

A review of the molecular and genetic foundations of Ankylosing Spondylitis

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### Abstract

Ankylosing Spondylitis (AS) is an autoimmune disease that occurs in an adult-onset or juvenile-onset form. The symptoms, severity, and prevalence of this disease vary according to sex, age, and race. AS has been strongly associated with the gene that codes for protein HLA-B27, a protein that misfolds in AS and causes stress on the Endoplasmic Reticulum (ER). The ER then sets off a chain of events leading to the observed AS symptoms. Other mutant genes such as Interleukin 23R (IL23R) and ER Amino Peptidase 1 (ERAP1) have been associated with AS, though not as prominent as HLA-B27. AS is thought to have a genetic basis that accounts for many cases of familial inheritance, though cases have been observed in patients with no family history of AS or with a lack of associated genes. There is much research needed to determine the etiology and map the behavior of Ankylosing Spondylitis.

## **A review of the molecular and genetic foundations of Ankylosing Spondylitis**

### **Introduction**

She rolled out of bed and gingerly stood, before, one step at a time, carefully shuffling to the door. Slowly making her way down the hallway she hunched her back, trying to relieve the stiff pain inhibiting her movements. If she is living with so much pain, why is she not on medication? She has a severe form of Ankylosing Spondylitis (AS), an autoimmune disease that causes inflammation of the joints of the body, mainly affecting the lower back and hips. Ankylosing Spondylitis can present in many different forms, depending on the age, gender, and race of the patient, though the same general tools are used to diagnose and treat it. There are many hypotheses concerning the mechanism of AS, though it has long been associated with mutation in HLA B27 and is currently treated with Non-Steroidal Anti-inflammatory Drugs (NSAIDs), anti-Tumor Necrosis Factor (TNF)  $\alpha$  and Disease Modifying Anti-Rheumatic Drugs (DMARDs). Ankylosing Spondylitis, an inheritable disease, continues to improve as we learn about its cause, presentation, mechanism, and treatment

### **Types of Ankylosing Spondylitis**

Ankylosing Spondylitis affects each patient in a unique way; the two broadest subcategories are Juvenile Onset AS and Adult-Onset AS (Brucke et al., 2005). Juvenile onset AS, in which the disease is expressed before the onset of puberty, displays somewhat different symptoms than adult onset AS. In a survey conducted by Brucke et al, it was found that juvenile onset AS manifested with more peripheral symptoms and often affected patients with greater severity, though it is unclear if this is due to increased disease duration or actual pathological differences. It is interesting to note that both Juvenile and Adult onset AS show similar proportions in both gender and race. Generally, about 60% of cases are male and around 90% of

cases are of Caucasian descent (Brucke et al., 2005). While most prevalent in Caucasian populations, a small percentage of AS patients come from other heritages such as African or Asian and once developed, the disease appears to run the same course with the same physiological symptoms around the globe. In each population, there is a trademark association with the gene coding for the HLA-B27 protein (Ghaffarpasand, Habibagahi, Heiran, & Nazarinia, 2009). For example, regions in Norway with a prevalent HLA-B27 genotype exhibit a greater number of AS cases; on the other side of the globe this same correlation is found in Han Chinese Families (Bakland, Gran, & Nossent, 2005; Cao, et al., 2009). While the presentation of AS may vary slightly by age and race, the same genetic mechanism is consistent behind every type of symptomatic display.

### **Clinical Evaluation of AS**

Diagnosing Ankylosing Spondylitis can be difficult. Most patients are diagnosed based on the severity and appearance of symptoms such as chronic lower back pain (AS accounts for at least 5% of chronic lower back pain), fatigue, and lack of mobility judged against standards such as the Assessment of SpondyloArthritis International Society (ASAS) Criteria for Spondyloarthropathy (Dougados, Gossec, & Rostom, 2010). According to the ASAS Criteria, patients should be considered for AS if they are under the age of 45 with chronic back pain or radiographic sacroilitis (inflammation of the sacroiliac joint). Other associated factors that are to be taken into consideration include uveitis, arthritis, psoriasis, a family history of AS, or increased C Reactive Protein levels (Drapé, n.d.). One study suggested that the rate of diagnosis of AS and general spondylarthrities could be improved by using specific criteria, including multiple regression analysis (analysis of risk factors such as family history and type of pains; Braun et al., 2011). An MRI is often used as a diagnostic tool to assess inflammation in the

