0.9-2.4)
AS I Ps IBD (n=46)	23 yrs	4.5	5.4	ns	1.5 (0.8-2.3)

Table 5: BASRI in primary vs secondary disease

	Matched pairs		
	AS I [mean (sd)]	**Pure AS [mean (sd)]**	
N	143	143	
M:F	3:1	3:1	
Disease duration	22 (+/- 9)	21 (+/-10)	
Age at onset	23.7 (+/- 8)	25.4 (+/-8)	
BASRI total	9.2 (+/- 3.8)	7.7 (+/- 3.4)	p<0.001
	AS Ps [mean (sd)]	**Pure AS [mean (sd)]**	
N	91	91	
M:F	2.5 : 1	2.5 :1	
Disease duration	21.5 (+/-9.6)	21.7 (+/-10.24)	
Age at onset	23.0	23.6	
BASRI total	8.7 (+/-3.5)	8.8 (+/- 3.7)	Ns
	Cohort comparison		
	AS IBD [mean (sd)]	**Pure AS [mean (sd)]**	
N	23	151	
Disease duration	21.3 (+/-10.3)	22.3 (9.2)	
BASRI total	8.7	8.3	Ns

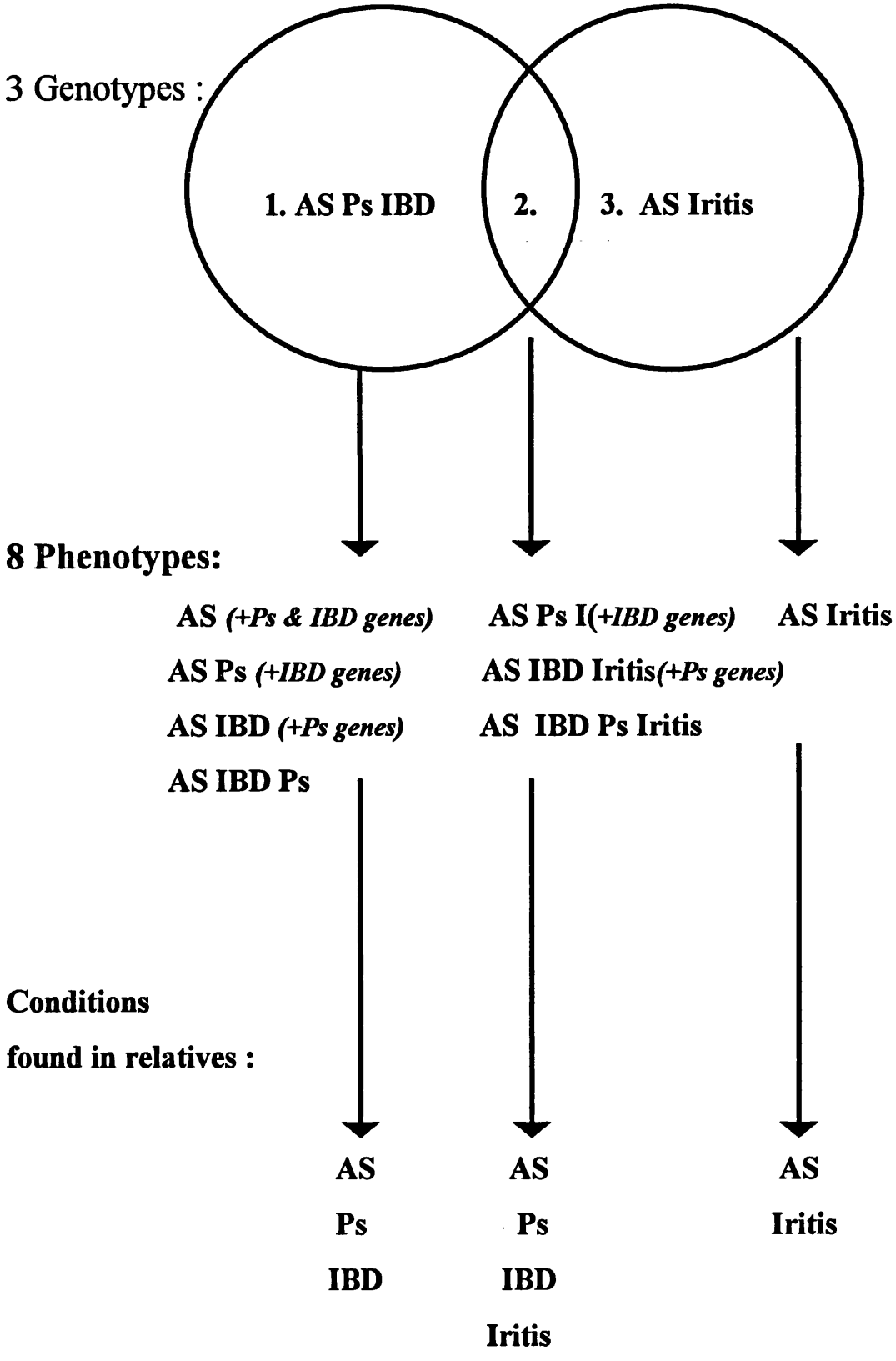


Figure 1: Relationship between AS and secondary disorders

Appendix 7 : In Press. Journal of Rheumatology

Ankylosing Spondylitis: Interaction between genes, joints, age at onset and disease expression.

SINÉAD BROPHY, ANDREI CALIN.

ABSTRACT. Objective: Ankylosing spondylitis (AS) is a chronic inflammatory disorder with symptom onset generally occurring in the late teens/ mid twenties. We have shown that in women, a younger age at onset enhances disease susceptibility in the next generation. Herein, we examine the influence of the age at symptom onset on phenotypic expression.

Methods: Patients were divided into cohorts according to age of symptom onset. The primary outcome measure was radiological progression (BASRI). Secondary measures were disease activity (BASDAI), function (BASFI), numbers undergoing AS-related surgery and percentage with secondary disorders.

Results: Age at onset had no significant effect on radiological progression [Young onset vs Late onset ; 8.0 : 8.6 respectively] disease activity [Young vs Late ; 4.4 :4.4], need for non-hip surgical intervention [9% : 8% respectively] or prevalence of secondary disorders [Iritis ; 40% :41%, Psoriasis ; 20% : 19% , IBD; 7.5% : 8.9%]. By contrast, there was a striking increase in prevalence of total hip replacement in those with juvenile onset [18% : 8% respectively ($p<0.001$)]. Regardless of age at onset, spinal progression determined radiologically, was greater in those with hip arthritis compared to those without [Young onset Hip invol vs non-hip invol. : 9.7 (2.4): 7.2 (3.0) respectively. $p<0.001$. Late onset. Hip invol vs non-hip invol. : 10.1 (2.5) :7.1 (3.0) respectively] . Function deteriorates with age [Young onset vs late onset ; 3.7 : 4.5 respectively $p<0.01$] .

Conclusion: 1) Hip disease (young or late onset) is a major prognostic marker for long term severe disease [Patients with hip disease have an increased spinal score by 2.5-3 points or 35%-40% more change]. 2) Hip involvement is more prevalent among patients with young age at onset. 3) Young onset patients without hip involvement do not have more severe disease. Thus, age at onset, itself, does not influence disease severity. 4) Since hip involvement and not age at onset is associated with worse outcome, patients with a young age at onset may be assumed to have an increased susceptibility load (ie genetic component or environmental trigger) rather than more severity genes. The lack of association between severity and age at onset implies that the determinants of susceptibility and severity are independent.

Key Indexing Terms: Ankylosing spondylitis, symptom onset, severity, outcome.

Introduction

Ankylosing spondylitis is a chronic inflammatory disorder with symptom onset generally occurring in the late teens/ mid twenties. A juvenile age at symptom onset (less than 16 yrs) has been found to correlate with increased disease severity [1,2,3]. In addition, hip involvement (and need for total hip replacement) is more often seen in those with juvenile onset [3,4] and hip involvement itself is a marker for more severe axial involvement [2,5] However, late onset, (after age 55) has also been reported to affect the clinical pattern of disease. Such patients are said to have more cervical pain, anterior chest wall involvement, aseptic osteitis [6] and shoulder involvement [7]. It can be assumed that the expression of AS results from a combination of severity and susceptibility genes. In women, a younger age at onset enhances disease penetrance in the next generation [8]. Therefore, patients with a younger age at onset may have an increased number of susceptibility factors and perhaps a different disease expression from those with late onset. Late onset individuals may carry fewer susceptibility and a different array of severity genes.

This study aims to examine the influence of age at symptom onset on disease expression as measured by radiological change (BASRI) [9], disease activity (BASDAI) [10], function (BASFI) [11], percentage undergoing AS-related surgery, and prevalence of secondary disorders (iritis, psoriasis, inflammatory bowel disease)

Methods

The Bath Ankylosing Spondylitis Database consists of 4741 patients (2.5:1 M:F). All were out-patients of the Royal National Hospital for Rheumatic Diseases (n=851) or were members of the National Ankylosing Spondylitis Society. Patients referred to the RNHRD had their diagnosis confirmed according to the New York Criteria. The NASS members are those who have received a positive diagnosis of ankylosing spondylitis from a specialist rheumatologist as

a result of an x ray. To validate the diagnosis in those patients recruited through NASS, one hundred and forty-six consecutive subjects were invited to attend an assessment clinic and all 146 were confirmed as having AS according to the same criteria. In addition, for 240 patients a confirmation was sought from the GP, and confirmed in 229 cases (95.4%) [ie AS with radiological evidence of sacroliitis]. We contacted the GP's of 120 Ps(AS) patients and 139 IBD(AS) patients. Of these, 77 (64%) and 112(81%) replied confirming the diagnosis of Ps and IBD in 65 (84%) and 108 (96%) cases.

Independent samples of patients were divided into cohorts according to age of symptom onset and were controlled for a) age now and b) disease duration now (McNemar's chi-squared). In addition, cohorts of juvenile onset (<16 yrs), teen onset (17-20 yrs), twenties (21-29 yrs), thirties (30-39) and late onset (40+ yrs) were compared. The primary outcome measure was radiological status as determined by BASRI. Secondary measures were disease activity (BASDAI), function (BASFI), numbers undergoing surgery and percentage with secondary disorders.

Results

Radiological progression [sacroiliac joints, hips, lumbar spine, cervical spine]; BASRI

Age at onset has no significant effect on radiological progression [Table 1 & Graph 1]. Radiological change is a factor of disease duration ie those with a young age at onset have more severe disease when compared to like aged late onset patients [Young onset: Late onset ; 10.0: 8.0 respectively (p=0.02)] However, disease duration matched pairs are comparable for radiological change [Young onset : Late onset ; 8.0 : 8.6]

Patients with hip disease and a young onset had comparable spinal disease to those with hip disease and a late onset [Table 1] . [Young : Late ; 9.0 :10.8 p=0.04 (corrected value not significant)]. Hip disease patients have more spinal change than non-hip patients [Table 2]

[Young onset - Hip disease : Non-hip disease ; 9.7 :7.2 respectively (p=0.0001). Late onset - Hip disease : Non-hip disease ; 10.13 : 7.1 p=0.0001]

Secondary outcome measures

Age at onset had no significant effect on disease activity, function, prevalence of secondary disorders, or need for surgery [Table 3 & Table 4]. At comparable age (ie age now [Table 2]), those with longer disease duration (ie young onset) have lower disease activity [Young onset : Late onset ; 4.0 : 4.3 respectively $p<0.02$], more iritis [Young onset : Late onset ; 50% : 40% $p<0.01$] and more surgical intervention [14% : 7% respectively $p<0.01$]. However, when matched for disease duration [Table 3], the disease activity [Young onset : Late onset ; 4.4 : 4.4] , prevalence of secondary conditions and need for surgery [9% : 8%] were all comparable for those with a young age at onset vs late age onset. When matched for disease duration the function was worse for the delayed onset males (ie older aged men) [Young onset : Late onset ; 3.6 : 4.5 respectively $p<0.01$]. However, at equivalent ages [Table1] the function is comparable regardless of disease duration (ie between young age at onset and delayed onset individuals).

Cohorts of juvenile onset (<16 yrs), young onset (17-20 yrs), twenties (21-29 yrs), thirties (30-39) and late onset (40+ yrs) were comparable for disease activity [Graph 2], function [Graph 3] and surgery [Graph 4].

Discussion:

Our data examines young onset compared to late onset and was not intended to analyse juvenile ankylosing spondylitis (JAS) as a separate entity. However, reviewing graphs 1-5 demonstrates that finding in young onset patients are applicable to juvenile AS subjects. The data suggest that age at first symptoms onset has no impact on disease severity. However, this must be seen in the context that hip disease is more prevalent among patients with juvenile

onset [THR rate - Juvenile vs non-juvenile : 18% : 8% respectively, $p<0.001$, Graph 5] and this phenomenon is known to be a predictor of more severe spondylitis [2,5]. This paradox [ie a) there is a link between age at onset and hip disease, b) hip disease is linked to increased severity but c) there is no link between age at onset and increased severity] may be explained on the basis that only a subgroup of young onset patients develop hip disease and only this cohort is at risk of more severe spondylitis. [In our study 21/68 (31%) of the young onset subjects and 13/68 (19%) in of the late onset patients had hip involvement as assessed by a radiograph and THR occurred in 9% and 4% respectively].

It is possible that the young developing hip may be more at risk of becoming affected than the adult hip. Thus, patients with young onset are more at risk of hip involvement (because of the juvenile hip). However, patients with hip disease and a young onset do not have more severe disease than subjects with a late onset and hip disease *[Figure 1]*. Hip involvement per se appears to be the relevant factor contributing to outcome.

The trigger for AS is thought to be a ubiquitous bacterium [12]. If this is so, then the age of onset of disease should be related to the genetic susceptibility load. Yet, this enhanced genetic susceptibly in young onset patients does not influence outcome, implying that the contributing genes for susceptibility and severity are independent of one another. If by contrast, the age of onset of a patient is governed by the timing of contact with an environmental trigger, the age when this happens appears to have no influence on the later disease development. Separate and unrelated severity factors must influence disease progression. This hypothesis has been supported by early findings identifing from a genome screen at least one area of significant linkage between severity and genetic factors.[13] In conclusion, there are three clearly distinct independent factors: the environment and both susceptibility and severity genes.

References

[1] Amor B et al. Predictive factors for the long-term outcome of spondyloarthropathies. J Rheumatol 1994; 21: 1883-7

[2] Claudepiere P et al. Predictive factors of spondyloarthropathy in North Africa Br J Rheumatol 1995; 34 (12): 1139-1145,

[3] Calin A, Elswood J. The natural history of juvenile-onset ankylosing spondylitis: a 24 year retrospective case control study. Br. J Rheumatol. 1988;27: 101-103

[4] Calin A, Elswood J. The relationship between pelvic, spinal and hip involvement in ankylosing spondylitis- one disease process or several ? Br. J Rheumatol. 1988;27 393-395.

[5] Calin, A, MacKay K, Santos H, Brophy S. A New Dimension to Outcome: Application of the Bath Ankylosing Spondylitis Radiology index. J Rheumatol. 1999;26 (4): 988-992.

[6] Caplanne D et al. Late onset spondylarthropathy: clinical and biological comparison with early onset patients. Ann Rheum Dis 1997;56 (3): 176-179

[7] Calin A, et al . Late onset ankylosing spondylitis: A distinct disorder ? Br J Rheumatol 1991;30: 69-70.

[8] Calin A, Brophy S. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. Lancet 1999; 354: 1687-1690

[9] Mackay K, Mack C, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI). A new, validated approach to disease assessment. Arthrits & Rheumatism. 1998; 41 (12): 2263-2270.

[10] Garrett S, Jenkinson T, Kennedy G, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylsoing spondylitis: The Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol. 1994:21 (12):2286-2291

[11] Calin A, Garrett S, Whitelock H, Kennedy G, O'Hea J, Mallorie P, Jenkinson T. A New approach to defining functional ability in ankylosing spondylitis: The development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol. 1994;21 (12):2281-2285.

[12] Uksila J, Toivanen P, Granfors K. Enteric infections and arthritis: bacteriological aspects. In: Calin A, Taurog T. Editors. The Spondylarthrides Oxford University Press 1998: 167-177.

[13] S Brophy, Hamersma J, Bradbury L, Timms A, Edwards S, Laval S, Wordsworth BP, Calin A, Cardon L, Brown M. Towards defining the genetic determinants of disease in ankylosing spondylitis. Rheumatol 2001 In Press (to be presented in April at British Society of Rheumatology, the Edinburgh conference)

Table 1: Young onset compared to Late onset in terms of radiological progression [ie Total = SI joint , Lumbar spine ,Cervical spine and Hips, Spinal = SI joint , Lumbar spine and Cervical spine].

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	p value (corrected)
Age Now (& Sex) Matched n=56 pairs			
BASRI-total* [Scale 2-16]	10.0	8.0	p=0.02 (ns)
BASRI-spine** [Scale 2-12]	8.7	7.6	p=0.02 (ns)
With hip involvement (n= 19 , 10)	9.7	10.0	ns
Without hip involvement (n=37,46)	8.2	7.1	ns
Disease Duration (& Sex) Matched n=68 pairs			
BASRI-total*	8.0	8.6	ns
BASRI-spine**	7.1	8.1	ns
With hip involvement (n=21, 13)	9.0	10.8	p=0.04 (ns)
Without hip involvement (n=47, 55)	6.3	7.5	p=0.06 (ns)

* including hips **without hips

Table 2 : Radiological hip disease vs non-hip disease patients : spinal severity score and age at onset of AS

	Hip disease	Non-hip disease	P value (corrected)
Young onset	n=81	n=148	
Disease duration	22.1 yrs	22.7 yrs	
Mean (sd)	9.7 (2.43)	7.2 (3.0)	p<0.001
Late onset	n=19	n=64	
Disease duration	15.2	14.1	
Means (sd)	10.13 (2.5)	7.1 (3.0)	p<0.001

Table 3: Influence of age at onset on outcome - Age Now and Sex matched

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	p value$
BASDAI			
Whole group (n=784)	4.0	4.3	p<0.02
Males (n=543 pairs)	3.7	4.2	p<0.01
Females (n=241 pairs)	4.5	4.4	Ns
BASFI			
Whole group (n=829)	4.4	4.3	Ns
Males (n=574 pairs)	4.3	4.3	Ns
Females (n=255 pairs)	4.8	4.3	p=0.03
Secondary Disorders			
Iritis (n=829 pairs)	50%	40%	p<0.01
Ps (n=807)	20%	20%	Ns
IBD (n=828)	7.5%	7.6%	Ns
Surgery			
Total surgery n=991	140 (14%)	67 (7%)	p<0.01

$ Corrected

Table 4: Influence of age at onset on outcome - Disease Duration and Sex Matched

	Age onset 0-21 (young onset)	Age onset 30+ (late onset)	P value$
BASDAI			
Whole group (n=784)	4.4	4.4	Ns
Males (n=543 pairs)	4.2	4.1	Ns
Females (n=241)	4.6	4.5	Ns
BASFI			
Whole group (n=762)	3.7	4.5	p<0.01
Males (n=546 pairs)	3.6	4.5	p<0.01
Females (n=216)	4.1	4.5	Ns
Secondary Disorders			
Iritis (n=788 pairs)*	280 (35.5%)	283 (36%)	Ns
Ps (n=777 pairs)**	110 (14.2%)	120 (15.4%)	Ns
IBD (n=810 pairs)***	61 (7.5%)	72 (8.9%)	Ns
Surgery			
Total surgery n= 924	86 (9%)	71 (8%)	Ns

$ Corrected

*Average age of onset of AS symptoms in iritis vs non iritis patients : 26.7 vs 26.9 (ns)

**Average age of onset of AS symptoms in psoriasis vs non-psoriasis patients : 27.7 vs 26.9 (ns)

***Average age of onset of AS symptoms in IBD vs non-IBD patients : 28.1 vs 27.0 (ns)

Impact of sex on inheritance of ankylosing spondylitis: a cohort study

Andrei Calin, Sinèad Brophy, David Blake

Summary

Background Ankylosing spondylitis is a genetically determined and commonly familial disorder. Men and women differ in their susceptibility to ankylosing spondylitis, with about 2·5 men affected for every woman with the disease. We investigated the influence of the sex of the index case on disease penetrance within families.