spine, especially around the sacroiliac joints (Dougados et al., 2010).. Two standards commonly used to assess the severity and progression of AS are the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Indices (BASFI). These were developed by six scientists in Bath, UK, working for the Royal National Hospital for Rheumatic Diseases (Calin et al., 1994). The BASDAI uses a scale from 1 to 10 with which patients answer six questions concerning the effects of the five major symptoms of AS. The BASFI evaluates the severity of AS according to its effects on daily activities. A score is then computed from the given answers for each of the indices (Calin, 2005). These tools were first published in 1994, replacing the previously used Dougados Articular Index (DAI) and Enthesis Index as a more reliable, simple method of evaluation (Calin, et al., 1994). There are many symptoms other than back pain that can point to a diagnosis of AS, the most of common of which is Acute Anterior Uveitis (AAU), which is essentially inflammation of the eye (Ausayakhun et al., 2006). The one factor that consistently is most useful in diagnosis of AS is the presence of HLA-B27, the strongest known indicator of susceptibility to AS. Because of the many evaluation tools available for the diagnosis of AS, patients are being diagnosed earlier in the course of the disease than in the past.

### **Familial Inheritance of AS**

Because of the genetic foundation of AS, there is a greatly increased risk of development of AS for family members of diagnosed individuals; it is important to note that some studies have found a greater correlation between mothers with AS passing the disease to their children than fathers (Bradbury et al., 2001). However, Lee, Reveille, & Weisman (2008) stated that female AS patients are just as likely to pass the disease to both sons and daughters though the disease is more prevalent in men. Whether the disease is inherited through the father or mother,

it is important to note that in general there is a strong case for the disease following familial lines (a logical conclusion if AS is caused by a genetic mutation), and in fact studies have traced inheritance through several populations, including the Icelandic population and Swedish population (Geirsson, Gudbjornsson, Kristjansson, 2010; Hemminki, Li, Sundquist, J., Sundquist, K., 2009; Lee et. al., 2008). While family history is one risk factor for AS, it was concluded in 2003 that a second risk factor is the age of the mother at the time of the birth of the child. If the mother was less than 30 years old when she gave birth, her child is more likely to develop AS. In fact, 95% of the studied AS patients were born to mothers younger than 30 years old. While this study did not include a very large or diverse sample (the patients surveyed were of only Mexican Mestizo descent), it was unusual for a disease to be associated with young motherhood. There was no correlation between birth order and prevalence of AS. (Arrelano et al., 2003). Despite the numerous studies concluding familial inheritance of AS, there are still many cases that seem to arise de novo in patients with no known risk factors. This would seem to indicate that there are also environmental conditions at work somehow in the development of Ankylosing Spondylitis. A better picture of the malfunctions and mechanisms in AS, through research, will lead to a greater understanding of the influence each factor has on AS.

### **AS in Women**

#### **Presentation of AS in Women versus Men**

Not only are cases of AS categorized by presentation in age and race, but also by gender. AS was originally considered *a man's disease*, but recently more women are being diagnosed and this stereotype is changing. It has been hypothesized that this perception may be due to the fact that women are less likely to be diagnosed with AS than men on first inspection, though it is important not to disregard the possibility that this difference is due to physical and/or

sociological differences. If social circumstances are at fault, lack of recognition and thus delayed diagnosis or the prominence of similar or related inflammatory diseases may be the cause of the greater severity of symptoms in women. Multiple sources confirm that diagnosis of AS generally takes noticeably longer for women. However, it is worth noting that this stereotype is changing, and the measured percentage of AS in women is now considered higher than it was in the past. (Husby & Gran, 1990; Lee et al., 2008). Women are less likely than men to utilize healthcare for AS (Chen, Chen, Chen, Chen, Ying-Ming, 2011).

Ankylosing Spondylitis has been observed to progress in a slightly different manner in women and men. Lee et al. (Lee et al., 2008) report that according to current literature, women show more peripheral arthritis and cervical pain, while men tend to develop more lumbar and thoracic pain and deformity). More interestingly, the genetic load theory hypothesizes that women must have a greater genetic burden in order for AS to be expressed. This may account for the increased prevalence of AS in men, since they can develop the disease with a lighter genetic load. (Lee et al., 2008). Women seem to be more affected in daily function and experience more fatigue due to AS, though cases in patients who are men generally are more severe (Davis, et al., 2007; Roussou & Sultana, 2010). Because AS is more studied in men since there are more documented cases, there is still much to be learned about how AS affects the female body.

### **Pregnancy and AS**

Pregnancy is a unique condition, and an added complication, when considering the effects of AS in women. There are special issues that must be addressed when discussing AS in women: the effects of pregnancy and hormone replacement therapy. While pregnancy may temporarily alleviate the effects of other diseases of the Rheumatoid arthritis family, this is not the case with AS. It was established in a study by Husby et al. in 1982 that the progression of AS



is relatively unaffected by pregnancy, and the pregnancy itself is at no increased risk because of the disease. However, there may be a post-partum flare within six months following delivery, though the exact reason for this is yet to be known (Lee et al., 2008). In an AS patient who is not pregnant, T-regulatory cells are abnormal and secrete increased amounts of pro-inflammatory cytokines and show decreased suppression of the secretion of Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ) and interferon  $\gamma$  (IFN $\gamma$ ) by T effector cells, molecules that are related to pain and inflammation. The exact malfunctional mechanism of AS will be discussed later. During a regular pregnancy, there is an increase in CD4+ and CD25+ T regulatory cells, the same cells that are mutated in AS, which secrete interleukin-10 and suppress cytokine expression. A patient with AS will also show an increase in these T regulatory cells, but the T regulatory cells will still malfunction. This continued malfunction explains why pregnancy does not alleviate the symptoms of AS. It does not appear that a developing unborn child is affected by this abnormality within the mother, but current research has yet to be fully accepted (Forger et al., 2009).

### **Hormone Replacement Therapy and AS**

Not only must pregnancy be discussed concerning AS in women, but there are also many hormonal differences to be evaluated. Currently there has only been one main study published addressing the effect of oral contraceptive pills and hormone replacement therapy on women with AS. The study evaluated a very small sample, but reached some interesting conclusions. In the study, Pre-menopausal women began taking oral contraceptives, and post-menopausal women took hormone replacement therapy. Both groups of women showed significant improvement in their peripheral arthritis and general improvement of symptoms. However, when removed from therapy, their symptoms returned with increased severity. This was a narrow study, with a small sample, but it demonstrates that the hormonal structure of

women may contribute to their disease and that hormone therapy is a good avenue for further research. (Jimenez-Balderas, Madero-Cervera, Mintz, Murrieta, & Tapia-Serrano, 1990).

### **The Genetic Basis of AS**

#### **HLA B27**

**HLA B27 mutation.** Through recent research, AS has been associated in varying degrees with several genes including HLA-B27, ERAP1, IL23R, IL-1 and many others, though HLA-B27 is consistently reported as exhibiting the strongest connection with AS (Brown, 2009). While only 5% of the general population has HLA B27, gene, 95% of AS patients possess it (Colbert, DeLay, Layh-Schmitt, & Sowders, 2009). It has been found that there is an increased risk of severe AS if homozygous for HLA-B27, and that HLA-B27 may contribute to a younger age at onset (Barnardo et al., 2006). In fact, as mentioned earlier, a positive HLA-B27 test is considered definitive in diagnosing AS in patients exhibiting the associated symptoms.

HLA stands for Human Leukocyte Antigen, which refers to a type of Major Histocompatibility Complex Class I (MHC Class I) receptor on the plasma membrane of a cell that presents molecules to CD8 + and the T cell receptor of T cells (also referred to as cytotoxic T cells). HLA/MHC Class I receptors are found on almost all cells in the body, and serve as markers for self identification as well and also present antigens (bound via complex with  $\beta$ 2 microglobulin) from within the cell to cytotoxic T cells (Kimball, 2011; Sheehan, 2004). The structure of the receptor itself, like all MHC class 1 receptors, consists of a heavy chain and light chain bound together and complexed with  $\beta$ 2 microglobulin. HLA B27 is a class of MHC receptors that have been strongly associated with autoimmune disease and spondylarthropathies, though what allele is discussed and how it malfunctions depends on the disease discussed (Brent & Kataria, 2004; Kimball, 2011). The HLA B27 class is encoded on chromosome 6 and includes

at least 50 allotypes (HLA B\*2701-2725), a great majority of which show association with AS (Cauli et al., 2010; Sheehan, 2004). The most widespread subtype is B\*2705, but all the subtypes differ from each other by a few amino acids (Sheehan, 2004). B\*2709 and B\*2706 alleles have been strongly associated with healthy individuals, while B\*2705 has been designated the main AS-associated allele (Bitti et al, 1995; Cauli et al., 2010). Allele presentation of the HLA B27 family has been found to differ according to ethnicity, which would account for the disproportionate percentages of AS in certain Caucasians, such as those of European or African descent, while only a small percentage of cases are of Italian or Sardinian heritage (Cauli et al., 2005).