Methods The ages at which 50% and 75% of patients were diagnosed with ankylosing spondylitis were ascertained from a database of 4400 cases. Index patients with children or siblings who were old enough to have obtained a diagnosis (50% and 75% rates) were assessed for prevalence of disease among relatives. Confirmation of diagnosis for affected relatives was sought for all offspring and a random 25% selection of siblings.

Findings Ankylosing spondylitis was more prevalent among children (odds ratio 1·9 [95% CI 1·2–3·0], p<0·005) and siblings (1·5 [1·2–1·9], p<0·0001) of female index cases than among those of male cases. Analyses restricted to index cases with a young age at onset (≤21 years) indicated that children of women had an even higher incidence of ankylosing spondylitis (7·2 [1·5–34], p=0·013) than did children of men at similar age at onset. 38% of children of female cases had disease compared with 8% of male cases. There was no difference in sex distribution among affected children or siblings of female patients with ankylosing spondylitis. By contrast, the sons and brothers of male patients had a higher prevalence of the disease (odds ratio 2·6 [1·4–5·2], p=0·003) than did daughters and sisters (1·7 [1·3–2·2], p<0·0001).

Interpretation The influence of female sex is greater than that of male sex in determining increased susceptibility to ankylosing spondylitis in children. The striking maternal effect is greatest for women with young age at onset, which is not seen in men. The sex ratio of affected children depends on the sex of the affected parent.

Lancet 1999; **354:** 1687–90

Royal National Hospital for Rheumatic Diseases, Bath BA1 1RL, UK
(A Calin FRCP, S Brophy BSc, Prof D Blake FRCP)

Correspondence to: Dr Andrei Calin
(e-mail: mpssb@bath.ac.uk)

Introduction

The genes of the HLA region control a variety of functions involved in immune responses and influence susceptibility to more than 40 diseases. Many HLA-associated diseases clearly involve heterogeneity in HLA components, as well as non-HLA genetic factors.[1] In the case of ankylosing spondylitis, susceptibility genes in the HLA region include B27, B60, and DR1. However, familial studies show that the HLA region explains only a third of the genetic influence on ankylosing spondylitis[2] and that other non-HLA genes are involved. Moreover, concomitant disorders associated with ankylosing spondylitis have been linked to different chromosomes, including chromosome 16, implicated in Crohn's disease,[3] uveitis,[4] and psoriasis.[5]

Disease expression differs in men and women, with about 2·5 men affected for every woman with the disease.[6] Men develop more severe spinal disease.[7] Women have more peripheral joint involvement.[8] These differences may be due to genetic factors in the sex chromosomes,[9] which would lead to a difference in the sex ratio of children and siblings who inherit the disease.

We investigated the influence of the sex of the index case of ankylosing spondylitis on disease inheritance among offspring and siblings, irrespective of HLA-B27 status.

Methods

Patients

We studied 4400 individuals with ankylosing spondylitis (3143 men, 1257 women), who were outpatients of the Royal National Hospital for Rheumatic Diseases (RNHRD) or members of the National Ankylosing Spondylitis Society (NASS) and had been recorded on our database. Patients referred to the RNHRD had their diagnosis confirmed according to the New York criteria.[10] All NASS members who completed our questionnaire were asked to complete the form only if they had received a positive diagnosis of ankylosing spondylitis by radiography from a specialist rheumatologist. To validate the diagnosis in patients recruited through NASS, 146 consecutive patients were invited to attend an assessment clinic. All 146 were confirmed as having ankylosing spondylitis according to the same criteria.[10] The family physicians of a further 330 NASS members were contacted to check whether their patient's disease had been confirmed radiologically. Of these, 306 (93%) had definite ankylosing spondylitis, 15 had arthritic diseases other than ankylosing spondylitis, three showed suspicious change on radiography, and for five patients the primary source material was unclear.

Analysis

The frequency distributions of age of onset and age at diagnosis of the 4400 individuals were calculated so that we could identify the ages at which 50% (ie, 30 years) and 75% (ie, 39 years) of patients should have obtained a diagnosis of ankylosing spondylitis (figure 1). The study then focused on 1192 individuals (879 men, 313 women) who had children and were older than 50 years (to select those with older children), and 2222 individuals (1615 men, 607 women) with siblings older than 39 years. Where there were several family members with ankylosing spondylitis, only one sibling per family was selected. The patients were asked

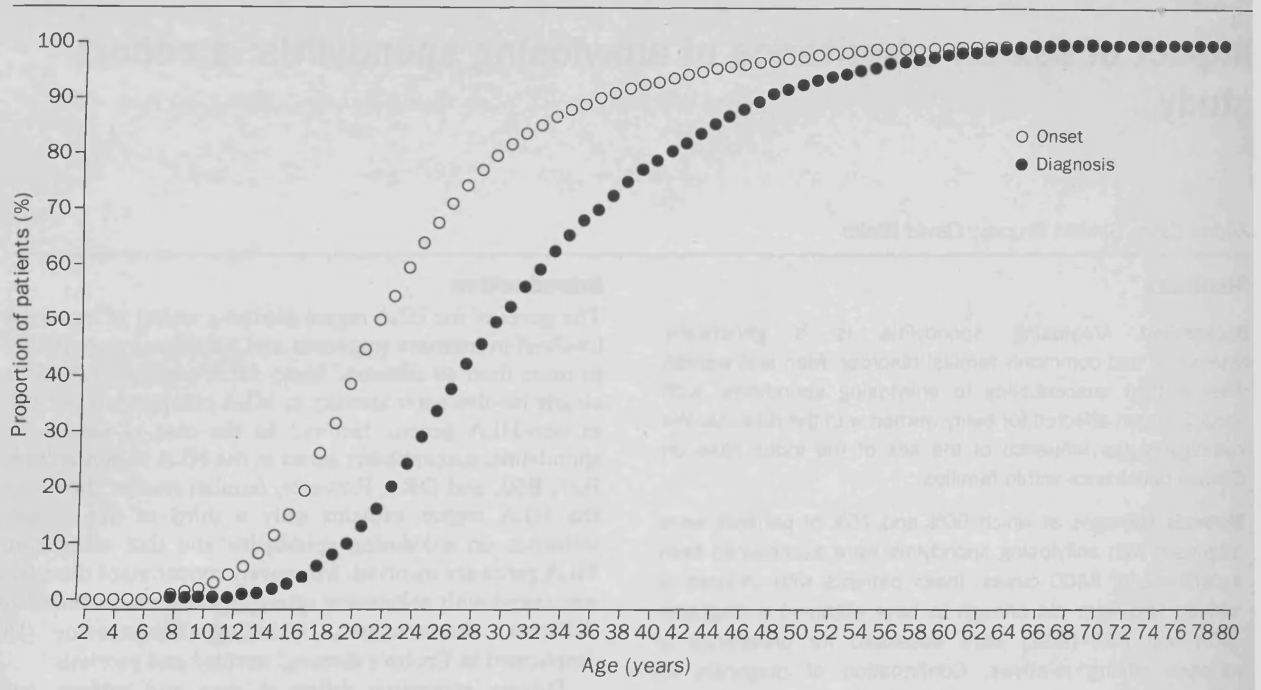


Figure 1: **Frequency distribution of age of onset and age of diagnosis of ankylosing spondylitis (actual data)**

by postal questionnaire whether any member of their family had ankylosing spondylitis and to state which relatives were affected. In each sample the sexes were compared to investigate transmission of the disease to sons and daughters or brothers and sisters.

All patients who had children older than 39 years who had ankylosing spondylitis were contacted to ensure that the child had a radiographically validated diagnosis. Of 29 families with a child older than 39 years with ankylosing spondylitis, all the affected offspring provided confirmation of diagnosis (ie, they were asked to confirm that a rheumatologist had made the diagnosis based on pelvic radiography). 100 (25%) patients who reported a sibling with ankylosing spondylitis were contacted to provide medical confirmation of diagnosis. We selected these patients using a random numbers table. Of those chosen, 18 could not be contacted (moved or died). 82 individuals were traced, of whom 78 (95%) had ankylosing spondylitis confirmed, two had no formal diagnosis but were still in the process of investigation for ankylosing spondylitis, and two did not have the disease.

The age of disease onset of fathers and mothers with ankylosing spondylitis was compared with the percentage of children with disease. To investigate the possibility of a uterine effect (eg, passage of pathogens or other agents across the placenta) in mothers with ankylosing spondylitis, we assessed whether the affected child was born before or after disease onset in the parent. The results for affected fathers were used as a control for when no uterine effect is present.

Statistical analysis

We analysed the data using χ^2 tests with Yates' continuity correction on SPSS (version 7.0). We used Fisher's exact test for small entries.

Results

The frequency distribution of age of diagnosis showed a mean 10-year delay from onset of symptoms to diagnosis (figure 1). Men and women did not differ in mean age at onset (24·5 [SD 10·1] *vs* 24·4 [10·5] years) or mean age at diagnosis (31·7 [11·5] *vs* 33·2 [11·4] years). The sex ratio of sporadic (simplex family) cases (74%) was 2·8, whereas the male/female ratio of familial (first-degree relatives, multiplex family) cases (22%) was 1·7 ($p<0·0001$). Further relatives (ie, second and third degree; 4%) had a ratio of 2·3.

The inheritance of ankylosing spondylitis among children of affected patients is shown in table 1. Overall, sons of men with ankylosing spondylitis were 2·5 times more likely than daughters to inherit the disease, and children of women with the disease were more likely to develop ankylosing spondylitis than were children of male patients (table 1). Women with a young age at onset were more likely to have children with disease than were men or women with older age at disease onset (table 1). Women

Index case	n	Sons	Daughters	Rate of inheritance (sons vs daughters)		Total	Rate of inheritance (father vs mother)		Ratio (M/F)
				Odds ratio (95% CI)	p		Odds ratio (95% CI)	p	
For children >30 years (50% diagnosis)									
Father	354	35/396 (8·8%)	13/376 (3·5%)	2·6 (1·4–5·2)	0·003	48/772 (6·2%)	..	..	2·5
Mother	173	23/185 (12·4%)	17/190 (8·9%)	1·4 (0·7–2·8)	0·3	40/375 (10·7%)	1·9 (1·2–3·0)	0·005	1·4
For children >39 years (75% diagnosis)									
Father	101	11/90 (12%)	5/92 (5%)	2·4 (0·8–7·2)	0·17	16/182 (8·8%)	..	..	2·25
Mother	53	9/45 (20%)	7/48 (15%)	1·5 (0·5–4·3)	0·6	16/93 (17%)	2·1 (1·0–4·5)	0·063	1·4
Age of onset ≤21 years and children >30 years									
Father	90	11/105 (10%)	4/99 (4%)	2·8 (0·9–9·0)	0·1	15/204 (7·3%)	..	..	2·6
Mother	41	7/49 (14%)	6/44 (14%)	1·0 (0·3–3·4)	1·0	13/93 (14%)	2·0 (0·9–4·5)	0·086	1
Age of onset ≤21 years and children >39 years									
Father	22	2/20 (10%)	1/19 (5%)	2·0 (0·2–2·4)	0·58	3/39 (8%)	..	..	1·9
Mother	9	4/7 (57%)	2/9 (22%)	4·7 (0·5–40·4)	0·3	6/16 (38%)	7·2 (1·5–34)	0·013	2·6

Table 1: **Inheritance of ankylosing spondylitis among sons and daughters of patients**

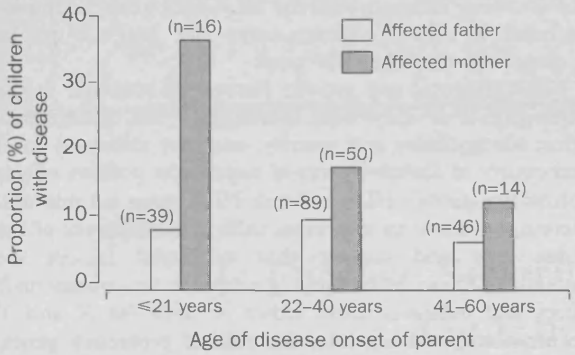


Figure 2: **Proportion of children older than 39 years with ankylosing spondylitis according to age of onset of parent**

aged 21 years or less at disease onset had a much higher proportion of affected children (38%) than women who were at least 25 years at disease onset (13%; odds ratio 4·0 [95% CI 1·1–14·0]; figure 2).

For women with an affected child, 74 (43%) mothers had onset of ankylosing spondylitis before birth of the affected child, whereas 48 (28%) women had onset of disease after birth of child and 26 (15%) had child and onset of disease in the same year (onset unknown in 25 [15%] cases). For men with an affected child, 205 (58%) had onset of ankylosing spondylitis before birth of an affected child, 103 (29%) had onset of disease after birth of an affected child, and 46 (13%) developed ankylosing spondylitis at the same time as the birth of their child (p=0·712).

For siblings older than 39 years of men with ankylosing spondylitis, brothers were 1·6 times more likely to have inherited the disease than sisters (table 2). For siblings older than 39 years of men with ankylosing spondylitis with young age of onset (≤21 years), brothers were twice as likely to have inherited the disease as sisters. In all cases, siblings of women were more likely to have the disease than siblings of men (table 2).

Discussion

Our study may have been biased because patients who have a relative with ankylosing spondylitis are more likely to join a self-help group, resulting in a greater number of patients with a positive family history. However, this would apply irrespective of the sex of the relative and should not influence the comparison of ratios for each sex affected by ankylosing spondylitis. The proportion of affected relatives in this study (11%) is similar to that of other studies.[11–13] A further issue is that certain subsets of individuals (ie, offspring of mothers with young age at onset) were small.

We did not systematically type patients or relatives for HLA-B27 status. Nevertheless, of the children randomly reviewed for HLA-B27 status, 92% (34 of 37) and 96% (113 of 118) of first-degree disease-concordant relatives of patients were HLA-B27 positive. Moreover, over 95% of patients carry this antigen and B27 is inherited as a co-dominant factor.[14] About 50–56% of siblings and children of patients are expected to be HLA-B27 positive. Thus, the risk for those carrying HLA B27 will be twice that of our estimates. The prevalence of ankylosing spondylitis among relatives may be a conservative estimate. Clearly, individuals with ankylosing spondylitis cannot be included in the study if they are undiagnosed, misdiagnosed, or have clinically silent disease. Although we ascertained whether patients had a diagnosis from their rheumatologist after pelvic radiography, we did not always review source material.

With rheumatoid disease, female susceptibility predominates. The patient's sex seems to be an important risk factor, with relatives of male patients having the greatest cumulative risk of rheumatoid arthritis.[15] However, we have shown that the opposite seems to be true for ankylosing spondylitis, which mainly affects men with relatives of female patients being more at risk. Women have a greater family history of ankylosing spondylitis than do men and this finding is reflected by our findings, which shows the sex ratio of sporadic (simplex family) cases compared with that of familial disease. Where the index case is male, female relatives are less susceptible than men to ankylosing spondylitis. By contrast, there is no significant difference in prevalence of ankylosing spondylitis among the relatives of either sex of female patients. The genetic load passed on by women with young age at onset seems to be even more striking. However, this finding is not seen among siblings. There are at least four possible explanations for these findings, none of which are mutually exclusive.

First is the genetic load effect. In general, women are less likely than men to develop ankylosing spondylitis. Thus, it remains possible that women with ankylosing spondylitis have to have a higher genetic load to develop the disease. A woman with ankylosing spondylitis has sufficient genetic susceptibility factors for disease expression. This could explain why her male and female children will have an equal chance of developing disease. By contrast, daughters of men with ankylosing spondylitis may not inherit enough genetic material to present clinical symptoms.

The second possibility is a uterine effect. The overall male/female sex ratio for ankylosing spondylitis is 2·5. However, 80% of these individuals have sporadic disease. For those with familial disease, a similar distribution is observed among the offspring of affected fathers. By contrast, the sex ratio for offspring of maternal cases is 1·0. Moreover, children of affected mothers have a higher risk of disease than do children of affected men. Mothers with ankylosing spondylitis may pass an environmental disease-inducing factor across the placenta. Such a uterine effect (ie, environmental overlay) may increase the risk of disease susceptibility in children who would not normally have

Index case	n	Brothers	Sisters	Rate of inheritance (brothers vs sisters)		Total	Rate of inheritance (male vs female)		Ratio (M/F)
				Odds ratio (95% CI)	p		Odds ratio (95% CI)	p	
Siblings >39 years									
Male	1615	153/1618 (9·46%)	90/1559 (5·77%)	1·7 (1·3–2·2)	<0·0001	243/3177 (7·65%)	..	..	1·6
Female	607	85/673 (12·6%)	57/613 (9·3%)	1·4 (1·0–2·0)	0·62	142/1286 (11·04%)	1·5 (1·2–1·9)	0·0001	1·4
Age of onset ≤21 and siblings >39 years									
Male	598	76/564 (13·5%)	40/594 (6·7%)	2·2 (1·4–3·2)	<0·0001	116/1158 (10·02%)	..	..	2·0
Female	220	43/260 (16·5%)	21/201 (10·4%)	1·7 (1·0–3·0)	0·07	64/461 (13·9%)	1·5 (1·0–2·0)	0·029	1·6

Table 2: **Inheritance of ankylosing spondylitis among brothers and sisters of patients**

	Father to son	Father to daughter	Mother to son	Mother to daughter
Actual data				
50% diagnosis*	9%	4%	12%	9%
75% diagnosis†	12%	5%	20%	15%
Extrapolated data				
100% diagnosis‡	~15%	~6%	~30%	~20%
HLA-B27 positive§	~30%	~12%	~60%	~40%

*Age at which 50% of patients are assumed to have received a diagnosis of ankylosing spondylitis. †Age at which 75% of patients are assumed to have received a diagnosis of anklyosing spondylitis. ‡Percentage of sons or daughters who will develop ankylosing spondylitis eventually based on linear extrapolation from actual data of 50% and 75%. §100% diagnosis based on HLA-B27 positive and negative cases; 100% diagnosis underestimates the value of HLA-B27-positive children by 50%.

Table 3: **Data and extrapolation of inheritance of ankylosing spondylitis to children**

developed ankylosing spondylitis. This effect may explain how the disease develops in female offspring. Women with a young age at onset may have a greater genetic susceptibility load than women developing the disease later in life. Therefore, children exposed to a uterine effect and carrying this larger genetic susceptibility factor would have an even higher prevalence of disease. Indeed, in the small number of women with a young age of onset, no less than 38% of children developed disease (ie, about 70–80% of HLA-B27-positive individuals; table 3). Nevertheless, for 28% of women onset of disease had not occurred before the birth of the affected child. Indeed, there is no difference in numbers of affected children born before, after, or during onset of ankylosing spondylitis among fathers and mothers. However, the time at which disease development is triggered remains unknown, and there may be many years of latency between precipitation of disease and symptom onset.

A third explanation is the influence of cytoplasmic or mitochondrial DNA. Children of affected fathers normally inherit disease at a male/female ratio of 2·5. That mothers with ankylosing spondylitis have more offspring with the disease than do men could be explained in part by additional non-nuclear cytoplasmic or mitochondrial genetic material that is operative only in women. Sons and daughters of affected mothers inherit susceptibility to ankylosing spondylitis equally because they both receive maternal mitochondrial DNA.