With these subtypes identified, the question is why B\*2709 is not associated with AS, and why B\*2705 and other alleles are associated. What amino acids mutate between each of the alleles and why does this cause HLA B27 to malfunction? HLA-B\*2705 has been found to differ from B\*2709 by just one mutation: His116Asp. Residue 116 lies on the floor of the F pocket of the HLA B27 peptide and is key to stability and binding to the C terminus of its ligand (Biddison, Carreno, Hansen, Taurog, & Winger, 1993; Bitti et al., 1995; Galocha & Lopez de Castro, 2010). It has been suggested that the His116Arg mutation causes HLA B27 to display a dual binding mode instead of a single conformation, thus changing the interaction with CD4+ and CD8+ T cells. One study examined the ability of pGR, a self peptide, to drive a cytotoxic T cell response in correlation to the dual binding capacity of B\*2709, finding that pGR ability to form two conformations has more pronounced in B\*2709 binding. They hypothesize that certain molecules such as pGR accessorize the B\*2709 mutation (Cauli et al., 2010). Not only have the residues of the F pocket of HLA B27 been indicated as important for binding, but also those of the B pocket. Not as much research has been done on the residues of the B pocket, which is

associated with binding and anchoring of the ligand to a HLA B27 molecules, and this is one avenue that is wide open for further research (Galocha & Lopez de Castro, 2010). Whether it is due to the His116Arg mutation or another structural difference, there is some aspect of B\*2709 that differs from B\*2705 that causes increased autoimmune response in AS patients.

**HLA B27 misfolding and accumulation.** While some scientists believe that a mutation of the HLA B27 molecule causes AS by changing binding interactions, others believe that the disease phenotype is actually caused by HLA B27 protein misfolding or folding too slowly (Cauli et al., 2010). One theory postulates that the HLA-B27 receptor misfolds by incorrectly forming disulfide bonds during its synthesis. It is suggested that the misfolding is due to over-expression and unique rearrangement in the variable region of the heavy chain in AS. Sequence and PCR analysis of the variable heavy chain gene segments showed unusual rearrangement of the gene sequence and Baek et al. (2010) hypothesized that this rearrangement would occur during B cell development. The misfolding might then lead to accumulation of the HLA-B27 protein bound to the Binding Immunoglobulin Protein (BiP) and an increase of stress in the Endoplasmic Reticulum (ER). BiP is a negative regulator of the unfolded protein response and normally keeps signal proteins in their inactive state. Increased stress, set off by signal proteins no longer bound to BiP, causes the ER to stimulate the unfolded protein response which leads to overproduction of the protein IL23R (Colbert, DeLay, Klenk, & Layh-Schmitt, 2010). With the overproduction of IL23R, activation of IL-17 producing T Helper cells (TH17 cells) ensues. These cells create proinflammatory cytokines that stimulate fibroblasts, macrophages, and other cells to synthesize IL-1, IL-6, TNF- $\alpha$ , NOS-2, metalloproteases, and chemokines, all proinflammatory mediators which lead to inflammation (Balog, et al., 2010; Colbert et al., 2010; Ishigame & Iwakura, 2006). The connection between HLA-B27 and the malfunctions within the

ER has been confirmed through a variety of methods, with consistent reports from all across the globe of aberrant HLA-B27 identified in AS cases (Burgos-Vargas et al., 2002).

The next step to understanding the connection between the genotype and phenotype of AS is to understand how HLA-B27 misfolding and/or mutation brings about the symptoms of AS. HLA B27 molecules are expressed on the surface of almost all cells, and interact with T cytotoxic as the T cytotoxic cells search for foreign cells/molecules invading the body (Kimball, 2011). The question is, how does a change in the HLA-B27 molecule presentation relate to the clinical symptoms of joint pain and inflammation, fatigue, and cartilage degradation? One theory proposes that chondrocytes have the ability to activate CD8+ T cells. CD8+ T cells which once activated will kill the offending chondrocyte via enzymes such as perforin and release cytokines that stimulate pain and inflammation by recruiting more T cells to the area. Note that in this process, not only would pain and inflammation ensue, but as chondrocytes are eliminated, weakening and eventually destroying joint cartilage. This explains the chronic fatigue and pain (due to a continually stimulated immune system) but also the long term spinal fusion (loss of cartilage discs between vertebrae) and immobilization of back and hip joints. A study Appel, Erben., et al., 2009 found that human chondrocytes (taking on the role of an antigen presenting cell) can indeed stimulate CD8+ T cells. Not only this, but it was found that there is increased expression of HLA-B27 in the chondrocytes surrounding points of arthritis in the femurs of AS patients. This increased expression of HLA-B27 in chondrocytes was associated with presence of a proinflammatory cytokine, interferon  $\gamma$  (IFN $\gamma$ ). The whole process could be set off by an initial injury or cartilage remodeling, which could cause micro-trauma. The micro-trauma would cause T cell involvement in the cartilage, which would bring about contact between CD8+ T cells and chondrocytes. The chondrocytes would then act as antigen

presenting cells, complexing HLA-B27 with CD8+ and thus cause inflammation and the release of the pro-inflammatory cytokine IFN $\gamma$ . IFN $\gamma$  would cause upregulation of HLA-B27 and the whole process begins again until the cartilage is completely gone.

**B Cells in AS.** Not only are the T cells of the immune system involved with AS, but more studies are starting to emerge concerning the role of B cells in AS. It has been noted that B cells are consistently found in areas of inflammation, and one trial targeting B cells was relatively effective (Appel, Braun et al., 2009; Baek et al., 2010). It has been hypothesized that B cells are involved in AS by antigen presentation with the mutated HLA-B27 receptor (Baek et al., 2010). Traditional theories concerning AS have focused on the cell mediated immunity breakdown because of the lack of unusual autoantibodies found. However, a recently developed technique, the Nucleic Acid Programmable Protein Array, allowed deeper analysis. It was found that there are specific autoantibody responses targeting connective tissue and skeletal proteins (Bowness et al., 2010). Not only that, but a separate study (Fu-cheng et al., 2009) evaluated the distribution of B cells in AS patients as they were treated with etanercept, an inhibitor of Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ). The mechanism of this drug will be discussed later. They found an unusual increase in B cells in the peripheral blood of AS patients (consistent with the location of their symptoms), a disproportion that did not change with treatment with etanercept). Both of these studies imply that there is some B cell involvement in the pathogenesis of AS and to understand the molecular changes in the body B cells must be further considered.

### **Innate Immunity and AS.**

Both B and T cells are part of the adaptive arm of the immune system, but the cells of the non-specific innate arm of immunity are also involved in the pathogenesis of AS. Natural Killer Cells (NK cells) interact with HLA receptors via polymorphic Killer Immunoglobulin-Like

Receptors (KIR); therefore, aberrant HLA-B27 receptors will also affect the response of NK cells. KIR3D11 and KIR3DS1 are both noted to bind to HLA-B, and KIR3DS1 is actually up-regulated in populations with AS (Armas et al., 2006; Jamil & Salim, 2011). NK cells, once stimulated, will not only destroy the cell presenting the mutated or foreign antigen, but may also stimulate CD28+ T cell proliferation, thus setting off the adaptive immune response. To make a connection with the discussion earlier, chondrocytes may be the antigen presenting cells that activate these NK and cytotoxic T cells (Armas et al., 2006; Appel, et al., 2009). Because of this interaction, it is highly plausible that NK cells contribute to the development of AS, as stimulated by HLA-B27. Thus, the effects of innate immunity must also be addressed when considering the full molecular picture of AS.

#### **Associated Diseases by Mechanism and Molecule**

HLA B27 is not unique to AS, there are many other diseases which are associated with a mutation on this receptor and that AS patients are at risk of developing. While it was discussed earlier that chondrocytes might act as non-professional antigen presenting cells, stimulating T cells and inflammation with their mutated HLA-B27 receptor. There is a suggestion that chondrocytes are not the only cells able to take on this capacity. Pancreatic endothelial cells, brain microglial cells are reported to have stimulated T cells via MHC complexes (note that HLA is a type of MHC complex as discussed earlier; Appel, et al., 2009). While these cells appear to malfunction in a comparable way to chondrocytes, there are many different MHC receptor subtypes involved and thus no correlation beyond similarity of mechanism can be concluded. However, they are worth mentioning because research concerning the mechanism of these diseases may be applicable to HLA-B27 and of AS.