Finally, the susceptibility genes might be on the X or Y chromosome. The X chromosome may carry susceptibility or protective genes and influence disease. Sons of affected mothers with early onset of disease have a 50% chance of developing ankylosing spondylitis, irrespective of HLA-B27 status. The healthy sons are probably those who have not inherited their mother's HLA B27. Of those with the antigen, there may be nearly 100% penetrance of disease. These sons will have inherited their mother's X chromosome. Daughters who develop disease may receive an X chromosome from their healthy father who carries the same recessive susceptibility gene. However, with an X-linked disease daughters of affected fathers should have a higher risk than sons, which is not supported by our data.

Alternatively, if the Y chromosome is involved, some of the susceptibility to ankylosing spondylitis may come from the Y chromosome. Therefore, men with this factor would have an extra susceptibility gene that cannot be inherited by women. The sons of affected men would be more likely to develop disease and the children of affected women would be equally likely to develop disease. This suggestion is supported by the finding that, in mice, the Y chromosome interacts with the HLA-B27 gene.[16] Peptides encoded by the Y chromosome are believed to be presented by the HLA-B27 gene.

Environmental and genetic factors are required in the pathogenesis of ankylosing spondylitis.[17] No doubt genes affect susceptibility and severity, and may influence onset and course of disease by way of negative or positive effects (protective genes). HLA and non-HLA genes are relevant. Herein, we show an important influence of the sex of the index case and suggest that additional factors are operative. These could include a uterine (environmental) effect and influence from either or both the X and Y chromosomes. Meanwhile, the role of protective genes, particularly in women, and the relation between such putative genes and sex remains an enigma.

Contributors

Andrei Calin prepared the study proposal, developed the database, and supported the work throughout the data collection, analysis, and preparation of the results. Sinèad Brophy was involved in designing the methods, collecting and analysing the data, and preparing the paper. David Blake was involved in the data analysis, preparing the discussion, and writing the paper.

Acknowledgments

The study was supported by a grant from the Coates Charitable Trust, Col W W Pilkington Trust, Bath Institute for Rheumatic Diseases, the National Ankylosing Spondylitis Society, and by an Integrated Clinical Arthritis Centre Award from the UK Arthritis and Research Council.

References

1 Thomson G. HLA disease associations: models for the study of complex human genetic disorders. *Clin Lab Sci* 1995; **32:** 183–219.
2 Brown M, Pile K, Gibson K, Kennedy L, Calin A. The HLA component of the genetic contribution to ankylosing spondylitis. *Br J Rheumatol* 1995; **34** (suppl 1): 76.
3 Cho Y, Fu Y. Confirmation of a susceptibly locus *IBDI* for Crohn's disease on chromosome 16. *Gasteroenterology* 1997; **112** (special issue): A948.
4 Tromp G, Kuivaniemi H, Rapael S, Alakokko L, Christiano A, Considine E. Genetic linkage of familial granulomatous inflammatory arthritis, skin rash and uveitis to chromosome 16. *Am J Hum Genet* 1996; **59:** 1097–107.
5 Nair R, Henseler T, Jenisch S, et al. Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Hum Mol Genet* 1997; **6:** 1349–56.
6 Calin A. Ankylosing spondylitis. *Clin Rheum Dis* 1985; **11:** 41–60.
7 Mackay K, Brophy S, Mack C, Calin A. Patterns of radiological axial involvement in 470 ankylosing spondylitis (AS) patients. *Arthritis Rheum* 1997; **40:** 561.
8 Calin A. Ankylosing spondylitis. In: Maddison P, Isenberg D, Woo P, Glass D, eds. Oxford textbook of rheumatology, vol 2. Oxford: Oxford Medical Publications, 1993.
9 Armstrong BD, MacFarlane D, Panayi G. Ankylosing spondylitis and Klinefelter's syndrome: does the X chromosome modify disease expression? *Br J Rheumatol* 1985; **24:** 277–81.
10 Bennett P, Burch T. Population studies of rheumatic diseases. Amsterdam: Excerpta Medica Foundation, 1968.
11 van der Linden S, Valkenburg H, de Jongh B, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. *Arthritis Rheum* 1984; **27:** 241–49.
12 Mfller P, Vinje O, Dale K, Berg K, Kåss E. Family studies in Bechterew's syndrome (ankylosing spondylitis). *Scand J Rheumatol* 1984; **13:** 1–10.
13 Calin A, Marder A, Becks E, Burns T. Genetic differences between B27 positive patients with ankylosing spondylitis and B27 positive healthy controls. *Arthritis Rhuem* 1983; **26:** 1460–64.
14 Calin A, Taurog J, eds. The spondylarthritides. Oxford: Oxford University Press, 1998.
15 Lynn AH, Kwoh CK, Venglish CM, Aston CE, Chakravarti A. Genetic epidemiology of rheumatoid arthritis. *Am J Hum Genet* 1995; **57:** 150–59.
16 Simmons W, Summerfield S, Roopenian D, et al. Novel HY peptide antigens presented by HLA-B27. *J Immunol* 1997; **159:** 2750–59.
17 Brown M, Kennedy G, MacGregor A, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997; **40:** 1823–28.

ARTHRITIS & RHEUMATISM
Vol. 44, No. 6, June 2001, pp 1396–1400
© 2001, American College of Rheumatology
Published by Wiley-Liss, Inc.

Is Disease Severity in Ankylosing Spondylitis Genetically Determined?

J. Hamersma,[1] L. R. Cardon,[2] L. Bradbury,[2] S. Brophy,[3] I. van der Horst-Bruinsma,[1] A. Calin,[3] and M. A. Brown[1]

Objective. **To assess the role of genes and the environment in determining the severity of ankylosing spondylitis.**

Methods. **One hundred seventy-three families with >1 case of ankylosing spondylitis were recruited (120 affected sibling pairs, 26 affected parent–child pairs, 20 families with both first- and second-degree relatives affected, and 7 families with only second-degree relatives affected), comprising a total of 384 affected individuals. Disease severity was assessed by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and functional impairment was determined using the Bath Ankylosing Spondylitis Functional Index (BASFI). Disease duration and age at onset were also studied. Variance-components modeling was used to determine the genetic and environmental components contributing to familiality of the traits examined, and complex segregation analysis was performed to assess different disease models.**

Results. **Both the disease activity and functional capacity as assessed by the BASDAI and the BASFI, respectively, were found to be highly familial (BASDAI familiality 0.51 [$P = 10^{-4}$], BASFI familiality 0.68 [$P = 3 \times 10^{-7}$]). No significant shared environmental component was demonstrated to be associated with either the BASDAI or the BASFI. Including age at disease onset and duration of disease as covariates made no difference in the heritability assessments. A strong correlation was noted between the BASDAI and the BASFI (genetic correlation 0.9), suggesting the presence of shared determinants of these 2 measures. However, there was significant residual heritability for each measure independent of the other (BASFI residual heritability 0.48, BASDAI 0.36), perhaps indicating that not all genes influencing disease activity influence chronicity. No significant heritability of age at disease onset was found (heritability 0.18; $P = 0.2$). Segregation studies suggested the presence of a single major gene influencing the BASDAI and the BASFI.**

Conclusion. **This study demonstrates a major genetic contribution to disease severity in ankylosing spondylitis. As with susceptibility to ankylosing spondylitis, shared environmental factors play little role in determining the disease severity.**

It is well established that genetic factors play a major role in susceptibility to ankylosing spondylitis. Heritability in twins is estimated to be >90% (1), and genes of the major histocompatibility complex, in particular HLA–B27, have been demonstrated, by both linkage and association methods, to be heavily involved (2–5).

Very little is known about the genetic control of disease severity in ankylosing spondylitis. Greater similarity of disease severity was observed in monozygotic, compared with dizygotic, twins, although no individual severity measure achieved statistical significance (1). A study of 42 sibling pairs revealed greater familiality of disease severity markers, including pain and disability indices and abnormalities on pelvic and spinal radiographs, but did not differentiate shared environmental factors from genetic factors (6). Few association studies

Supported by the National Ankylosing Spondylitis Society (UK). Ms Bradbury and Dr. Brown's work was supported by the Arthritis Research Campaign. Ms Brophy's work was supported by the Arthritis Research Campaign, the John Coates Charitable Trust, and the Colonel W. W. Pilkington Trust. Dr. Calin's work was supported by the John Coates Charitable Trust and the Colonel W. W. Pilkington Trust.

[1]J. Hamersma, I. van der Horst-Bruinsma, PhD, M. A. Brown, MD, FRACP: University Hospital Vrije Universiteit, Amsterdam, The Netherlands; [2]L. R. Cardon, MD, PhD, L. Bradbury: Wellcome Trust Centre for Human Genetics, Oxford, UK; [3]S. Brophy, A. Calin, MD, FRCP: Royal National Hospital for Rheumatic Diseases, Bath, UK.

Address correspondence and reprint requests to M. A. Brown, MD, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington OX3 7BN, UK.

Submitted for publication September 8, 2000; accepted in revised form January 25, 2001.

of candidate genes and disease severity in ankylosing spondylitis have been performed, and to date, all have yielded negative results. Genetic associations with particular disease characteristics have been reported, although no reported association has been widely replicated (for review, see ref. 7).

In recent years, specific disease activity and outcome measures have been designed for ankylosing spondylitis, to allow easy and reproducible assessment of disease severity. In particular, Calin and colleagues have developed a suite of validated, self-administered measures of ankylosing spondylitis disease activity and functional effects. These include the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (8), Bath Ankylosing Spondylitis Functional Index (BASFI) (9), Bath Ankylosing Spondylitis Global Index (10), Bath Ankylosing Spondylitis Metrology Index (11), and Bath Ankylosing Spondylitis Radiology Index (12). The aim of the current study was to test the hypothesis that disease severity, as assessed by the indices BASDAI and BASFI, is genetically determined.

PATIENTS AND METHODS

Patients. Probands of families with >1 member affected with ankylosing spondylitis were recruited from the Royal National Hospital for Rheumatic Diseases Ankylosing Spondylitis database. Ankylosing spondylitis was defined according to the modified New York diagnostic criteria (13). Affected family members were contacted by telephone and the diagnosis confirmed by a semistructured interview that was conducted by 1 of us (JH, LB, or MAB). All patients were then sent a self-report questionnaire to complete, which included scores for disease activity and function on the BASDAI and BASFI; in addition, patients were asked to detail the date at onset of the ankylosing spondylitis symptoms and the date of diagnosis. More than 90% of the individuals who were approached to participate in the study completed the questionnaire.

One hundred seventy-three families were identified with 384 affected family members. Of these families, 146 had affected first-degree relatives only, including 120 sibling pairs and 26 parent–child pairs. Twenty families had affected first and second-degree relatives, and 7 families had affected second-degree relatives.

Blood samples were obtained from 240 of the 384 affected family members, and these were typed for HLA–B27 bypolymerase chain reaction/sequence-specific primer analysis (14).

Statistical methods. Familiality and heritability of the traits evaluated quantitatively on the BASDAI and BASFI were assessed using the Pedigree Analysis (PAP) statistical package (15). Familiality refers to the proportion of the overall trait variance due to shared environmental and genetic factors. Heritability refers to the proportion of the overall trait variance ascribable to the genetic variance. PAP uses a variance-components approach to assess the relative contributions of genetic and environmental effects in the trait studied. Using the known genetic relationship between family members, and assuming greater environmental sharing between first-degree relatives than with more distantly related individuals or with unrelated individuals, the genetic and environmental variances of the trait are calculated. The total trait variance is assumed to be the sum of the genetic variance and shared and random environmental variances. The correlations between genotypic and environmental factors and the gene–environment interaction are assumed to be negligible; violation of these assumptions could lead to over- or underestimation of heritability, respectively.

Potential covariates studied included age at onset of symptoms, age at diagnosis, and disease duration from age at symptom onset. The heritability of the BASDAI independent of the BASFI, and vice versa, was assessed by calculating BASDAI or BASFI heritability estimates with BASFI or BASDAI incorporated as a covariate.

Ascertainment differences between closely and distantly related affected individuals were assessed by analysis of covariance using the program SuperAnova, version 1.11 (Abacus Concepts, Berkeley, CA) to compare the age at disease onset, diagnostic delay, BASDAI scores, and BASFI scores between probands and non-probands. The linear association between variables was studied by correlation coefficient analysis, using the same software package.

Complex unified segregation analysis (16,17) was performed using the package PAP. A unified model consisting of environmental effects and a genetic component consisting of a biallelic major gene and polygenes was fitted to the family data, against which other genetic and environmental models were compared. Parameters estimated in this model included the frequency of the major gene alleles, f(A) and f(B), the mean trait values associated with the major gene genotypes, μ(AA), μ(AB), and μ(BB), 3 transmission parameters, τAA, τAB, and τBB, which represent the probability of transmission of the major gene genotypes, a single standard deviation, σ, for all genotype means, and a residual multifactorial familial component, h^2. Environmental covariates were not included in this analysis because they had been shown to have no significant influence on the BASDAI or BASFI heritability (see below).

Comparisons of models were performed by likelihood ratio tests, in which the difference of the $-2 \times \log_e$ likelihood of the models was asymptotically distributed as the χ^2 value (with degrees of freedom equal to the difference in the number of parameters estimated in the 2 models). A significant value of this statistic indicates that a more general model provides a better fit to the data than does the comparison model. Submodels of the unified model fitted to the data included a codominant/Mendelian model (τAA, τAB, and τBB set to 1.0, 0.5, and 0.0, respectively, with genotypic means independently estimated), dominant and recessive models (both models, τAA, τAB, and τBB set to 1.0, 0.5, 0.0, respectively; recessive model, μ[AA] and μ[AB] equal, μ[BB] fixed; dominant model, μ[AA] free, μ[AB] and μ[BB] equal), and polygenic and environmental models (estimated by equating all transmission probabilities and all genotypic means, and setting the residual familial component h^2 to zero for the environmental model). Because the Mendelian/codominant, dominant, and recessive models fitted the observed data, the presence of a residual

Table 1. Segregation models of the Bath Ankylosing Spondylitis Disease Activity Index*

Model	f(A)	μ(AA)	μ(AB)	μ(BB)	σ	h^2	−2lnL	χ^2	df	*P*
1. General	0.371	2.775	2.337	5.792	1.432	0.681	1,650.178			
2. Mendelian codominant	0.730	2.227	5.251	6.962	1.378	0.024	1,653.893	3.715	3	0.3
3. Dominant	0.765	2.405	5.679	5.679	1.464	0.181	1,654.694	0.801	1	0.4
4. Recessive	0.370	2.439	2.439	5.738	1.462	0.251	1,655.160	1.267	1	0.3
5. Restricted Mendelian/codominant	0.729	2.220	5.240	6.961	1.377	0	1,653.9	0.007	1	0.9
6. Restricted dominant	0.760	2.379	5.647	5.647	1.642	0	1,655.23	1.57	1	0.2
7. Restricted recessive	0.359	2.400	2.400	5.679	1.460	0	1,656.264	1.104	1	0.3
8. Polygenic	–	3.758	3.758	3.758	2.178	0.512	1,673.368	23.19	3	3.7×10^{-5}
9. Environmental	–	3.769	3.769	3.769	2.179	0	1,687.843	37.665	4	1.3×10^{-7}

* Model 2 is compared with model 1, models 5–7 are compared with models 2–4, respectively, and all other models are compared with model 2. f = frequency; A and B = major genes; μ = mean trait value; σ = standard deviation; h^2 = residual multifactorial familial component; −2lnL = likelihood ratio; df = degrees of freedom.

polygenic contribution was investigated by comparing these models with models fitted with no residual polygenic contribution (restricted models).

RESULTS

Of the 384 affected individuals studied, 36% were female. The median age was 50 years (range 18–86 years) and the median age at onset of symptoms was 21 years (range 7–63 years). The median disease duration was 24 years (range 1–64 years). The mean (±SD) BASDAI was 3.8 ± 2.2 and the BASFI was 3.8 ± 2.7. Psoriasis was reported by 13% of the affected individuals, and inflammatory bowel disease by 8%. Of the 240 patients typed for HLA–B27, all were found positive.

There was no evidence of ascertainment bias. Probands and non-probands had similar disease severity as assessed by either the BASDAI or the BASFI (controlled for disease duration). There was no difference in the mean diagnostic delay (probands mean ± SD 7.6 ± 0.6 years, non-probands 9.1 ± 0.6 years).

No correlation was observed between the BASDAI and disease duration, whereas a significant correlation was observed between the BASFI and disease duration ($r = 0.18$, $P = 0.0004$). No correlation was observed between the age at disease onset and either the BASFI or the BASDAI. There was no effect of sex on the age at disease onset, the BASDAI, or the BASFI.

Marked familiality and heritability were observed for both the BASFI and the BASDAI. For the BASFI, familiality was 0.68 ($P = 3 \times 10^{-7}$, 95% confidence interval [95% CI] 0.42–0.94) and heritability was nearly identical (0.68; $P = 3 \times 10^{-7}$, 95% CI 0.44–0.92). This indicates virtually no shared environmental effect on the BASFI. The BASDAI similarly showed high familiality (0.51; $P = 10^{-4}$, 95% CI 0.25–0.77) and identical heritability. Neither familiality nor heritability estimates for the age at disease onset were significant (0.2 [$P = 0.14$] and 0.18 [$P = 0.2$], respectively). Inclusion of age at symptom onset and disease duration as covariates, either singly or together, made no difference to these assessments, with negligible correlation (0.00 for BASDAI versus either age at symptom onset or disease duration, 0.01 for BASFI versus age at symptom onset, 0.04 for BASFI versus disease duration).

A strong genetic correlation was observed between the BASDAI and the BASFI (correlation coefficient 0.9), suggesting the possibility of shared determinants of these 2 measures. The presence of significant residual heritability for each measure independent of the other (BASDAI residual heritability [considering BASFI as a covariate] 0.36; BASFI residual heritability [considering BASDAI as a covariate] 0.48) suggests that some genes influence disease activity and function differentially.

Results of the segregation analyses are presented in Tables 1 and 2. These results show that for both the BASDAI and the BASFI, the polygenic and environmental models are strongly excluded (models 8 and 9). For both the BASDAI and the BASFI, the codominant, dominant, and recessive models could not be rejected (models 2, 3, and 4), and these models are therefore considered to be consistent with the observed data. Note that models 3 and 4 produced conceptually equivalent findings, with the major gene allele B being either rare and dominant (model 3—equivalent to allele A being common but recessive) or common and recessive (model 4—equivalent to allele A being rare but dominant). Considering the major gene effect observed in the absence of residual polygenic contribution (models 5–7 compared with models 2–4, respectively, in Tables 1 and 2), there was no significant evidence of a residual

Table 2. Segregation models of the Bath Ankylosing Spondylitis Functional Index*

Model	f(A)	μ(AA)	μ(AB)	μ(BB)	σ	h^2	−2lnL	χ^2	df	*P*
1. General	0.793	2.041	6.526	8.708	1.387	0.290	1,756.948			
2. Mendelian/codominant	0.797	2.066	6.607	8.731	1.406	0.247	1,757.533	0.585	3	0.9
3. Dominant	0.812	2.152	6.949	6.949	1.496	0.332	1,760.659	3.126	1	0.08
4. Recessive	0.420	2.151	2.151	6.940	1.496	0.378	1,757.972	0.439	1	0.5
5. Restricted Mendelian/codominant	0.214	2.058	6.579	8.813	1.393	0.000	1,758.787	1.254	1	0.3
6. Restricted dominant	0.815	2.164	6.991	6.991	1.497	0.000	1,763.805	3.146	1	0.08
7. Restricted recessive	0.425	2.162	2.162	6.995	1.493	0.000	1,761.733	3.761	1	0.052
8. Polygenic	–	3.765	3.765	3.765	2.724	0.681	1,833.563	76.030	3	2×10^{-16}
9. Environmental	–	3.782	3.782	3.782	2.726	0.000	1,859.927	102.394	4	3×10^{-21}

* Model 2 is compared with model 1, models 5–7 are compared with models 2–4, respectively, and all other models are compared with model 2. In estimating the parameters of the general model, the value of τAA always hit the boundary of 1.0, and was therefore fixed at this value to estimate the other parameters. See Table 1 for definitions.

polygenic component for either the BASDAI or the BASFI, with models including such a component not being significantly different from those without.

DISCUSSION

This study demonstrates that in ankylosing spondylitis, disease severity is largely genetically determined, and that shared environmental factors play little role in determining either disease activity measured at any one point in time or functional incapacity. Although the genetic factors involved in determining disease activity and functional impairment overlap, some genes affect either disease activity or functional impairment, but not both. This is similar to the findings from twin studies of disease susceptibility, which have also shown that heritability of ankylosing spondylitis itself is extremely high. However, those studies were not sufficiently powerfull to demonstrate the heritability of disease severity scores (1).

Questionnaire-based assessment of disease severity is clearly subject to many potential sources of error, which would diminish the power of heritability studies and lead to underestimation of familiality and heritability. More objective measures, such as metrology (11) and radiology indices (12), have been developed, but insufficient family data are currently available for use in such a study. This study indicates, however, that the BASDAI and the BASFI are sufficiently robust to detect significant genetic effects, and therefore could be used for gene-mapping studies.

The results of the segregation analysis suggest that there is a major genetic effect influencing both the BASDAI and the BASFI. The codominant, dominant, and recessive models could not be differentiated and were all quite similar, with significant dominance-variance apparent. For all Mendelian models, exclusion of a residual polygenic component did not significantly alter the model likelihood. Estimated genotypic means and allele frequencies for the major gene models are given in Tables 1 and 2. The polygenic and environmental models were strongly excluded for both disease severity measures.

Few candidate gene association studies have been performed for disease severity or disease features in ankylosing spondylitis, in contrast to an abundance of genetic studies on disease susceptibility. A study of British Caucasian cases (18) found no association between HLA–B27, –B60, or –DR1 and disease severity assessed by a composite disease severity index (the Bath Ankylosing Spondylitis Disease Severity Index [19]). Other studies have addressed associations with HLA–B27 (20) and *LMP2* (21), and similarly reported no association with disease severity scores. Homozygosity for HLA–B27 also appears not to significantly influence the severity of ankylosing spondylitis (22). These negative findings suggest that the genes determining the severity of ankylosing spondylitis may be encoded outside of the major histocompatibility complex. In the current study, it is known that all typed cases were B27 positive, but whether they were B27 heterozygotes or homozygotes is unknown. It is unlikely that more than a small proportion of the cases would be B27 homozygotes, since the allele frequency of B27 in this population is only 4%, and therefore, unless B27 homozygosity had a marked influence on disease severity, it would be unlikely to explain the segregation study findings. The findings of previous association studies suggest that if B27 has any effect on disease severity, it is likely to be small. Therefore, although the current findings may be explained by an effect of B27 homozygosity on disease severity, it is more likely that another gene is involved.

No other studies have addressed the heritability

of disease activity or functional impairment. Several associations with complications of ankylosing spondylitis have been reported, such as associations with iritis and peripheral arthritis and with the age at symptom onset (for review, see ref. 7), but there is little consensus among these studies and no gene has conclusively been demonstrated to be involved. The current study suggests that further studies of the genetic determination of disease severity are indicated, and that significant genetic effects can be assessed using the easily administered self-report questionnaires, the BASDAI and the BASFI. Practically, this study also indicates that because disease severity and functional impairment show a consistent pattern within families, the severity of disease in secondarily affected family members can be roughly predicted from the severity of previously affected family members. Identifying the actual genes involved would obviously improve the precision and reliability of such predictions.

ACKNOWLEDGMENT

The authors would like to thank the National Ankylosing Spondylitis Society (UK) for their assistance and support of this research.

REFERENCES

1. 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.
2. Brown MA, Pile KD, Kennedy LG, Campbell D, Andrew L, March R, et al. A genome-wide screen for susceptibility loci in ankylosing spondylitis. Arthritis Rheum 1998;41:588–95.
3. Schlosstein L, Terasaki PI, Bluestone R, Pearson C. High association of an HL-A antigen W27, with ankylosing spondylitis. N Engl J Med 1973;288:704–5.
4. Brewerton DA, James DC. The histocompatibility antigen (HL-A 27) and disease. Semin Arthritis Rheum 1975;4:191–207.
5. Rubin LA, Amos CI, Wade JA, Martin JR, Bale SJ, Little AH, et al. Investigating the genetic basis for ankylosing spondylitis: linkage studies with the major histocompatibility complex region. Arthritis Rheum 1994;37:1212–20.
6. Calin A, Elswood J. Relative role of genetic and environmental factors in disease expression: sib pair analysis in ankylosing spondylitis. Arthritis Rheum 1989;32:77–81.
7. Brown MA, Wordsworth BP. Predisposing factors to spondyloarthropathies. Curr Opin Rheumatol 1997;9:308–14.
8. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286–91.
9. Calin A, Garrett S, Whitelock H, Kennedy LG, O'Hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281–5.
10. Jones SD, Steiner A, Garrett SL, Calin A. The Bath Ankylosing Spondylitis Patient Global Score (BAS-G). Br J Rheumatol 1996; 35:66–71.
11. Jenkinson TR, Mallorie PA, Whitelock HC, Kennedy LG, Garrett SL, Calin A. Defining spinal mobility in ankylosing spondylitis (AS): the Bath AS Metrology Index. J Rheumatol 1994;21:1694–8.
12. MacKay K, Mack C, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI): a new, validated approach to disease assessment. Arthritis Rheum 1998;41:2263–70.
13. 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.
14. Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J, et al. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 1996;55: 268–70.
15. Hasstedt SJ. Variance components/major locus likelihood approximation for quantitative, polychotomous, and multivariate data. Genet Epidemiol 1993;10:145–58.
16. Morton NE, MacLean CJ. Analysis of family resemblance. III. Complex segregation of quantitative traits. Am J Hum Genet 1974;26:489–503.
17. Elston RC, Stewart J. A general model for the genetic analysis of pedigree data. Hum Hered 1971;21:523–42.
18. Brown MA, Kennedy LG, Darke C, Gibson K, Pile KD, Shatford JL, et al. The effect of HLA–DR genes on susceptibility to and severity of ankylosing spondylitis. Arthritis Rheum 1998;41:460–5.
19. Taylor AL, Balakrishnan C, Calin A. Reference centile charts for measures of disease activity, functional impairment, and metrology in ankylosing spondylitis. Arthritis Rheum 1998;41:1119–25.
20. Khan MA, Kushner I, Braun WE. Comparison of clinical features in HLA–B27 positive and negative patients with ankylosing spondylitis. Arthritis Rheum 1977;20:909–12.
21. Maksymowych WP, Adlam N, Lind D, Russell AS. Polymorphism of the LMP2 gene and disease phenotype in ankylosing spondylitis: no association with disease severity. Clin Rheumatol 1997;16: 461–5.
22. Khan MA, Kushner I, Braun WE, Zachary AA, Steinberg AG. HLA-B27 homozygosity in ankylosing spondylitis: relationship to risk and severity. Tissue Antigens 1978;11:434–8.

Appendix 10

Am. J. Hum. Genet. 68:000–000, 2001

Whole-Genome Screening in Ankylosing Spondylitis: Evidence of Non-MHC Genetic-Susceptibility Loci

S. H. Laval,[1,*] A. Timms,[1] S. Edwards,[1] L. Bradbury,[1] S. Brophy,[2] A. Milicic,[1] L. Rubin,[3] K. A. Siminovitch,[4] D. E. Weeks,[5] A. Calin,[2] B. P. Wordsworth,[1] and M. A. Brown[1]

[1]Spondyloarthritis and Bone Disease Research Group, Wellcome Trust Centre for Human Genetics, Headington, United Kingdom; [2]Royal National Hospital for Rheumatic Diseases, Bath, United Kingdom; [3]Division of Rheumatology, St. Michael's Hospital, and [4]Mount Sinai Hospital, Department of Medicine, University of Toronto, Toronto; and [5]Department of Human Genetics, University of Pittsburgh, Pittsburgh

Ankylosing spondylitis (AS) is a common inflammatory arthritis predominantly affecting the axial skeleton. Susceptibility to the disease is thought to be oligogenic. To identify the genes involved, we have performed a genomewide scan in 185 families containing 255 affected sibling pairs. Two-point and multipoint nonparametric linkage analysis was performed. Regions were identified showing "suggestive" or stronger linkage with the disease on chromosomes 1p, 2q, 6p, 9q, 10q, 16q, and 19q. The MHC locus was identified as encoding the greatest component of susceptibility, with an overall LOD score of 15.6. The strongest non-MHC linkage lies on chromosome 16q (overall LOD score 4.7). These results strongly support the presence of non-MHC genetic-susceptibility factors in AS and point to their likely locations.

Introduction

Ankylosing spondylitis (AS) is the second-most-common cause of inflammatory arthritis worldwide, with a prevalence of 1/1,000–3/1,000 in white populations (Calin 1998). It is characterized by inflammation in the spine and sacroiliac joints, causing initial bone and joint erosion and subsequent ankylosis. Arthritis affecting peripheral joints, particularly the hips, occurs in 40% of cases, and inflammation may also involve extraarticular sites such as the uvea, tendon insertions, aorta, lungs, and kidneys. Genetic factors were implicated in the etiology of the disease long ago, with the demonstration of high disease familiality (de Blecourt et al. 1961). The sibling recurrence-risk ratio is 82 (Brown et al. 2000*b*), and heritability, assessed by twin studies, is >90% (Brown et al. 1997). The recognition of the association of B27 with AS confirmed the importance of heritable factors in the disease (Brewerton et al. 1973; Schlosstein et al. 1973) and remains one of the strongest disease associations of any inflammatory human disease. In most populations that have been studied, the prevalence of AS is strongly correlated with the prevalence of the main disease-susceptibility gene, HLA-B27 (B27). Only a few families have been reported in which AS segregates independently from B27 (van der Linden et al. 1975; Gladman et al. 1986; Deshayes et al. 1987; Woodrow 1988; Brown et al. 1996; Said-Nahal et al. 2000) and only rare cases of familial B27-negative AS have been reported (Rubin et al. 1994; Skomsvoll et al. 1995), suggesting that B27 is almost essential for the inheritance of AS within families. However, only 1%–5% of B27-positive individuals develop AS, and there is increasing evidence to suggest that other genes must also be involved. B27-positive relatives of AS patients have a recurrence risk of the disease that is 5.6–16 times greater than that of B27-positive individuals in the population at large, implying the presence of non-B27 shared familial risk factors (Calin et al. 1983; van der Linden et al. 1983). Recurrence-risk modeling in AS rejects single-gene and polygenic models. Oligogenic models with between three and nine genes operating in addition to B27 fit the observed pattern of recurrence risks in relatives of patients with AS (Brown et al. 2000*b*). A major non-B27 contribution to susceptibility to AS is suggested by the greater concordance rate in MZ twins (63%) than in B27-positive DZ twin pairs (23%) (Brown et al. 1997).

A preliminary whole-genome screen in 105 affected-sibling-pair families with AS demonstrated strong linkage to the MHC locus (LOD 8.1) but also identified several other regions with moderate evidence of linkage (Brown et al. 1998). Six regions lying on chromosomes 2p, 2q, 3p, 10q, 11p, and 16q achieved LOD scores >1.0, with the peak non-MHC linkage occurring on

Received December 4, 2000; accepted for publication February 9, 2001; electronically published February 27, 2001.

Address for correspondence and reprints: Dr. Matthew A. Brown, Spondyloarthritis and Bone Disease Research Group, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, OX3 7BN, United Kingdom. E-mail: mbrown@well.ox.ac.uk

* Present affiliation: School of Biochemistry and Genetics, University of Newcastle upon Tyne, Ridley Building, Claremont Place, Newcastle NE1 7RU, United Kingdom.

© 2001 by The American Society of Human Genetics. All rights reserved.
0002-9297/2001/6804-00XX$02.00

chromosome 16q (LOD 2.6). Candidate-gene studies have also implicated the gene cytochrome P450 2D6, lying on chromosome 22q13.1, in susceptibility to AS (Beyeler et al. 1996; Brown et al. 2000*a*) and suggest the involvement of more than one MHC gene (Hohler et al. 1998; Laval et al. 2000).

Since this preliminary study, we have continued to recruit additional families (screen 2) and have reinvestigated our initial families (screen 1) with a denser marker map. The results of these screens have been analyzed both separately and as a combined cohort consisting of 185 families containing 255 affected sibling pairs (see table 1). Results of the X-chromosome mapping have been presented elsewhere (Hoyle et al. 2000).

Subjects and Methods

Families with AS

This project was approved by the Central Oxford Research Ethics Committee (approval CM95.061) and the University of Toronto Ethics Committee. AS was defined according to the modified New York diagnostic criteria (van der Linden et al. 1984). All patients had been seen by a qualified rheumatologist, and the diagnosis of AS had been confirmed. To further confirm the diagnosis, all cases either were examined or were interviewed by telephone by one of us (L.B., M.A.B., or L.R.). In patients with atypical histories or for whom radiographs had not been performed previously, pelvic and lumbosacral spine radiographs were obtained, and attending physicians were contacted to confirm the diagnosis. All family members were typed for HLA-B27 (see below), and all the affected individuals were B27-positive. All family members were of white descent. Among participants affected with AS, the mean age at time of recruitment was 46 years (range 16–86 years), 63% were male, and 44% reported having had iritis, 16% psoriasis, and 9% inflammatory bowel disease.

Two cohorts of families were recruited (screens 1 and 2—see table 1 for family details). Six families included in our initial publication have been excluded from the current study because of changes of diagnosis or paternity (outlined below). Although both screens have similar numbers of affected sibling pairs, there are significant differences between the two cohorts. Screen 2 has fewer families (86) than screen 1 (99) and fewer affected individuals (210) than screen 1 (235). Blood samples were available from a higher proportion of parents in screen 1 families (62% of all parents) than of parents in screen 2 families (51% of all parents). Thus, it is likely that the power to detect linkage was higher for the screen 1 families than the screen 2 families.

Table 1

Summary of Families Included in Genome Screen

	Screen		
No. of	1	2	1 and 2
Families:			
Overall	99	86	185
With two affected sibs	87	69	156
With three affected sibs	7	9	16
With four affected sibs	2	1	3
With five affected sibs	...	1	1
With sibling pairs in two generations	2	5	7
With a sibling trio in one generation and a sibling pair in another	...	1	1
With sibling trios in two generations	1	...	1
With other first- or second-degree relatives	21	18	39
With both parents	50	29	79
With one parent	22	29	51
With no parents	27	28	55
Sibling pairs overall	130	125	255
Affected individuals	235	210	445
Unaffected individuals	187	186	373

Genotyping

All individuals were typed for HLA-B27 by PCR-SSP (Brown et al. 1996). Screen 1 families were genotyped for 505 autosomal microsatellite markers, including 259 markers from the Medical Research Council (United Kingdom) set (Reed et al. 1994), 5 markers lying within the MHC locus (62A, 82-1, 82-2, T2, and D3A) (Hsieh et al. 1997), and 241 additional markers from the Applied Biosystems Prism Linkage Mapping Set Version 2 (LMSv2) marker set (PE Biosystems). Screen 2 families were genotyped for 367 markers from the LMSv2 marker set (not including markers excluded by error-checking procedures). The markers were amplified by methods reported elsewhere (Brown et al. 1998), pooled in sets of 15–20 markers, and products were separated by electrophoresis in 6% polyacrylamide gels using ABI 373 semiautomated sequencers. Products were sized using the program GENESCAN 672, version 1.1 (PE Biosystems), and genotypes were assigned semiautomatically using the program GENOTYPER, version 1.1 (PE Biosystems).

Statistical Analysis

To minimize data errors, extensive checking procedures were employed. Mendelian inheritance of markers was checked manually within GENOTYPER, and the program GAS, version 2.0 (A. Young, unpublished), was used to convert the size data into discrete allele numbers. Consistency of allele assignment was ensured by use of a control sample on each gel and by comparison of allele distributions between screens. The program PEDCHECK (O'Connell and Weeks 1998) then was used to

screen all data for previously undetected inconsistencies of Mendelian inheritance. The relationship between pedigree members was then examined, using the results of >90 markers, by means of the program SIBERR (Ehm et al. 1998). This program identifies misspecification of probable MZ twins, half-siblings, and unrelated individuals as full sibling pairs by comparison of the number of alleles shared IBD at unlinked loci with null hypothesis expectations. One previously unrecognized MZ twin pair was identified by this program and was removed from the analysis. This pair was identical at 181 of 182 alleles checked (0.5% genotyping-error rate). Excess recombination events between markers, a finding suggestive of genotyping error, was screened for, by calculation of recombination distances, using the program SIMWALK2 (Sobel and Lange 1996). Of the 527 markers used, 3 were removed from the screens as a result of this analysis. Finally, non-Mendelian errors were investigated further using the program SIBMED (Douglas et al. 2000). SIBMED identifies likely genotyping errors and marker mutations by calculating the posterior probability of an error for each sibling-pair–marker combination. The prior probability of genotype error was set at 1%, and genotypes of sibling-pair–marker combinations with a posterior probability of error of >50% were checked. Among the 614,929 genotypes scored, 51 genotype errors (0.008%) were identified by this program and were removed from the analysis.

Allele frequencies were calculated, from all scored genotypes, by means of the program DOWNFREQ (J. Terwilliger). Marker positions were obtained from public databases (either the Whitehead Institute for Biomedical Research database or GeneMap'99).

Multipoint analysis theoretically is more accurate in identifying the position of maximum linkage and has greater power to identify linkage but is susceptible to biases that are less important in two-point analysis. Multipoint analysis depends critically on the correct marker order and intermarker distances. Genotyping errors in two-point analysis affect only the marker involved, whereas, in multipoint analysis, they can also affect surrounding markers. Therefore, both analyses are presented.

Two-point nonparametric affected-sibling-pair linkage analysis was performed using the program ANALYZE (Satsangi et al. 1996). Multipoint nonparametric linkage analysis was performed using the ALL statistic of the program GENEHUNTER-PLUS (Kong et al. 1997). IBD sharing by affected sibling pairs was determined using GENEHUNTER, version 2.0 (Pratt et al. 2000), and confidence intervals for locus-specific λ values were determined by methods reported elsewhere (Cordell and Olson 1997). The contribution of each locus to the overall sibling recurrence risk was calculated assuming a sibling recurrence-risk ratio of 82 (Brown et al. 2000*b*) and either additive (Norman et al. 1998) or multiplicative (Risch 1987) interaction between loci. "Suggestive" and "significant" linkage are defined, according to published recommendations for affected-sibling-pair nonparametric analysis, as LOD $\geq$ 2.2 and LOD $\geq$ 3.6, respectively (Lander and Kruglyak 1995).