While some diseases are similar in mechanism to HLA-B27, there are many that are specific to HLA-B27 and thus often found concurrent with AS. Acute Anterior Uvitis (AAU), is short term inflammation of the eye that occurs in about 40% of AS patients sometime during their lifetime (Rosenbaum & Smith, 2002). Though the molecular mechanism is unclear, it appears that AAU is caused by increased TNF $\alpha$ , which would be part of the cytokine cascade activated by HLA-B27. AAU is treatable and often recedes with Anti-TNF $\alpha$  medication and steroids (Elewaut & Matucci-Cerinic, 2009). Other diseases associated with AS include type II Palmoplantar Psoriasis, Upper Lobe Fibrosis, Aortic Regurgitation, and Crohn's Disease. A study in November of 2010 discovered a significant overlap between genes associated with AS and those associated with Crohn's disease (Sheehan, 2004). It is logical that there might be a connection between these two disorders in that they both are chronic inflammatory diseases, just in different locations (joints vs. intestines; Cruyssen et al, 2010).

There are multiple links between intestinal inflammation and arthritis, in fact recent studies have connected gut-mediated inflammation with arthritis, even suggesting that arthritis could be set off by infection with intestinal microbes and thus arthritic symptoms might be controlled by diet (Bourne et al., 1985; Cederholm, Johansson, Kokkonen, Sundstrom, & Wallberg-Johsson, 2011; Ebringer & Rashid, 2011). Rashid stated, "An extensive amount of studies...have shown that *Klebsiella pneumonia* microorganisms could be suggested as the most likely etiopathogenetic triggers for AS and Crohn's disease based on the molecular mimicry mechanism..." (Ebringer & Rashid, 2011, p.1). As reviewed by Rashid, there is considerable evidence for the connection between AS and bowel inflammation. Up to 70% of AS patients show some type of bowel inflammation, and there is a strong connection between HLA-B27 and many types of gut disease. The evidence also runs in the opposite direction: many patients



diagnosed with Crohn's disease or another type of bowel inflammation later develop a form of spondylarthropathy. Because of the connection between gut inflammation and AS, the possibility is then raised that the microbes responsible for digestive inflammation could heighten the symptoms of AS. The main microbe suggested to have an effect is *Klebsiella pneumonia* (Ebringer & Rashid, 2011). Schwimbeck et al. found a shared sequence between the *Klebsiella* and HLA-B27 genes, and both genes induce a similar response in rats (Oldstone, Schwimbeck, & Yu, 1987). In fact, a rabbit immunized by Avakian et al. with HLA-B27 + lymphocytes cross reacted with *Klebsiella* (Avakian, Ebringer, Entwistle, & Welsh, 1980). Rashid summarized the proposed connection between HLA-B27 and microbes such as *Klebsiella*, stating,

In patients with AS...we suggest that *Klebsiella* infection in the bowel... primarily causes production of anti-*Klebsiella* antibodies which can also bind to the cross-reactive self antigens like HLA-B27 and collagen fibers in the joints with release of further new antigens on the surface of damaged tissue. These new antigens are responsible for prolonged or continuous production of autoantibodies and further damages to the articular tissues with a perpetuation of the disease process (Ebringer & Rashid, 2011, pg5).

The connection between bowel inflammation and AS is a strong one, and a better understanding of the mechanism of this connection may elucidate the mystery behind when and why AS manifests in some HLA-B27 negative patients and not in some HLA-B27 positive patients. Better treatments and even the possibility of preventative techniques may lie behind this connection.

Bowl inflammation is not the only disease that is prevalent in the AS population. Upper lung fibrosis is another disease that is strongly associated with HLA-B27. Multiple studies have

documented decreased lung capacity in patients with AS and a prevalence of upper lung fibrosis. Some attribute this to thoracic cage fixation, though this theory is highly disputed (Feltelius, Hedenstrom, Hillerday, & Hallgreen, 1986; Hickling, Parkin, & Robinson, 1982; Jessamine, 1968). Finally, AS patients are at increased risk of cardiovascular problems, including Aortic regurgitation, atrioventricular and intraventricular blocks (Momeni, Taylor, & Tehrani, 2011). Not only are AS patients at risk for these diseases, but because of the pathology of their disease there are complications in normal molecular pathways. For example, it was found that patients with AS have impaired Nitric Oxide (NO) metabolism and are at risk for osteoporosis (Alacacioglu et al., 2009; Arends, Brouwer, Bruyn et al., 2011). It is possible that the appearance of decreased NO metabolism is actually due to elevated NO production. Koncz et al. noted in 2003 that TCR stimulation led to increased  $Ca^{2+}$  levels, which then caused NO production. Because AS has continuing TCR stimulation by HLA-B27, a chronic increase in cellular NO concentration might be expected. Ankylosing Spondylitis affects more than just the joint of the body, and patients with AS are at risk to develop pulmonary, cardiac, and digestive complications (Koncz, Nagy, & Perl, 2003).