Results

Two-point results for markers achieving LOD scores of 1.0 in screens 1 and 2 and the combined results are listed in table 2. Twenty-eight markers from 14 regions achieved LOD scores of $\geq$1.0 in screen 1. Among these, three markers (D6S276, D10S185, and D16S422) from three regions achieved LOD scores of $\geq$1.0 in screen 2. Strong linkage with the MHC locus in this set was observed, with a peak LOD score of 6.9. Outside of the MHC locus, "significant" linkage was observed with marker D9S1826 (LOD 3.9). "Suggestive" linkage was observed for markers D10S597 (LOD 2.4) and D16S289 (LOD 2.7). In screen 2, 22 markers from 11 regions achieved LOD scores of $\geq$1.0. Of these, three markers (D6S276, D10S185, and D16S422) from three regions achieved linkage with LOD 1.0 in screen 1. Strong linkage was again observed with the MHC locus (LOD 4.8). "Significant" linkage was only observed with the microsatellites in the region of the MHC locus. Outside of the MHC locus, "suggestive" linkage was observed with markers D7S519 (LOD 2.6), D19S414 (LOD 2.5), and D19S420 (LOD 3.58). Considering the combined data across both screens, 34 markers from 14 regions achieved LOD scores of $\geq$1.0. Five markers from four regions achieved "suggestive" linkage (markers D1S255 [LOD 2.2], D9S288 [LOD 2.3], D9S1682 [LOD 2.3], D9S1826 [LOD 2.8], and D16S422 [LOD 3.3]).

The results of the multipoint analysis are given in figure 1. For most loci, there are only minor differences between the two-point and multipoint LOD scores, but, for a small number of loci, quite marked differences were observed. For the MHC locus, the peak LOD scores obtained in screen 1, 2, and the combined screen, respectively, were 7.8, 8.1, and 15.6. The λ value for this locus overall was 5.2 ($z_0 = 0.048$, $z_1 = 0.5$, $z_2 = 0.45$, where z_n is the probability of sharing n alleles identical by descent). The contribution of the MHC locus (λ = 5.2, 95% CI 3.0–9.0) to the recurrence-risk ratio in AS is either 37%, under a multiplicative disease model, or 6.8%, under an additive disease model. Multipoint analysis demonstrated greater evidence of linkage on chromosomes 2, 5, and 16 than did two-point analysis and showed less evidence of linkage on chromosomes 1 and 7. On chromosome 1, the peak multipoint linkages were at 60 cM from the p telomere, with LOD scores in screens 1 and 2 and the combined analysis being 0.6, 1.1, and 1.7, respectively. On chro-

Table 2

Two-Point Linkage Results for Screens 1 and 2 and the Combined Data Set, Using the Program ANALYZE

Chromosome and Marker	Distance From p Telomere (cM)	Screen 1		Screen 2		Screens 1 and 2	
		LOD	*P*	LOD	*P*	LOD	*P*
1:							
D1S199	47.7	1.2	.01	.0	.41	.5	.07
D1S255	66.6	1.7	.0029	.6	.046	2.2	.0007
D1S197	78.3	1.0	.014	.0	.5	.5	.074
D1S484	173.9	1.0	.016	.4	.083	1.4	.0053
D1S2836	290.1	.6	.045	.7	.032	1.3	.007
2:							
D2S391	73.8	1.0	.017	.4	.083	1.3	.0073
D2S337	84.1	1.3	.0079	.1	.23	1.1	.014
D2S160	127.4	1.1	.011	.2	.18	1.1	.011
D2S347	135.7	.7	.034	.4	.076	1.2	.011
D2S335	182.5	1.2	.0083	.0	.5	.5	.069
D2S157	212.6	1.6	.0034	...	...	1.6	.0034
3:							
D3S1300	79	1.1	.012	.0	.5	.4	.092
D3S1314	218.3	.0	.5	1.4	.0061	.6	.044
5:							
D5S400	174.3	.0	.5	1.4	.0056	.6	.044
6:							
D6S309	13.6	.0	.5	1.5	.0043	.8	.026
D6S470	17.7	.1	.26	3.1	.000079	2.2	.00066
D6S289	29.55	.8	.028	1.9	.0017	2.5	.00033
D6S422	35.7	.9	.02	2.9	.00012	***3.6***	***.000023***
D6S276	44.8	1.8	.0022	***4.8***	***.0000013***	***6.5***	***$<10^{-6}$***
82II	44.95	***6.9***	***$<10^{-6}$***	...	...	***6.9***	***$<10^{-6}$***
HLADRA	46.05	***3.9***	***.000011***	...	...	***3.9***	***.000011***
D6S291	49.8	1.2	.0097	...	...	1.2	.0097
D6S1610	53.9	1.5	.0042	...	...	1.5	.0042
D6S257	80	1.0	.015	.7	.033	1.7	.0024
D6S460	90	.6	.045	2.0	.0012	2.4	.00041
7:							
D7S519	70.5	.0	.5	2.6	.00025	.6	.045
8:							
D8S1784	116.8	.7	.042	.8	.024	1.5	.0043
D8S514	128.9	.5	.068	.6	.054	1.0	.014
9:							
D9S288	8.8	1.6	.003	.7	.037	2.3	.00058
D9S286	16.8	.5	.066	1.0	.014	1.5	.0044
D9S161	50.3	.6	.043	.7	.032	1.4	.006
D9S283	93.2	.7	.039	1.2	.01	1.8	.0019
D9S1682	132.9	.5	.068	2.1	.001	2.3	.0006
D9S1826	160.2	***3.9***	***.000013***	.0	.33	2.8	.00016
10:							
D10S185	123.3	1.0	.016	1.1	.012	2.1	.0009
D10S192	131.2	1.5	.0041	.2	.2	1.1	.011
D10S597	137.6	2.4	.00043	.1	.23	1.8	.0022
D10S190	147.2	1.0	.015	.1	.29	.8	.025
11:							
D11S922	3.2	1.1	.014	...	...	1.1	.014
D11S935	49.6	1.1	.011	.0	.5	.6	.054
15:							
D15S165	20.2	.0	.5	1.6	.003	.4	.078
16:							
D16S3068	46.6	1.3	.0068	.0	.5	.0	.39
D16S515	90.2	1.8	.0022	.0	.5	1.1	.014
D16S516	98.3	.8	.028	.4	.095	1.1	.012
D16S422	109.1	1.4	.0049	1.9	.0015	3.3	.000044
D16S289	122.1	2.7	.0002	...	...	2.7	.0002
17:							
D17S831	6.6	.0	.32	1.2	.0093	1.0	.014
19:							
D19S226	41.7	.3	.14	1.2	.009	1.3	.0065
D19S414	53.2	.0	.5	2.5	.00037	.7	.042
D19S220	61.4	.0	.48	1.8	.0021	1.1	.013
D19S420	66	.0	.44	3.58	.000025	2.0	.0012
D19S902	76.2	.4	.096	1.7	.0025	1.9	.0016
D19S571	87.7	.3	.13	1.4	.0058	1.4	.0057
21:							
D21S266	49.9	1.1	.011	.4	.092	1.4	.0052

NOTE.—Markers scoring LOD ≥1.0 in any screen are presented. Results achieving "significant" linkage (LOD ≥3.6) are indicated in boldface italics, and those achieving "suggestive" linkage (LOD ≥2.2 and <3.6) are underlined.

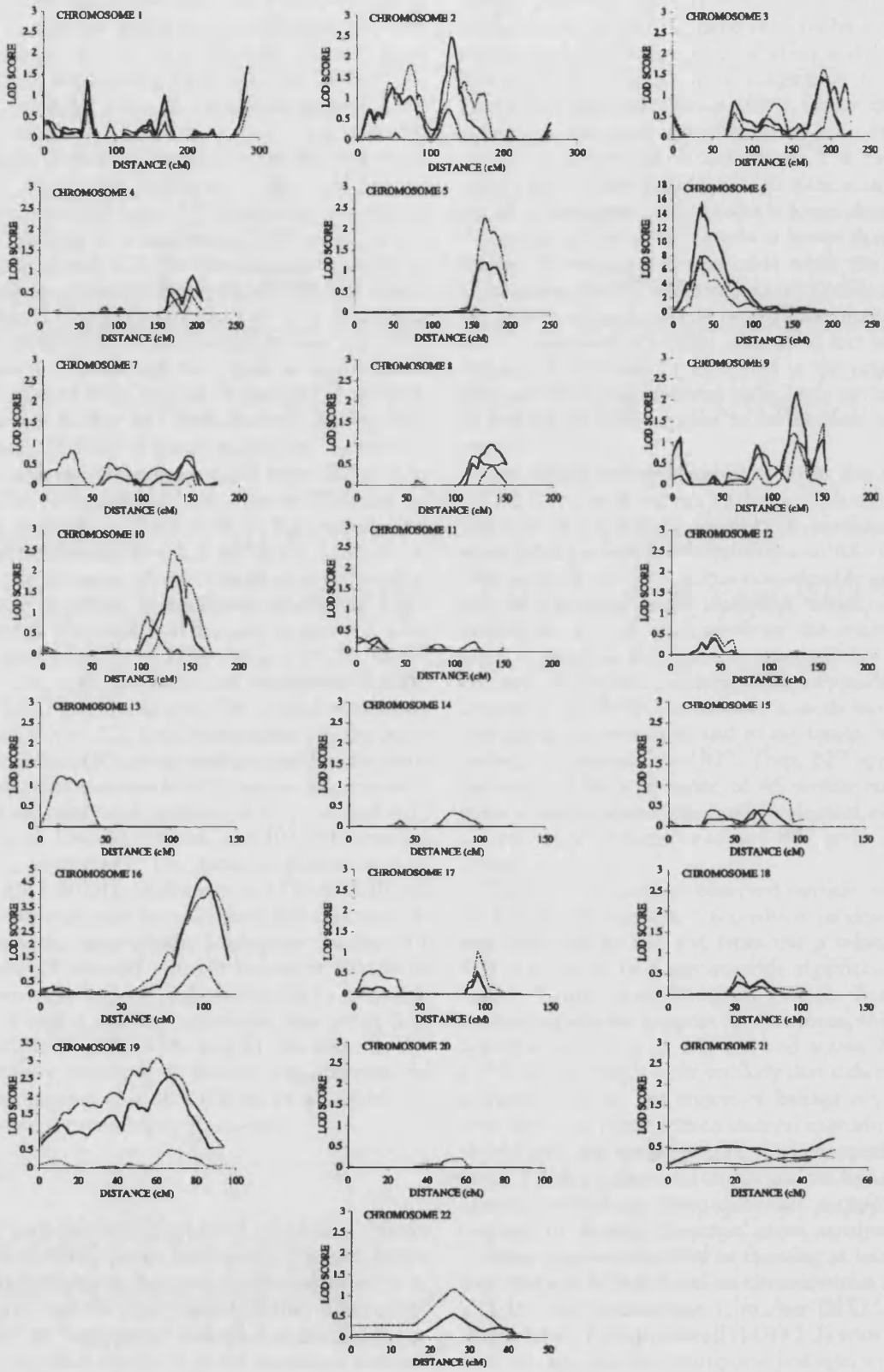


Figure 1 Multipoint results for screens 1 and 2 and the combined data set using the program GENEHUNTER PLUS. Screen 1 results are given with a dotted line, screen 2 with a dashed and dotted line, and the combined results with an unbroken line.

from the p telomere. The IL-1/IL-1 RA complex is encoded in this region, and association studies of the IL-1 RA gene previously have reported association of alleles of an IL-1 RA VNTR with AS (McGarry et al. 2000; Van der Paardt et al. 2000). On chromosomes 9q and 10q, there were substantial differences in maximum points of linkage for screens 1 and 2. On chromosome 9q, although the maximum points of linkage by multipoint analysis were similar for screens 1 and 2, by two-point analysis there were substantial differences. In screen 1, marker D9S1826 achieved significant linkage (LOD 3.9; position 160.2 cM), with no support in screen 2. In screen 2, marker D9S1682, located at 132.9 cM, achieved a LOD score of 2.1 in screen 2, but a LOD of only 0.5 in screen 1. On chromosome 10, the peak position of linkage differed in screens 1 and 2 by 25 cM. By two-point analysis, one marker lying in this region (D10S192, at 131.2 cM) had good support in both screens, achieving LOD scores of 1.1, 1.0, and 2.1 in screens 1 and 2 and in the combined data set, respectively. It is a well-recognized characteristic of linkage analyses that the maximum position of linkage may vary between genome screens (Roberts et al. 1999), and, therefore, one locus may produce the different point of maximum linkage results in this region in the two screens. Further linkage mapping on chromosomes 9 and 10 may help define better the true regions of linkage in the different data sets. The region on chromosome 19 that achieved "suggestive" linkage in screen 1 (marker D19S420; LOD 3.58) achieved only minor support in screen 2 (peak multipoint LOD score 0.5), and further studies will be required to confirm this finding.

Replication of linkages in complex genetic diseases has often proved unreliable, resulting in confusion as to which linkages are true- or false-positive findings. Reasons for this include use of insufficiently stringent thresholds for identification of loci, genetic heterogeneity, and inadequate power of replication studies. To achieve 80% power to detect a locus of magnitude $\lambda = 1.8$ in a complex genetic disease (equivalent to the magnitude of the chromosome 16q locus in this screen), even at a low significance threshold of LOD 1.0, will require studying 200 affected sibling pairs (with both parents available for genotyping, using a 10-cM marker map). The low power of linkage studies to replicate small genetic effects must be considered in comparisons between the results of future genetic studies and those presented here.

No other genomewide scans have been reported in AS, but scans have been completed in both psoriasis and inflammatory bowel disease, which are clinically related to AS. AS frequently complicates inflammatory bowel disease, and subclinical bowel inflammation is present in the majority of AS cases (Mielants et al. 1991). Strong linkage of chromosome 19 similar to that observed in our screen 2 was recently identified in a genomewide screen in inflammatory bowel disease (Rioux et al. 2000). The two other main IBD linkages (IBD1, located in the pericentromeric region of chromosome 16, and IBD2, located on chromosome 12) showed no evidence of linkage with AS in this study. Genomewide linkage studies in psoriatic skin disease have identified replicated linkages on chromosomes 1cen-q21, 3q21, 4q, 6p (PSORS1, the MHC region), 17q, 19p13, and 20p (Enlund et al. 1999; Samuelsson et al. 1999; Lee et al. 2000). Of these regions, we identified strong linkage at the MHC locus and on chromosome 19, weak linkage in chromosome 1q, and no linkage on chromosomes 3q21, 4q, and 17q. The peak linkage in both psoriasis and inflammatory bowel disease lies on chromosome 19p (Lee et al. 2000; Rioux et al. 2000), but, as in our own study, the area of linkage is quite broad, and considerable overlap exists. A genomewide screen has recently been reported in families with psoriatic arthritis (Samuelsson et al. 1999). Arthritis in psoriasis is clinically heterogeneous, and only a proportion of cases suffer from a spondyloarthritis similar to AS. Nonetheless, there are some overlaps with the findings presented here. Specifically, regions on chromosomes 1q, 5q, and 15p showed evidence of linkage to both diseases, adding support to our findings.

The ultimate proof that genes other than HLA-B27 are involved in AS awaits identification of the actual genes involved. The linkage results presented in this study represent an important advance in that search. This study presents strong evidence of the involvement with AS of genes localized on chromosomes 1p, 2q, 6p, 9q, 10q, 16q, and 19q. Further linkage mapping, positional candidate, and linkage disequilibrium studies are under way to define these regions better and to identify the actual genes involved.

Acknowledgments

The authors wish to thank the members of the National Ankylosing Spondylitis Society (United Kingdom) and the study families for their support of and participation in this project. We would also like to thank the staff at the following centers who assisted in recruiting families for the study: University Hospital, Birmingham (Dr. P. Jobanputra and S. Brailsford); Addenbrookes Hospital, Cambridge (Professor H. Gaston, C. Richards); Glasgow Royal Infirmary (Professor R. Sturrock, S. Armstrong); Norfolk and Norwich Hospital (Professor D. Scott); The King's Mill Centre, Mansfield (Dr. D. Walsh); Amersham Hospital (Dr. R. Stevens); Warwick Hospital (Dr. C. Marguerie, J. Gladwin-Geoghegan); Staffordshire Rheumatology Centre (Dr. A. Hassell); Princess Margaret Hospital Swindon (Dr. E. Price); West Suffolk Hospital (Dr. A. Nicholls); and York District Hospital (Dr. M. Roman). This study was supported by the Arthritis Research Campaign (United Kingdom). S.H.L. was supported by the Oliver Bird

Fund of the Nuffield Foundation. S.B. and A.C. received additional support from the Coates Foundation Trust, and the Colonel W. W. Pilkington Charitable Trust. L.A.R. was supported by the Arthritis Society of Canada and the Medical Research Council of Canada. K.A.S. was supported by the Medical Research Council of Canada.

Electronic-Database Information

The accession number and URLs for data in this article are as follows:

GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap99/ (for marker positions)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for AS [MIM 106300])
Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu/ (for marker positions)

References

Beyeler C, Armstrong M, Bird HA, Idle JR, Daly AK (1996) Relationship between genotype for the cytochrome P450 CYP2D6 and susceptibility to ankylosing spondylitis and rheumatoid arthritis. Ann Rheum Dis 55:66–68

Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD (1973) Ankylosing spondylitis and HL-A 27. Lancet 1:904–907

Brown MA, Edwards S, Hoyle E, Campbell S, Laval S, Daly AK, Pile KD, Calin A, Ebringer A, Weeks DE, Wordsworth BP (2000*a*) Polymorphisms of the CYP2D6 gene increase susceptibility to ankylosing spondylitis. Hum Mol Genet 9: 1563–1566

Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, Taylor A, Calin A, Wordsworth P (1997) Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 40: 1823–1828

Brown MA, Laval SH, Brophy S, Calin A (2000*b*) Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. Ann Rheum Dis 59:883–886

Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J, Wordsworth BP, Cornelis F (1996) HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 55:268–270

Brown MA, Pile KD, Kennedy LG, Campbell D, Andrew L, March R, Shatford JL, Weeks DE, Calin A, Wordsworth BP (1998) A genome-wide screen for susceptibility loci in ankylosing spondylitis. Arthritis Rheum 41:588–595

Calin A (1998) Ankylosing spondylitis. In: Maddison PJ, Isenberg DA, Woo P, Glass DN (eds) Oxford textbook of rheumatology, vol. 2. Oxford, Oxford University Press, pp 1058–1070

Calin A, Marder A, Becks E, Burns T (1983) Genetic differences between B27 positive patients with ankylosing spondylitis and B27 positive healthy controls. Arthritis Rheum 26:1460–1464

Cordell HJ, Olson JM (1997) Confidence intervals for relative risk estimates from affected-sib-pair data. Genet Epidemiol 14:593–598

de Blecourt J, Polman A, de Blecourt-Meindersma T (1961) Hereditary factors in rheumatoid arthritis and ankylosing spondylitis. Ann Rheum Dis 20:215–223

Deshayes P, Demangeon S, Cavelier B, Le Loet X, Daragon A, Ropartz C (1987) Genetic study of a family of subjects with ankylosing spondylitis. Rev Rhum Mal Osteoartic 54: 171–174

Douglas JA, Boehnke M, Lange K (2000) A multipoint method for detecting genotyping errors and mutations in sibling-pair linkage data. Am J Hum Genet 66:1287–1297

Ehm M, Wagner M (1998) A test statistic to detect errors in sib-pair relationships. Am J Hum Genet 62:181–188

Enlund F, Samuelsson L, Enerback C, Inerot A, Wahlstrom J, Yhr M, Torinsson A, Riley J, Swanbeck G, Martinsson T (1999) Psoriasis susceptibility locus in chromosome region 3q21 identified in patients from southwest Sweden. Eur J Hum Genet 7:783–790

Gladman DD, Urowitz MB, Anhorn KA, Chalmers A, Mervart H (1986) Discordance between HLA-B27 and ankylosing spondylitis: a family investigation. J Rheumatol 13:129–136

Hohler T, Schaper T, Schneider PM, Meyer zum Buschenfelde KH, Marker-Hermann E (1998) Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. Arthritis Rheum 41:1489–1492

Hoyle E, Laval SH, Calin A, Wordsworth BP, Brown MA (2000) The X-chromosome and susceptibility to ankylosing spondylitis. Arthritis Rheum 43:1353–1355

Hsieh S-L, March RE, Khanna A, Cross SJ, Campbell RD (1997) Mapping of 10 novel microsatellites in the MHC class III region: application to the study of autoimmune disease. J Rheumatol 24:220–222

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Laval SH, Bradbury L, Darke C, Brophy S, Calin A, Brown MA (2000) The role of HLA-DR genes in ankylosing spondylitis complicating inflammatory bowel disease. Rheumatology 39:64

Lee Y-A, Rüschendorf F, Windemuth C, Schmitt-Egenolf M, Stadelmann A, Nürnberg G, Ständer M, Wienker TF, Reis A, Traupe H (2000) Genomewide scan in German families reveals evidence for a novel psoriasis-susceptibility locus on chromosome 19p13. Am J Hum Genet 67:1020–1024

McGarry F, Neilly J, Sturrock R, Field M (2000) A polymorphism within the interleukin 1 receptor antagonist (IL-1RA) gene is associated with ankylosing spondylitis (AS). Rheumatology 39:63

Mielants H, Veys EM, Goemaere S, Goethals K, Cuvelier C, De VM (1991) Gut inflammation in the spondyloarthropathies: clinical, radiologic, biologic and genetic features in relation to the type of histology: a prospective study. J Rheumatol 18:1542–1551

Norman RA, Tataranni PA, Pratley R, Thompson DB, Hanson RL, Prochazka M, Baier L, Ehm MG, Sakul H, Foroud T, Garvey WT, Burns D, Knowler WC, Bennett PH, Bogardus C, Ravussin E (1998) Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. Am J Hum Genet 62:659–668

O'Connell JR, Weeks DE (1998) PedCheck: a program for

identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266

Pratt SC, Daly MJ, Kruglyak L (2000) Exact multipoint quantitative-trait linkage analysis in pedigrees by variance components. Am J Hum Genet 66:1153–1157

Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SC, Kawaguchi Y, Cordell HJ, Balfour KM, et al. (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA (2000) Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. Am J Hum Genet 66: 1863–1870

Risch N (1987) Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 40:1–14

Risch N (1990) Linkage strategies for genetically complex traits. II. The power of affected relative pairs. Am J Hum Genet 46:229–241

Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS (1999) Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65:876–884

Rubin LA, Amos CI, Wade JA, Martin JR, Bale SJ, Little AH, Gladman DD, Bonney GE, Rubenstein JD, Siminovitch KA (1994) Investigating the genetic basis for ankylosing spondylitis: linkage studies with the major histocompatibility complex region. Arthritis Rheum 37:1212–1220

Said-Nahal R, Miceli-Richard C, Berthelot JM, Duche A, Dernis-Labous E, Le Blevec G, Saraux A, Perdriger A, Guis S, Claudepierre P, Sibilia J, Amor B, Dougados M, Breban M (2000) The familial form of spondylarthropathy: a clinical study of 115 multiplex families. Arthritis Rheum 43: 1356–1365

Samuelsson L, Enlund F, Torinsson A, Yhr M, Inerot A, Enerback C, Wahlstrom J, Swanbeck G, Martinsson T (1999) A genome-wide search for genes predisposing to familial psoriasis by using a stratification approach. Hum Genet 105: 523–529

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger J, Lathrop G, Bell J, Jewell D (1996) Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7, and 12. Nat Genet 14:199–202

Schlosstein L, Terasaki PI, Bluestone R, Pearson C (1973) High association of an HL-A antigen W27, with ankylosing spondylitis. N Engl J Med 288:704–705

Skomsvoll JF, Ostensen M, Romberg O, Anda S (1995) HLA-B27 negative ankylosing spondylitis in a father and a son. Scand J Rheumatol 24:321–322

Sobel E, Lange K (1996) Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 58:1323–1337

van der Linden J, Cats A, Keunig J, Van Rood J, Wuisman J (1975) HL-A 27 and ankylosing spondylitis. Ann Rheum Dis Suppl 34:53

van der Linden S, Valkenburg H, Cats A (1983) The risk of developing ankylosing spondylitis in HLA-B27 positive individuals: a family and population study. Br J Rheumatol 22:18–19

van der Linden S, Valkenburg HA, Cats A (1984) Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 27:361–368

Van der Paardt M, Crusius B, Van der Horst-Bruinsma I, Garcia-Gonzalez M, Baudoin P, Kostense P, Dijkmans B, Pena A (2000) Interleukin-1B and Interleukin-1 receptor antagonist gene polymorphism in ankylosing spondylitis. Arthritis Rheum 43:S264

Woodrow JC (1988) Genetics of the spondarthropathies. Baillieres Clin Rheumatol 2:603–622

Appendix 11

Exercise in Ankylosing Spondylitis: How Much Is Optimum?

HELENA SANTOS, SINEAD BROPHY, and ANDREI CALIN

ABSTRACT. *Objective.* To evaluate how much exercise patients with ankylosing spondylitis perform on a regular basis, to analyze demographic and clinical variables that might influence adherence to exercise, and to determine the effect of exercise on disease activity and function.

Methods. We analyzed 4282 patients who completed a self-administered questionnaire regarding physical exercise. To determine the relationship between exercise, demographic data, and clinical variables, patients who exercise 2 to 4 hours and 10 or more hours per week were compared to nonexercising controls matched for age, sex, and disease duration.

Results. Most of the patients reported 2 to 4 hours of exercise or no exercise. The group who performed moderate exercise had improved function and lower disease activity ($p < 0.001$, $p < 0.015$, respectively). The group who performed intensive exercise had improved function, but no difference in disease activity was found ($p = 0.033$, $p = 0.394$, respectively). Adherence to a regular exercise regime is associated with rheumatologist followup, beliefs in the benefits of exercise, and a higher education level.

Conclusion. This study suggests there is an optimum duration for exercise performed over a weekly period. Consistency, rather than quantity, is of most importance. Individuals most likely to follow this regime attend a rheumatologist, believe that exercise is of benefit, and are in the higher education category. Those who are less educated and followed by general practitioners should be targeted. (J Rheumatol 1998;25:2156–60)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS EXERCISE ADHERENCE
FUNCTION DISEASE ACTIVITY

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disorder mainly affecting the axial skeleton and the peripheral joints. For the individual, the natural history of the disease is poorly defined. Some have minimal symptoms with only pelvic involvement, while others have widespread disease that results in poor functional outcome[1].

There is no cure for the disorder. Moreover, we are uncertain whether the longterm course of the disease can be altered but, despite this, most patients can be adequately managed[2-4].

Drug therapy for symptom control in patients with AS has largely been confined to the use of nonsteroidal antiinflammatory drugs (NSAID) and second line agents like sulfasalazine, which have some activity on peripheral joints but less effect on the spine itself. Although drug therapy with NSAID can be effective, the cornerstone of medical management is therapeutic exercise with the prime objective of maintaining normal posture and activity. This is now an essential part of a patient's treatment[1].

From the Epidemiology Department, Royal National Hospital for Rheumatic Diseases, Bath, UK.

Supported by the National Ankylosing Spondylitis Society, the John Coates Charitable Trust, and the Col. W.W. Pilkington Trust.

H. Santos, MD, Visiting Research Fellow; S. Brophy, BSc, Research Assistant; A. Calin, MD, FRCP, Consultant Rheumatologist.

Address reprint requests to Dr. A. Calin, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL, UK.

Submitted December 23, 1997 revision accepted May 19, 1998.

Many kinds of physical therapy can be considered fo patients with AS: supervised individual therapy, unsuper vised self-administered individual exercise, and supervise group therapy[5]. Short term improvement, following inpa tient and outpatient treatment regimes, has been observed i a number of centers[6-16]. There remains some doubt whethe this improvement is sustained in the longer term. In add tion, there is the bias that the act of followup may create th Hawthorne effect[17], encouraging patients to do more exe cise.

Our aim was to evaluate how much exercise patients pe form on a regular basis, to analyze demographic and clinic variables that might influence adherence to exercise, and assess the effect of exercise on disease activity and functio

MATERIALS AND METHODS

The database. The Bath Ankylosing Spondylitis Data Base consisted 4365 subjects. These were outpatients referred to the Royal Natio Hospital for Rheumatic Diseases or members of the National Ankylosi Spondylitis Society. The diagnosis of AS has been substantiated in a se rate study[18].

Study population. Altogether, 4282 patients (M:F = 3057:1221; dise duration = 21.3 ± 0.2 years) completed a self-administered questionna regarding physical exercise. Patients were asked "For how many hours week on average have you taken part in sports, AS exercises or hydroth apy during the last three months?" Patients were given 5 options: 0 ho (Group 0), 1 hour (Group I), 2-4 hours (Group II), 5-9 hours (Group I 10 or more hours (Group IV). Those who did not answer the question w excluded from the study (n = 83). The time delay between diagnosis

start of exercising was very varied and ranged from immediately at time of diagnosis (for some younger patients), to not following any form of exercise after 50 years of disease.

Data processing and statistical methods. To determine the relationship between physical exercise, demographic data, and clinical variables, patients who exercised 2 to 4 hours and 10 hours were compared to the nonexercising group. Group II (2 to 4 hours exercise) was matched for sex, age, and disease duration with Group 0 (n = 553). Group IV (10 or more hours exercise) was matched for sex, age, and disease duration with Group 0 (n = 272). The matched pairs were analyzed for demographic and clinical status. This included marital status, employment status, education level, medical followup (rheumatologist or general practitioner), delay in diagnosis, medication/no medication, and family history of AS. Beliefs in the benefits of exercise were assessed by asking the patients to record on a separate 10 cm visual analog scale (VAS) the effectiveness that exercise has on relieving their symptoms. Clinical status was assessed by self-administered validated instruments: (1) the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) consists of 6 VAS (each 10 cm) referring to symptoms of AS related to the previous week: fatigue, spinal pain, peripheral joint pain or swelling, tender areas, and morning stiffness (quantity and "quality"). A mean of the 2 stiffness VAS are added to the other 4 values and the overall mean represents the final BASDAI score[19]; (2) the Bath Ankylosing Spondylitis Functional Index (BASFI) consists of 10 questions assessing functional ability related to the previous week and recorded on a 10 cm VAS. A final score is obtained by calculating the mean of the 10 responses[20]; (3) the Bath Ankylosing Spondylitis Global Score is a single item question regarding a patient's sense of well being over the last week and the last 6 months, also recorded on a 10 cm VAS[21].

Statistical analysis was performed using the SPSS package. Paired t tests or McNemar's chi-squared tests were used in analysis.

RESULTS

In total, 915 (21%) patients were in Group 0, 836 (20%) were in Group I, 1491 (35%) were in Group II, 647 (15%) were in Group III, and 393 (9%) patients were in Group IV (see Figure 1). There were proportionally more men in the higher exercise groups and more women in the lower exercise groups.

Group II (2 to 4 hours exercise). Group II [M:F = 408:145 (ratio 2.8:1)] compared to matched controls (n = 553 pairs) had improved function and lower disease activity ($p < 0.001$, $p < 0.015$, respectively; Table 1). There was no difference in the global well being between the 2 groups.

Delay in diagnosis, family history, and marital and employment status were comparable (Table 2). A significantly greater number of patients from Group II were followed by a rheumatologist ($p < 0.001$), had a higher education level ($p = 0.005$), and took more medication ($p < 0.001$). Controlling for medication, disease activity and function were still significantly better for group II ($p = 0.017$, $p = 0.001$, respectively).

Group IV (10 or more hours exercise). Group IV [M:F = 220:52 (ratio 4.2:1)] had improved functional status compared to matched controls ($p = 0.033$; Table 3). The delay in diagnosis was significantly greater in Group IV ($p = 0.003$), as was the number of nonmarried individuals ($p < 0.04$) and the number of patients followed by a rheumatologist ($p < 0.001$; Table 4).

Employment, education level, family history, medication, disease activity, and global well being were comparable.

DISCUSSION

We assessed physical exercise of patients with AS and explored the effect in terms of disease activity, function, and

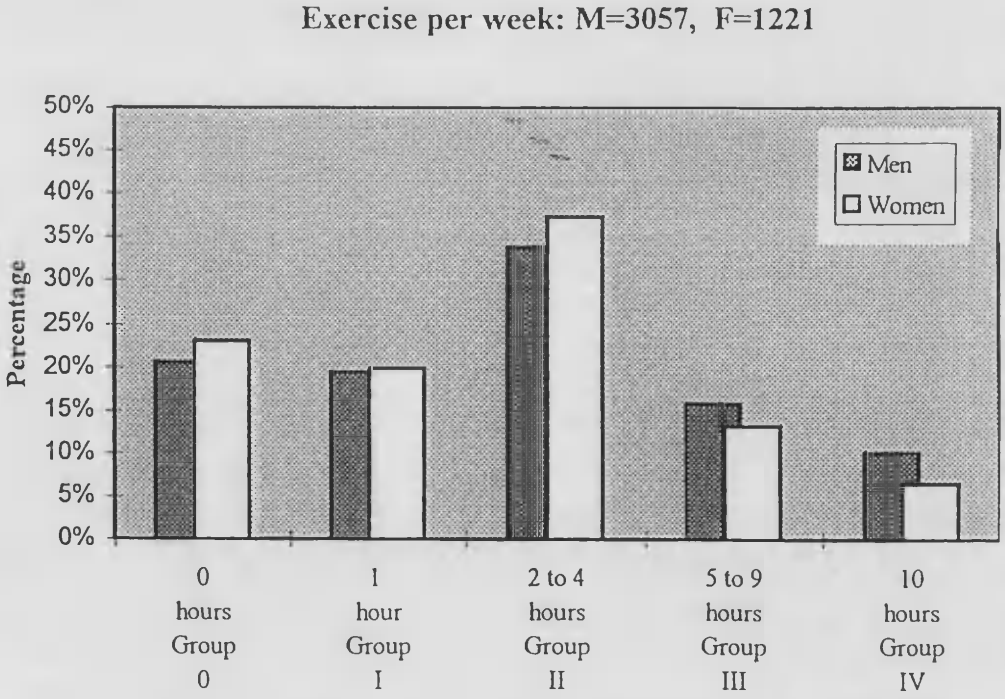


Figure 1. Distribution of the number of hours of exercise per week in men and women (%).

Table 1. Comparison of self-assessment outcome measures between Group 0 and Group II measured on a 0–10 scale.

	Group II	Group 0	p	Mean differnce ± SEM (95% CI)
Total, n	1460	893		
Study group, n	553*	553*		
BASDAI	3.8 ± 0.09	4.2 ± 0.1	0.015	0.33 ± 0.13 (0.2–0.6)
BASFI	3.9 ± 0.1	4.5 ± 0.12	< 0.001	0.59 ± 0.16 (0.3–0.9)
BAS-G (1 week)	4.2 ± 0.17	4.3 ± 0.12	0.686	0.08 ± 0.21 (–0.3–0.4)
BAS-G (6 months)	4.8 ± 0.12	4.8 ± 0.13	0.880	0.004 ± 0.18 (–0.3–0.3)

Group 0: no exercise; Group II: 2 to 4 h of exercise per week.
*Matched for current age, sex, and disease duration.

Table 2. Comparison of demographic and clinical variables between Group II and Group 0.

	Group II	Group 0	p
Total, n	1460	893	
Study group, n	553*	553*	
Current age, yrs, mean ± SD	47.3 ± 11.6	47.2 ± 11.6	0.150
Age at onset, yrs, mean ± SD	23.8 ± 7.9	23.6 ± 7.9	0.111
Duration of disease, yrs, mean ± SD	23.0 ± 11.9	23.0 ± 11.9	0.157
Delay in diagnosis, yrs, mean ± SD	9.6 ± 9.7	8.8 ± 9.6	0.141
Married, %	71	67	0.200
Employed, %	60	60	0.725
High education level, %	42	27	0.005
Rheumatologist care, %	93	86	< 0.001
Family history of AS, %	25	29	0.222
Co-medication, %	82	73	0.001
Efficacy of exercise, mean ± SD	5.2 ± 2.4	3.4 ± 3.0	< 0.001

Group 0: no exercise; Group II: 2 to 4 h of exercise per week.
*Matched for current age, sex, and disease duration.

Table 3. Comparison of self-assessment outcome measures between Group 0 and Group IV measured on a 0–10 scale.

	Group IV	Group 0	p	Mean difference ± SEM (95% CI)
Total, n	381	893		
Study group, n	272*	272*		
BASDAI	4.3 ± 0.13	4.1 ± 0.14	0.394	0.18 ± 0.2 (–0.6–0.2)
BASFI	4.0 ± 0.17	4.5 ± 0.17	0.033	0.56 ± 0.2 (0.024–1.0)
BAS-G (1 w)	4.7 ± 0.18	4.3 ± 0.2	0.148	0.44 ± 0.3 (–0.13–1.0)
BAS-G (6 m)	5.1 ± 2.6	4.8 ± 2.9	0.284	0.34 ± 0.28 (–0.2–0.9)

Group 0: no exercise; Group IV: 10 or more h of exercise per week.
*Matched for current age, sex, and disease duration.

Table 4. Comparison of demographic and clinical variables between Group IV and Group 0.

	Group IV	Group 0	p
Total, n	381	893	
Study group, n	272*	272*	
Current age, yrs, mean ± SD	46.4 ± 12.6	46.4 ± 12.6	1.00
Age at onset, yrs, mean ± SD	24.4 ± 8.5	24.1 ± 8.5	0.422
Duration of disease, yrs, mean ± SD	22.0 ± 12.9	22.0 ± 12.9	0.706
Delay in diagnosis, yrs, mean ± SD	9.6 ± 10.1	7.4 ± 7.8	0.003
Married, %	64	70	0.040
Employed, %	52	59	0.118
High education level, %	32	31	0.389
Rheumatologist care, %	92	84	0.001
Family history of AS, %	24	24	1.00
Co-medication, %	77	71	0.137
Efficacy of exercise, mean ± SD	6.8 ± 2.3	3.4 ± 3.2	< 0.001

Group 0: no exercise; Group IV: 10 or more h of exercise per week.
*Matched for current age, sex, and disease duration.

well being. We also tried to identify factors associated with adherence to longterm exercise. The response rate was excellent in this study. However, we do recognize that the patients were from a highly motivated group and many were members of the national self-help group, the National Ankylosing Spondylitis Society.

One potential defect of the study is the cross sectional design allowing assessment at only one point. As with all studies of this design it is impossible to show a specific group has improved in any aspect.

Our definition of exercise was very general, including sports, specific AS exercises, and hydrotherapy. However, the accuracy of the self-reported exercise frequency is hard to judge, and the intensity is unknown. The relative value of different forms of exercise cannot be determined. Some of the patients reporting no exercise would have performed regular housework, walked short distances, and helped with all the usual household tasks. All these activities, although not strictly classified as formal exercise, are in themselves forms of exercise and patients would in fact be stretching, bending, and lifting. However, all the groups would be performing these daily activities.

Most of the subjects reported either 2 to 4 hours of exercise per week (35%) or no exercise (21%). There were more men in Group IV and predominantly more women in Group 0 (Figure 1). Most of the research relating to exercise in AS has been carried out in Europe using inpatient programs of 2–4 weeks[2,8,10,12-16]. The majority of these studies did not include functional measurements. However, they reported improvement in metrology[8-10,13-16] and short term benefits in symptom control[7]. Those that used functional measures reported that after the inpatient programs those who continue exercising had improved function[7,22].

We found that individuals who do 2–4 hours of exercise a week have lower disease activity and improved function in comparison to nonexercising patients. This may imply that exercise improves disease activity and function or it may show that patients with lower disease activity and good function are able to do more exercise. It may be that the amount of time spent exercising during a weekly period can be used as a rough indicator for disease activity. The study also found that patients who performed intensive daily exercise had improved function, but the disease activity did not improve. This suggests an optimum exercise duration, with most benefit being derived from moderate consistent exercise. However, further study would be needed to investigate which comes first, whether the improved disease activity means there is less discomfort and pain when moving, therefore improving function. Or exercise improves function first, and from this, disease activity is improved. If the former is true then better medical control of the disease may enhance exercise adherence.