### **Other Associated Genes**

Ankylosing Spondylitis not only manifests with many different complications, but the genotype behind the phenotype includes many genetic associations beyond HLA-B27. These associations include KIR (previously discussed receptor of NK cells), Interleukin 1 (IL1), Endoplasmic Reticulum Associated Aminopeptidase 1 (ERAP1), Transforming Growth Factor Beta 1 (TGFB1), and other HLA genes.

**HLA genes.** HLA B27 is not the only HLA subtype to be associated with AS, though it is most definitely the most prevalent mutation. HLA-DR81 and HLA-B15 have also been shown to

be linked to AS. They are also prevalent in diseases with similar mechanisms to AS such as IBS and Crohn's disease. These associations are stronger in patients with AS who are HLA-B27 negative. Because the receptors they encode are of the same general family as AS, it is logical that they would malfunction in a similar way (Burgos-Vargas et al., 2002). AS is a complicated disease and there are cases that arise in HLA-B27 negative patients, which would indicate that there are many other factors at work to create the phenotype seen.

**IL1.** Interleukin 1 has long been known to be a pro-inflammatory cytokine and has been identified with susceptibility to AS, though its effect on disease phenotype has yet to be determined (Aggarwal, et al., 2008; Greenwood, et al., 2008). Not only is IL1 associated with AS, but it has also been strongly implicated in psoriasis, a secondary complication common in those with AS (Gladman et al., 2006; Greenwood et al., 2008). One problem yet to be resolved with this association is that it is not consistent throughout different races; while all demonstrate an association with the IL-1 gene cluster polymorphism, the exact polymorphic sites vary (Gu & Wu, 2007).

**ERAP 1.** As with IL-1, while the association with ERAP has been confirmed, the exact manner of malfunction has yet to be clearly identified. (Gladman, et al., 2009) It has been observed that there are single nucleotide polymorphisms, including rs27434 in the regulatory sequence of ERAP1 genes of patients with AS that could be indirectly affecting the expression of AS by not correctly regulating the ERAP1 expression ( Appleton et al., 2009). However, it has also been noted that ERAP1 does not influence the IL-1 cytokine receptors and thus there is still much research to be done on the defect in ERAP1 (Chiu, Haroon, Inman, Tsui & Tsui 2010).

**TGFβ1.** The association with TGFβ1 is not as strong as that of ERAP1, HLA, and IL-1. However, it is somehow involved in the disease and was weakly associated with a younger age

of onset. There is a possibility that some of the surrounding genes on chromosome 19 are also involved in AS. It is not surprising that this gene would be at least weakly linked since it is involved in many processes from inflammation and bone remodeling to fibrosis (Bradbury et al., 2004).

## **Treatments for AS**

### **Anti-Inflammatory Drugs (NSAIDs)**

Now that the basic mechanism of AS is moderately understood, western medicine is able to utilize medication to prevent and relieve the symptoms and progression of AS. There are many different medications that can be used to treat AS, including NSAIDs, DMARDs, and anti-TNF  $\alpha$  drugs. NSAIDs are non-steroidal anti-inflammatory drugs and mainly treat the symptoms but not the cause of AS. These medications inhibit the COX 2 (Cyclo-oxygenase 2) enzyme in the body. There are two COX enzymes. COX 1 is found in normal tissues and is involved in the general maintenance of tissue. COX 2 is only activated in inflammation. Both of these enzymes are part of the Arachidonic Acid Pathway, which converts Arachidonic acid to prostaglandins (Gotlieb, 2005). Prostaglandins promote fever, inflammation, pain, and blood clotting (Goldsby, Kindt, & Osborne, 2007). By binding COX2 and inhibiting it, NSAIDs inhibit the production of prostaglandins and thus inhibit the painful inflammatory symptoms of AS. However, one thing to remember with this treatment, as with any treatment that changes the biochemical reactions of the body, is that there are also other effects. For example, with NSAIDs side effects would include blood thinning. Some commonly recognized NSAIDs include ibuprofen and aspirin (Gotlieb, 2005).