Exercise has been reported to improve well being[23]. However, this study did not find an improvement in the patients' global well being with increased exercise. This was possibly a reflection of a difference in study design. Previous studies used an intensive course and assessed well being post treatment[12]. Therefore there is a point of intervention against which patients can be assessed to improve or deteriorate. This study, on the other hand, asked patients for their subjective opinion of their day to day well being. Patients who exercise regularly will have a better function, but they may not report any improvement or change in global well being.

It has been suggested that compliance to exercise may be influenced by unmarried status[24]. This study did not find marital status to influence regular moderate exercise; however, the intensive exercise group were generally unmarried.

In our study, employment status and family history of AS did not influence adherence to exercise.

Adherence with a regular exercise regimen is associated with rheumatologist followup, beliefs in the benefits of

exercise, and a higher education level (degree, diploma, higher national diploma).

Rheumatologists may be more aware than general practitioners of the benefits of exercise and therefore encourage and motivate the patient in the importance of regular moderate exercise.

Patients who practice regular exercise had strong belief in its efficacy. This finding is supported by studies that suggest beliefs in the benefits of exercise would differentiate exercisers from nonexercisers[24].

Patients who performed moderate exercise had a higher education level. It has been suggested that higher occupational status, which is associated with higher education, correlates with lower disease severity. Education may have an effect on an individual's sense of self-efficacy, encouraging the patients to play a more active role in the control and the treatment of their disease by exercising, rather than relying purely on medical intervention[25].

This study suggests that moderate, regular exercise may be beneficial for both functional status and disease activity. However, intensive longterm exercise may not be as valuable for function or disease activity. Therefore there may be an optimum duration for exercise over the period of a week; however, this study does not attempt to identify where this optimum level lies. It appears that consistency, not quantity, is of most importance. Those individuals most likely to follow this regime attend a rheumatologist, believe that exercise is of benefit, and are in the more highly educated category. Therefore, the less educated and those followed by general practitioners should be preferentially targeted.

REFERENCES

1. Calin A. Ankylosing spondylitis and its management. Rheumatol Pract 1994;1:14-6.
2. Koh W-H, Pande I, Samuels A, Jones SD, Calin A. Low dose amitriptyline in ankylosing spondylitis: A short term, double blind, placebo controlled study. J Rheumatol 1997;24:2158-61.
3. Calin A. Can we define the outcome of ankylosing spondylitis and the effect of physiotherapy management [editorial]. J Rheumatol 1994;21:184-5.
4. Bakker C, Hidding A, Van der Linden S, Van Doorslaer E. Cost effectiveness of group physical therapy compared to individualized therapy for ankylosing spondylitis. A randomized controlled trial. J Rheumatol 1994;21:264-8.
5. Gall V. Exercise in the spondyloarthropathies. Arthritis Care Res 1994;7:215-20.
6. Hidding A, Van der Linden S, De Witte L. Therapeutic effects of individual physical therapy in ankylosing spondylitis related to duration of disease. Clin Rheumatol 1993;12:334-40.
7. Kraag G, Stokes B, Groh J, Helewa A, Goldsmith C. The effect of comprehensive home physiotherapy and supervision on patients with ankylosing spondylitis - A randomized controlled trial. J Rheumatol 1990;17:228-33.
8. Helliwell PS, Abbott CA, Chamberlain MA. A randomised trial of three different physiotherapy regimes in ankylosing spondylitis. Physiotherapy 1996;82:85-90.
9. Russell P, Unsworth A, Haslock I. The effect of exercise on ankylosing spondylitis - A preliminary study. Br J Rheumatol 1993;32:498-506.
10. Viitanen JV, Lehtinen K, Suni J, Kautiainen H. Fifteen months' follow-up of intensive inpatient physiotherapy and exercise in ankylosing spondylitis. Clin Rheumatol 1995;14:413-9.
11. Kraag G, Stokes B, Groh J, Helewa A, Goldsmith CH. The effect of comprehensive home physiotherapy and supervision on patients with ankylosing spondylitis - An 8-month followup. J Rheumatol 1994;21:261-3.
12. Band D, Jones SD, Kennedy LG, et al. Which patients with ankylosing spondylitis derive most benefit from an inpatient management program? J Rheumatol 1997;24:2381-4.
13. Pearcy MJ, Wordsworth BP, Portek I, Mowat AG. Spinal movements in ankylosing spondylitis and the effect of treatment. Spine 1985;10:472-4.
14. Tomlinson MJ, Barefoot J, Dixon AJ. Intensive in-patient physiotherapy courses improve movement and posture in ankylosing spondylitis. Physiotherapy 1986;75:6-9.
15. O'Driscoll SL, Jayson MIV, Baddeley H. Neck movements in ankylosing spondylitis and their responses to physiotherapy. Ann Rheum Dis 1978;37:64-6.
16. Viitanen JV, Suni J, Kautiainen H, Liimatainen M, Takala H. Effect of physiotherapy on spinal mobility in ankylosing spondylitis. Scand J Rheumatol 1992;21:38-41.
17. Roethlisberger FJ, Dickson WJ. Management and the worker. Cambridge, MA: Harvard University Press; 1939.
18. Kennedy LG, Edmunds L, Calin A. The natural history of ankylosing spondylitis. Does it burn out? J Rheumatol 1993; 20:688-92.
19. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: The Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286-91.
20. Calin A, Garrett S, Whitelock H, et al. A new approach to functional ability in ankylosing spondylitis: The development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281-5.
21. Jones SD, Steiner A, Garrett SL, Calin A. The Bath Ankylosing Spondylitis Global score (BAS-G). Br J Rheumatol 1996;35:66-71.
22. Hidding A, Van der Linden S, Gielen X, de Wite L, Dijkmans B, Moolenburgh D. Continuation of group therapy is necessary in ankylosing spondylitis. Arthritis Care Res 1994;7:90-6.
23. Stenström CH. Therapeutic exercise in rheumatoid arthritis. Arthritis Care Res 1994;7:190-7.
24. Stenström CH, Arge B, Sundbom A. Home exercise and compliance in inflammatory rheumatic diseases - A prospective clinical trial. J Rheumatol 1997;24:470-6.
25. Roussou E, Kennedy LG, Garrett S, Calin A. Socioeconomic status in ankylosing spondylitis: Relationship between occupation and disease activity. J Rheumatol 1997;24:908-11.

Appendix 12 : Submitted to Journal of Rheumatology

Predictors of Long-term Outcome in Ankylosing Spondylitis

Michele Doran, Sinead Brophy, Kirsten MacKay, Gordon Taylor, Andrei Calin.

ABSTRACT

Objective: To determine predictors of long-term outcome in ankylosing spondylitis (AS).

Methods: Data were collected on constitutional and environmental factors that may predict outcome in AS in 311 patients (252 (81%) male). Univariate statistics and multivariable regression analysis were used to identify factors correlated with disease outcome, which was defined in terms of radiological (Bath Ankylosing Spondylitis Radiology Index (BASRI)) and functional status (Bath Ankylosing Spondylitis Functional Index (BASFI)).

Results: Disease duration, sex and iritis are independently associated with BASRI and account for 23% ($p<0.001$) of variation in radiological scores (BASRI-t), a measure which includes the hip joint in the score. Radiological hip involvement is significantly associated with higher scores of spinal radiological change (BASRI-s) ($p<0.001$). Cigarette smoking, radiological status and Bath Ankylosing Spondylitis Disease Activity Score (BASDAI) are independently associated with and account for 50% of variability in functional status ($p<0.001$).

Conclusions: Much of the variability in disease severity in AS remains unexplained. Furthermore, all but one of the factors associated with outcome in this study are inherent. This suggests that genetic factors have a greater influence than environmental factors on radiological progression and disability in AS. It may, however, be possible

to improve long-term functional outcome in AS by targeting of high-risk individuals early in the disease course with more aggressive management strategies and encouraging smoking cessation in all AS patients.

Key indexing terms: ankylosing spondylitis; outcome; radiology; function

Appendix 13 : In Press. Journal of Rheumatology

Birth Order and Ankylosing spondylitis : no increased risk of developing ankylosing spondylitis among first-born children.

SINEAD BROPHY[1], GORDON TAYLOR[1+2], ANDREI CALIN[1]

Abstract *: Objective* In the HLA-B27 transgenic mouse model the first litters have been shown to have a higher percentage of diseased offspring than later litters. In addition, first born children (n=162) have also been shown to have a higher risk of AS than later born children. Our study aims to examine this effect of birth order using similar methods but larger numbers.

Methods: Patients from the Bath AS database [n=4517 (M:F 2.5:1)] were examined according to position of birth within the family unit. Chi-squared analysis was used to examine if AS was more prevalent among first born children than later born children.

Results : The first born child was not significantly more likely to suffer from AS than later born children (p=0.295). [Observed compared to expected : 1607 (36%) compared to 1641.13 (36%) for first born children and 2910 (64%) compared to 2876.3 (64%) for later born children, respectively]. There was no biological gradient (ie inverse correlation between birth order and disease risk).

Conclusion : There was no statistically significant effect of birth order based on our data. Findings suggesting a birth order effect may be skewed as it is possible that those parents who do have AS will be less likely to have a large family and yet it is their offspring who will be at greatest risk of developing disease. This will affect the data as those children born into a large family (ie high birth order children) will be at a lower risk of AS than any child born into a small but family history positive unit.

Key words : Birth order, ankylosing spondylitis.

1 Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL.

2. School of Postgraduate Medicine, University of Bath, Bath. BA2 7AY.

Sinead Brophy BSc PhD, Research Assistant,

Gordon Taylor BSc, PhD. Biostatistician.

Andrei Calin MD FRCP, Consultant Rheumatologist.

Work attributed to Epidemiology Department, Royal National Hospital for Rheumatic Diseases.

Address reprint requests to : Andrei Calin, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath. BA1 1RL, UK. Tel : 01225 465941. E-mail : andrei.calin@virgin.net.

Supported by grants from the Arthritis and Rheumatism Council, National Ankylosing Spondylitis Society, John Coates Charitable Trust and Col. WW Pilkington Trust.

INTRODUCTION:

Ankylosing spondylitis is a systemic disease occurring in genetically predisposed individuals exposed to specific environmental triggers. In the HLA-B27 transgenic mouse the first litters have a higher percentage of diseased offspring than later litters [1]. This could be explained either by: a) the age of the mother (older maternal age decreases transmission of disease to the next generation) or b) the birth order of the offspring (ie environmental influence) has an influence on susceptibility to disease. Previous work has shown, that in humans the maternal age does not appear have an effect on inheritance of disease [2]. (Maternal age of onset of symptoms of AS, on the other hand, does influence prevalence of AS among the children of women [3]). However, it has been shown using 162 AS patients, that the number of first born children with AS is significantly higher than would be expected. [4]. Herein, we examine this hypothesis , that birth order of the child may effect the prevalence of disease, using the similar methods on 4313 patients.

METHODS:

The Bath ankylosing spondylitis database of 5623 patients was used (M:F 2.5:1). These patients were either out-patients of the Royal National Hospital for Rheumatic Diseases (RNHRD) or were members of the National Ankylosing Spondylitis Society (NASS). Patients referred to the RNHRD (n=1874) had their diagnosis confirmed according to the New York Criteria. To estimate the validity of the diagnosis of ankylosing spondylitis among the 3749 patients recruited through NASS, several samplings were performed. First, 146 consecutive patients were invited to attend an assessment clinic. All 146 were confirmed as having ankylosing spondylitis according to the same criteria. Second, the family physicians of a further 330 NASS

members were contacted to check whether their patient's disease had been confirmed radiologically. Of these 307 (93%) had definite ankylosing spondylitis, 15 had arthritic diseases other than ankylosing spondylitis, three showed suspicious change on radiograph, and for 5 patients the primary source material was unclear. Thus, of 5623 patients on the database 264 (4.7%) individuals [ie a maximum of 7% of 3764 NASS patients] may not have had definite AS. Thirdly, the radiographs of the SI joints were requested and scored on 90 subjects, all of whom were found to have evidence of sacroliitis of grade 2 or more. Taken together, the various samplings suggest that approximately 4% (ie 23 of 566 patients sampled using the three methods) of NASS recruits may not actually have AS.

All adopted patients were removed from the study (n=54) and patients from one child families (n=1013) were not included.

χ^2 was performed on position of birth compared to family size using SPSS. Patients were grouped as first-born versus not first-born. After stratification by family size, the number of children with AS who were expected to be first-born on the basis of an equal distribution within birth order was computed.

In the first analyses only confirmed cases of AS (ie Royal National Hospital for Rheumatic Diseases patients) were analysed. Of 1874 subjects; 670 were not included due to adoption/only child/incomplete data.

In the second analysis all confirmed or possible AS (ie 4% of the sample may not have definite AS) were included. Of 5623 subjects : 1013 were single child families/ 56 incomplete data/ 37 adopted.

RESULTS :

Demographic date: The AS children, born into family sizes of 2-9 children were comparable for sex ratio and disease duration. [Table 1]. AS patients from families of seven or eight children were older than those from 2, 3, or 4 child families [50 vs 45 respectively p=0.001] and they therefore had the disease for longer. [22.5 vs 21 years respectively p=0.001] [Table 1]. Of the 4517 patients studied 741 (16.4%) had a sibling with AS.

Birth Order: Chi squared test on first born compared to later born children: When observed values of confirmed AS patients who were first born were compared to those observed for later born children, the first born child is not more likely to develop disease [p=0.9]. [Table 2].

When all patients were included (confirmed AS and not confirmed) there was still no effect of birth order [p=0.3] [Table 3]

DISCUSSION:

Taking the AS population as a whole there is no significant effect of birth order. Factors which may have an influence on birth order would obviously include abortion, miscarriage and stillbirth. Only live births are included in this analysis. It is possible that families of 4 or 5 children have more miscarriages and the children may be of a much later birth order (ie 6th or 7th) than recorded. In addition, this study did not attempt to validate the absence of AS in apparently unaffected siblings and it is possible that asymptomatic AS is present among some of the sample. However, it is unlikely to affect the results of the study unless first born child were more likely to have milder (asymptomatic) disease.

Recent research by Baudoin et al [4] shows an increased risk of AS among first born children. In 40 families with 2 children , 26 of the AS affected children were first born, whereas the number expected was 20, a surplus of 30%. This study, does not substantiate these findings. It is possible that the discrepancy between the Baudoin findings and those present here are due to the difference in numbers. The difference of 6 children in the expected compared to the observed translates to 3.7% of the total patient sample being first born instead of later born. This difference seems to disappear with larger number. In addition, there are many factors which make comparisons between families difficult. For example, we know that genetic susceptibility is required to develop AS and that women with early onset AS are more likely to pass on the disease to their children [3]. Logically, women who suffer symptoms of AS would be less likely to have a large family. Therefore, only women with no family history of AS will be likely to have families of 4-5 children. Thus, any child born into a large family is automatically at a lower risk of AS than any child born into a small family. This means that children born 4th or 5th (higher birth order) may appear to have a lower risk when compared to the population. However, when compared to first born children in a larger family they do not actually have a lower risk [Table 2 and 3].

Both this study and Baudoin et al only examined multi-sibling families. Future work may examine if AS is more prevalent in 'only' children (by definition first born, with no sibs) than expected. This could be done by estimating how many only children with HLA-B27 there are in the population (ie HLA frequency X only child frequency) and then compare the prevalence of AS in this group with the prevalence of AS in the entire HLA B27 positive population.

In summary, previous work using a mouse model and examining 162 patients suggested a birth order effect in AS. However, using similar methods on 4517 patients we were not able to substantiate these findings. Thus, there does not appear to be a birth order effect operable in AS.

REFERENCES:

1 Weinreich S, Hoebe , Ivanyi P. Maternal age influences risk for HLA-B27 associated ankylosing enthesopathy in transgenic mice. Ann Rhuem Dis 1995; 54: 754-6.

2 Raza K, Kennedy G, Calin A. Maternal age and the risk of developing ankylosing spondylitis. Ann Rheum Dis 1997; 56: 209-210.

3. Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. The Lancet 1999; 354: 1687-1690.

4. Baudoin P, van der Horst-Bruinsma E, Dekker-Saeys A, Weinreich S, Bezemer P, Dijkmans B. Increased risk of developing ankylosing spondylitis among first born children. Arthritis Rheum 2000; 43 (12): 2818-2822.

Family size

	Family of 2 (n=1785)	Family of 3 (n=1330)	Family of 4 (n=706)	Family of 5 (n=492)	Family of 6 (n=106)	Family of 7 (n=46)	Family 8 (n=32)	Family of 9 (n=14)	p value
Age of AS child (sd.)	45 (13)	45 (13)	45 (13)	47 (12)	45 (10)	51 (11)	49 (11)	45 (9)	p=0.001
Sex ratio of child	2.3 : 1	2.3 : 1	2.3 : 1	2.3 : 1	2.0:1	3.0:1	1.5:1	1.8:1	Ns
Disease duration of child (sd)	21 (14)	21 (13)	20 (13)	21 (13)	20 (11)	23 (13)	22 (13)	23(12)	Ns
Age at onset of child (sd.)	24 (11)	24 (11)	25 (11)	25 (10)	25 (9)	27 (10)	27 (10)	22 (10)	p=0.001

Table 2 : Birth order of confirmed AS individuals within the family.

	Family Size							Total	
Birth Order	2	3	4	5	6	7	8	**Observed**	**Expected**
1	215 (48%)	127 (36%)	54 (27%)	30(18%)	2 (6%)	1 (11%)	1 (20%)	**430**	**429.23**
2	232 (52%)	110 (32%)	55(28%)	27(17%)	9 (27%)	2 (22%)	0	**435**	**429.23**
3		112 (32%)	40 (20%)	32(20%)	5(15%)	2 (22%)	0	**191**	**205.73**
4			48 (24%)	41(25%)	10(30%)	1 (11%)	0	**100**	**89.43**
5				34(21%)	4 (12%)	0 (0%)	2 (40%)	**40**	**40.18**
6					3 (9)	1 (11%)	1 (20%)	**5**	**7.38**
7						2 (22%)	1 (20%	**3**	**1.88**
8							0	**0**	**0.6**
Total	447	349	197	164	33	9	5	**1204**	**1203.66**
AS child first born	215	127	54	30	2	1	1	**430 (35.7%)**	**429.23 (35.6%)**
AS child not first born	232	222	143	134	31	8	4	**774 (64.3%)**	**774.43 (64.4%)**
								$\chi^2 = 0.006$*	
								p=0.967	

* First born child compared to later born children

Table 3 : Birth order of AS individuals (confirmed and suspected) within the family.

	Family Size										Total	
Birth Order	2	3	4	5	6	7	8	9	10	11	**Observed**	**Expected**
1	865 (49%)	447 (34%)	186 (26%)	92 (19%)	7 (7%)	4 (9%)	5 (16%)	1 (7%)	0	0	**1607**	**1641.13**
2	920 (525%)	462 (35%)	176 (25%)	84 (17%)	20 (19%)	9 (20%)	1 (3%)	1 (7%)	1 (33%)	0	**1674**	**1641.13**
3		421 (32%)	190 (27%)	86 (18%)	16 (15%)	9 (20%)	5 (16%)	2 (14%)	0	1(33%)	**730**	**748.63**
4			154 (22%)	104 (21%)	26 (25%)	10 (22%)	2 (6%)	2 (14%)	0	0	**298**	**305.33**
5				126 (26%)	21 (20%)	7 (15%)	5 (16%)	0	1 (33%)	0	**160**	**128.83**
6					16 (15%)	5 (11%)	3 (9%)	1 (7%)	0	0	**25**	**30.43**
7						2 (4%)	5 (16%)	4 (29%)	1 (33%)	1 (33%)	**13**	**12.77**
8							6 (19%)	2 (14%)	0	0	**8**	**6.17**
9								1 (7%)	0	0	**1**	**2.17**
10									0	1 (33%)	**1**	**0.57**
11										0	**0**	**0.27**
Total	1785	1330	706	492	106	46	32	14	3	3	**4517**	**4517.43**
AS child first born	865	447	186	92	7	4	5	1	0	0	**1607 (36%)**	**1641.13 (36%)**
AS child not first born	920	883	520	400	99	42	27	13	3	3	**2910 (64%)**	**2876.3 (64%)**
											$\chi^2 =$ 1.09* p=0.295	

* First born child compared to later born children

Ann Rheum Dis 2000;59:883–886

Appendix 14
Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis

M A Brown, S H Laval, S Brophy, A Calin

Abstract

Objectives—It has long been suspected that susceptibility to ankylosing spondylitis (AS) is influenced by genes lying distant to the major histocompatibility complex. This study compares genetic models of AS to assess the most likely mode of inheritance, using recurrence risk ratios in relatives of affected subjects.

Methods—Recurrence risk ratios in different degrees of relatives were determined using published data from studies specifically designed to address the question. The methods of Risch were used to determine the expected recurrence risk ratios in different degrees of relatives, assuming equal first degree relative recurrence risk between models. Goodness of fit was determined by χ^2 comparison of the expected number of affected subjects with the observed number, given equal numbers of each type of relative studied.

Results—The recurrence risks in different degrees of relatives were: monozygotic (MZ) twins 63% (17/27), first degree relatives 8.2% (441/5390), second degree relatives 1.0% (8/834), and third degree relatives 0.7% (7/997). Parent-child recurrence risk (7.9%, 37/466) was not significantly different from the sibling recurrence risk (8.2%, 404/4924), excluding a significant dominance genetic component to susceptibility. Poor fitting models included single gene, genetic heterogeneity, additive, two locus multiplicative, and one locus and residual polygenes (χ^2 >32 (two degrees of freedom), $p<10^{-6}$ for all models). The best fitting model studied was a five locus model with multiplicative interaction between loci (χ^2=1.4 (two degrees of freedom), p=0.5). Oligogenic multiplicative models were the best fitting over a range of population prevalences and first degree recurrence risk rates.

Conclusions—This study suggests that of the genetic models tested, the most likely model operating in AS is an oligogenic model with predominantly multiplicative interaction between loci.

(*Ann Rheum Dis* 2000;59:883–886)

Spondyloarthritis Research Group, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, OX3 7BN, UK
M A Brown
S H Laval

Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath, BA1 1RL, UK
S Brophy
A Calin

Correspondence to:
Dr Brown
mbrown@well.ox.ac.uk

Accepted for publication
10 April 2000

The importance of genes encoded within the major histocompatibility complex (MHC) in the aetiology of ankylosing spondylitis (AS) has been well established by association[1 2] and linkage studies.[3 4] Few families have been reported in which AS has occurred in the absence of HLA-B27 (B27) or in which AS segregates separately from B27, suggesting that the inheritance of AS depends on the presence of B27.

There are considerable data suggesting that B27, though essential, is not sufficient to explain the genetic epidemiology of AS. Only a small proportion of B27 positive subjects develop AS, suggesting the presence of other susceptibility factors. Twin studies demonstrating high heritability of susceptibility to AS (97%) indicate that these factors are primarily genetic.[5] The concordance rate for B27 positive dizygotic twin pairs (24%) is considerably lower than for MZ twins (63%), suggesting the presence of other genes influencing disease susceptibility. Gene mapping studies have implicated other genes, both within the MHC (for example, HLA-B60 and HLA-DR1) and distantly encoded (CYP2D6), which are likely to have small effects on disease susceptibility.[6 7] Linkage studies in affected sibling pair families have also identified several non-MHC regions likely to contain further susceptibility genes.[3]

The recurrence risk in relatives of affected subjects (the proportion of relatives of an affected subject who develop the disease themselves) is determined by the number of genes involved, the magnitude of their individual effects, and the manner of their interaction. Risch has reported a non-parametric approach to studying different genetic models to assess likely modes of inheritance in genetic diseases employing familial recurrence risk ratios.[8] In this study Risch's approach has been used to investigate the likely genetic models operating in AS.

Methods

Recurrence risk rates in different degrees of relatives of white patients with AS were determined using the results of all studies published in peer reviewed journals. Studies were included if they had either complete ascertainment of a population or proband ascertainment with careful control against ascertainment bias. Case reports or studies without specific measures to avoid ascertainment bias were not included. Table 1 outlines the studies meeting these criteria and their findings.

The population prevalence of AS was set at 0.1% (95% confidence interval 0.03 to 0.24%) from the studies of van der Linden and colleagues.[9] This study was used because it was determined using screening methods similar in sensitivity to those employed by the recurrence risk studies. Alteration in the population prevalence directly influences the recurrence risk ratio (recurrence risk/population prevalence),

Table 1 Recurrence risk of ankylosing spondylitis in different degrees of probands of affected subjects

Study	Relationship	MZ twins	Siblings	Parent-child	First degree	Second degree	Third degree
de Blécourt[18]	Number affected	—	24	5	29	8	7
	Number studied	—	401	191	592	834	997
	Recurrence risk (%)	—	6.0	2.6	4.9	1.0	0.7
Brown[5]	Number affected	6	4	—	4	—	—
	Number studied	8	32	—	32	—	—
	Recurrence risk (%)	75	12.5	—	12.5	—	—
Calin[11]	Number affected	—	363	32	363	—	—
	Number studied	—	4298	275	4298	—	—
	Recurrence risk (%)	—	8.4	11.6	8.4	—	—
Carter[19]	Number affected	—	10	—	10	—	—
	Number studied	—	169	—	169	—	—
	Recurrence risk (%)	—	5.9	—	5.9	—	—
Emery[20]	Number affected	1	0	—	0	—	—
	Number studied	2	4	—	4	—	—
	Recurrence risk (%)	50	0	—	0	—	—
Jarvinen[21]	Number affected	3	3	—	3	—	—
	Number studied	6	20	—	20	—	—
	Recurrence risk (%)	50	15	—	15	—	—
Moesman[20]	Number affected	7	—	—	—	—	—
	Number studied	11	—	—	—	—	—
	Recurrence risk (%)	63.6	—	—	—	—	—
Total	Number affected	17	404	37	441	8	7
	Number studied	27	4924	466	5390	834	997
	Recurrence risk (%)	63.0	8.2	7.9	8.2	1.0	0.7
	(95% CI)	(42 to 81)	(7.4 to 9.0)	(5.6 to 10.7)	(7.4 to 8.9)	(0.2 to 1.7)	(0.1 to 1.4)
	Recurrence risk ratio	630	82	79	82	10	7

and different studies have reported different population prevalences. A recent study reporting a population prevalence of 1.0% used magnetic resonance imaging (MRI) scanning to screen for cases, a considerably more sensitive method than has been used previously in studies either of population prevalence or recurrence risk.[10] It would be expected that the use of MRI scanning, or a similarly sensitive screening modality, in studies of recurrence rates would observe higher recurrence rates than those previously reported. Therefore models using prevalences in the range 0.1%–0.5% were tested against the observed data, but not as high as 1.0% as no comparable recurrence risk data are available.

Risch defines the recurrence risk ratio in relatives of type R (λ_R) as λ_R=recurrence risk in relatives of type R/population prevalence. In single gene models, $\lambda_R - 1$ falls by half with each increase in distance of relationship to the proband, such that:

$$\lambda_1 - 1 = 2(\lambda_2 - 1) = 4(\lambda_3 - 1).$$

Where there is no dominance variance component to disease susceptibility (see below), this relation can be extended to MZ twins, such that:

$$\lambda_{MZ} - 1 = 2(\lambda_1 - 1).$$

This relation is also true for polygenic models with additive interaction between loci, and approximately for genetic heterogeneity models (where the same phenotype may result from different susceptibility genes).

In polygenic multiplicative models, in which there is multiplicative interaction between large numbers of loci, the recurrence risk ratio falls by the square root with each increase in distance of relationship, such that:

$$\lambda_{MZ} = \lambda_1^{\,2}$$
$$\lambda_2 = \lambda_1^{\,0.5}$$
$$\lambda_3 = \lambda_2^{\,0.5} = \lambda_1^{\,0.25}$$

Dominance variance is the component of genetic susceptibility due to interaction between alleles of a particular locus. Whereas there is a dominance variance component to the genetic correlation between siblings, this is not the case between parents and children, who can only share one copy of any gene identical by descent. No significant difference was noted between parent-child and sibling recurrence risks (7.9% *v* 8.2%, χ^2=0, odds ratio = 1.0, 95% confidence interval 0.7 to 1.4), indicating that any dominance variance component in AS must be small. In the largest study examining both types of first degree relatives, the parent-child rate was actually greater than the sibling recurrence rate, a finding not consistent with any genetic model.[11] The dominance and additive variance components to susceptibility can be calculated from the parent-child and sibling recurrence risk ratios assuming a single locus model, the model most affected by misspecification of dominance variance. The dominance variance component is calculated to be only 4% of the total variance. The estimated MZ recurrence risk ratio for a single locus model assuming dominance variance is 130, not significantly different from the figure estimated assuming no dominance variance (125). Therefore for all further calculations it was assumed that there is no significant dominance variance component in AS.

When a combination of these equations is used, any variety of genetic model can be studied. The models studied were single locus/additive/genetic heterogeneity, polygenic multiplicative, two locus multiplicative, one locus and residual polygenic multiplicative component, and five loci with multiplicative interaction and residual polygenic multiplicative component. More complex models of genetic interaction may exist but are not considered in this study. The models considered broadly reflect the likely models operating, and greater definition of the models, though increasing the precision of the estimates would not add greatly to the findings presented.

For the two locus multiplicative and one locus with residual polygenes models, one locus was assumed to be HLA, which in AS has a recurrence risk value (λ_{HLA}) of 3.6.[12] Oligogenic models using λ_{HLA} of 3.6, and 2–10 loci

Table 2 Observed and expected recurrence risk ratios for different genetic models assuming no dominance variance component and a population prevalence of 0.13%

	Observed	*Single locus*	*Polygenic multiplicative*	*Two locus multiplicative*	*HLA, residual polygones*	*Five locus multiplicative*
MZ twins	630	163	6694	282	3202	780
First degree relatives	82	82	82	82	82	82
Second degree relatives	10	41	9	27	11	15
Third degree relatives	7	21	3	11	4	5

with equal λ and a residual polygenic component were fitted against the observed data. The best fitting oligogenic models had no residual polygenic component, and the best fitting such model had five genes, consisting of HLA and four genes each with λ=2.2. The first degree relative recurrence risk ratio was fixed at the observed figure of 82 for all models.

Goodness of fit of models was compared with the observed data by χ^2 analysis comparing the expected and observed number of affected MZ, second and third degree relatives for each model. For each model, the expected number of affected subjects for each degree of relationship was calculated from the predicted recurrence risk ratio and the number of subjects with that degree of relationship actually studied.

There is a clear bias in AS between the sexes, with men more commonly affected than women. Most available recurrence risk data are not specific for one sex, and therefore the effects of the sex of the subject were not considered in this study. It is assumed that the inheritance of AS in men and women follows similar genetic models, an assumption supported by the available recurrence risk data.[11]

Results

Table 2 shows the expected recurrence risk ratios for each genetic model. The results clearly demonstrate that single gene, polygenic multiplicative, one locus (HLA) with residual polygenes models, and the two locus multiplicative model match the observed data poorly (χ^2=129, 90, 32, 36; $p<10^{-6}$, $<10^{-6}$, $<10^{-6}$, $<10^{-6}$) respectively. The expected recurrence risk ratios for MZ twins under the polygenic multiplicative and one locus (HLA) are extremely high, but these models accurately estimate the recurrence risk ratio in more distant relatives. The single gene and two locus multiplicative models underestimate the MZ recurrence risk ratio and greatly overestimate the second degree relative recurrence risk ratios.

The best fitting model is an oligogenic multiplicative model with a small residual polygenic effect. The five locus with residual polygenes is not significantly different from the observed data (χ^2=1.4, p=0.5), and estimates the recurrence risk in MZ twins, second and third degree relatives quite closely. Models with 3–9 genes fitted the observed data ($p<0.05$), but three gene models can be excluded as the magnitude of the genes involved (λ=4.8) would be larger than the MHC (λ=3.6). The presence of genes of this magnitude has been excluded by whole genome linkage studies, which clearly indicate that the MHC is the major susceptibility locus in AS.[3]

These findings were largely unaffected by the population prevalence chosen. Increasing the population prevalence alters the recurrence risk ratios for all types of relatives, but the models fitting the data were similar for a range of population prevalences. Oligogenic multiplicative models of 2–6 loci in addition to the MHC fit the observed recurrence risk rate data for population prevalences from 0.1 to 0.2% (data not shown). With a population prevalence of 0.5%, non-oligogenic models (monogenic, HLA and polygenic residual genes, and the polygenic model) were rejected, but the ability to differentiate between oligogenic models with different numbers of genes was poor, and models with 20 genes fitted the data similarly to those with only two (data not shown). The first degree relative recurrence risk rate varies between 0% and 25% in various studies, but in studies including more than 100 relatives, the rate is between 4.9% and 8.4%. Oligogenic multiplicative models are still favoured where lower first degree relative recurrence risk rates are chosen, but models with a larger number of genes are permitted. For example, if the true first degree relative recurrence risk is 50, then only oligogenic multiplicative models with two to 14 loci fit the observed data. Conversely, where higher first degree relative recurrence risk rates are employed, models with fewer genes are permitted. Overall, however, the finding that oligogenic multiplicative models are the best fitting is sustained over a range of population prevalence and first degree recurrence risk rates.

Discussion

This study suggests that the likely genetic model in AS is an oligogenic model with multiplicative interaction between loci. The number of loci involved and their individual magnitude is of great importance in determining the feasibility of mapping such genes. Genes of magnitude $\lambda \leq 1.3$ require extremely large sample sizes to be identifed by either linkage studies in affected sibling pair families or by linkage disequilibrium mapping.[13] The finding that single gene and polygenic models (with or without a contribution from the MHC region) were excluded by this study is therefore of great relevance. The data predict the existence of genes lying distant to the MHC of sufficient magnitude to be tractable to positional cloning.

The precise number of genes involved cannot be accurately modelled by such studies, particularly where the population prevalence increases relative to the recurrence rate, as the power to discriminate between multilocus models is small, with all such models giving

similar predictions. However, a model with only five genes models the observed data quite closely, and models with three to nine genes were consistent with the observed data. Models with 10 or more genes interacting multiplicatively fail to model accurately the observed recurrence risks, significantly overestimating the MZ twin recurrence risk ratio and underestimating the third degree relative recurrence risk ratio (data not shown). Oligogenic multiplicative models with two to four loci model the observed data closely, but only if the recurrence risk for each locus is fairly high (non-HLA loci $\lambda \geq 2.8$). Five locus models estimated the recurrence risk well with non-MHC loci of magnitude $\lambda=2.2$. Genes of this magnitude are sufficiently large to be identified by current linkage-mapping techniques.

Although there are data supporting the autosomal dominant role of B27 in AS,[14 15] the finding that the sibling recurrence risk ratio is not significantly greater than the parent-child recurrence risk ratio suggests that there is not a great dominance variance component to susceptibility to AS. This might be explained if there was a substantial non-MHC genetic component to AS, or, alternatively, if the MHC acted codominantly. If the latter situation is the case, then either B27 does not act dominantly, or there are other MHC susceptibility genes either operating in trans relative to B27, or acting recessively. HLA-B60 is one gene that in white populations acts in trans with B27 to increase susceptibility to AS,[2 16] but there is increasingly strong evidence of other MHC susceptibility genes in AS, which may explain the apparent lack of a strong dominance genetic effect in this disease.[17]

Although this study supports the presence of non-MHC genetic susceptibility in this common rheumatic condition, the ultimate proof of this theory will be the isolation of the genes involved. Perhaps the most important conclusion from the modelling presented here is that the genes involved are likely to be of sufficient magnitude to be identifiable by current gene mapping techniques.

This study was funded by the Arthritis Research Campaign. Steven Laval is funded by the Oliver Bird Fund, Nuffield Foundation; Sinead Brophy by the Colonel WW Pilkington Charitable Trust; and Dr Andrei Calin by the Royal National Hospital for Rheumatic Diseases NHS Trust.

1 Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A27. Lancet 1973;i:904–7.
2 Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J, *et al.* HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 1996;55:268–70.
3 Brown MA, Pile KD, Kennedy LG, Campbell D, Andrew L, March R, *et al.* A genome-wide screen for susceptibility loci in ankylosing spondylitis. Arthritis Rheum 1998;41:588–95.
4 Rubin LA, Amos CI, Wade JA, Martin JR, Bale SJ, Little AH, *et al.* Investigating the genetic basis for ankylosing spondylitis. Linkage studies with the major histocompatibility complex region. Arthritis Rheum 1994;37:1212–20.
5 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.
6 Beyeler C, Armstrong M, Bird HA, Idle JR, Daly AK. Relationship between genotype for the cytochrome P450 CYP2D6 and susceptibility to ankylosing spondylitis and rheumatoid arthritis. Ann Rheum Dis 1996;55:66–8.
7 Brown MA, Campbell S, Pile KD, Calin A, Ebringer A, Weeks DE, *et al.* Polymorphisms of the CYP2D6 gene increase susceptibility to ankylosing spondylitis [abstract]. Arthritis Rheum 1998;41:S355.
8 Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 1990;46:222–8.
9 van der Linden S, Valkenburg H, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals: a family and population study. Br J Rheumatol 1983;22:18–19.
10 Braun J, Bollow M, Remlinger G, Eggens U, Rudwaleit M, Distler A, *et al.* Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. Arthritis Rheum 1998;41:58–67.
11 Calin A, Brophy S, Blake D. Impact of sex on inheritance of ankylosing spondylitis: a cohort study. Lancet 1999;354: 1687–90.
12 Laval S, Timms A, Bradbury L, Edwards S, Rubin L, Siminovitch K, *et al.* Whole genome screening in ankylosing spondylitis confirms presence of major non-MHC susceptibility loci on chromosomes 7, 10, 16 and 19 [abstract]. Rheumatology 2000;suppl 1:83.
13 Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273:1516–17.
14 Suarez AM, Russell AS. B27 homozygosity and ankylosing spondylitis. J Rheumatol 1987;14:302–4.
15 Arnett FC Jr, Schacter BZ, Hochberg MC, Hsu SH, Bias WB. Homozygosity for HLA-B27. Impact on rheumatic disease expression in two families. Arthritis Rheum 1977;20:797–804.
16 Robinson WP, van der Linden S, Khan MA, Rentsch HU, Cats A, Russell A, *et al.* HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27+ patients. Arthritis Rheum 1989;32:1135–41.
17 Hohler T, Schaper T, Schneider PM, Meyer zum Buschenfelde KH, Marker-Hermann E. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. Arthritis Rheum 1998;41:1489–92.
18 de Blécourt J, Polman A, de Blécourt-Meindersma T. Hereditary factors in rheumatoid arthritis and ankylosing spondylitis. Ann Rheum Dis 1961;20:215–23.
19 Carter N, Williamson L, Kennedy L, Brown M, Wordsworth B. Sibling recurrence risk in ankylosing spondylitis. Rheumatology (Oxford) 2000;39:445.
20 Lawrence J. *Rheumatism in populations.* London: Heinemann, 1977:282–324.
21 Jarvinen P. Occurrence of ankylosing spondylitis in a nationwide series of twins. Arthritis Rheum 1995;38: 381–3.