### **Disease Modifying Anti-Rheumatic Drugs (DMARDs)**

Not only do NSAIDs inhibit the Arachadonic Acid Pathway, but another genre of drugs used to treat AS, DMARDs (disease modifying anti-rheumatic drugs), also work on this pathway. There are 3 main types of DMARDs: Methotrexate, Corticosteriods, and Anti-TNF $\alpha$ . Not much is known about Methotrexate except that it has some effect on the TNF pathway and inhibits dihydrofolate reductase, an enzyme in the folic acid pathway. Corticosteriods, however, work on the Arachadonic Acid pathway. Instead of inhibiting COX2 they work a little earlier in the pathway by inhibiting phospholipase A2. Phospholipase A2 is responsible for synthesizing the Arachidonic Acid, which is then converted by COX 2 to prostaglandins, which stimulate inflammation and pain. In this way, Corticosteriods are an effective treatment of the inflammatory symptoms of AS. While DMARDs are a moderately effective treatment for AS symptoms, it takes anti-TNF medications to actually slow the progression of the disease (Matsumoto et al., n.d.).

### **Anti-TNF $\alpha$ Medications**

Anti-TNF medications are generally the most effective medication against the progression of AS. While DMARDs and NSAIDs inhibit the prostaglandin producing pathway, anti-TNF medications bind the inflammatory mediator TNF $\alpha$  as it is produced from activated T cells, stopping it from stimulating inflammation. TNF $\alpha$ , when secreted, activates endothelial cells, which increase production of cell adhesion molecules and recruits lymphocytes, activates more monocytes which then activate more T cells, and also activate fibroblasts. The activated fibroblasts secrete more inflammatory mediators, including IL1, which leads to bone and cartilage destruction. Anti-TNF medications inhibit TNF $\alpha$  and in doing so prevent the signals that lead to degradation and inflammation from being transmitted. Thus, Anti-TNF $\alpha$  drugs slow the progression of AS and have even been known to put it in remission (Guzman, 2009). There

are many types of Anti-TNF medications, including Entanercept, Infliximab, and Adalimumab. Entanercept (brand name Enbrel) contains human TNF receptor protein stabilized by a Fc fragment of human IgG. Infliximab is a mixture of human and mouse anti-TNF $\alpha$ . Adalimumab is an anti-TNF $\alpha$  monoclonal antibody. All of these variations of anti-TNF medications bind to TNF $\alpha$  and inhibit it from stimulating inflammation and cartilage degradation .

Anti-TNF medication effectiveness has been documented by several studies to be a very effective treatment, though the efficacy depends on the patient. Lower BASFI scores and younger age at baseline were connected to a more effective response, though women are less likely to gain remission of AS through use of TNF therapy. The levels of C reactive protein, a protein that indicates inflammation, was decreased in patients using anti-TNF $\alpha$  monoclonal antibodies, a decrease that tends to be greater in men than women. Anti-TNF $\alpha$  treatment can be terminated for a variety of reasons, the most common of which is lack of response to therapy and lack of peripheral arthritis (Arends, Brouwer, Groen et al., 2011). Not only does Anti-TNF $\alpha$  medications decrease inflammation (as evidenced by decreased C reactive protein) and prevent joint damage, but it also positively changes the composition of HDL, increasing cardiovascular health. While the full effects of anti-TNF $\alpha$  monoclonal antibodies, both positive and negative, are still be researched, it has been established as one of the most effective drugs against spondyloarthropathies with positive effects (De Vries et al., 2009).

### **Negative Side Effects of Anti-TNF $\alpha$**

The main negative side effect of Anti-TNF medications is that they also cause immunosuppression; this side effect is much more serious than the anti-coagulant properties of aspirin and AS sufferers on Anti-TNF medications are extremely susceptible to all types of infection and sometimes even cancer. The most dangerous infection for those on

immunosuppressant medications is tuberculosis. If a healthy patient has been infected with tuberculosis, it has been contained in granules by the body. The infection never truly goes away, and putting that patient on immunosuppressant drugs later in life will cause the granules to be released and the infection active once again. Not only are patients on immunosuppressants susceptible to TB, but they have a decreased ability to fight all infections, from the common cold to the flu. While there are many positive outcomes to using anti-TNF $\alpha$  medications, there are also other effects such as immunosuppression that need to be considered. (Guzman, 2009)

### **Lifestyle Changes and the Control of AS Symptoms**

Medications are not the only way to challenge the progression of AS; there are some basic lifestyle changes that have been shown to lead to overall improvement in symptoms. As mentioned earlier, AS is strongly associated with changes in the bowel, and recently it has been suggested that symptoms can be treated by a change in diet. A low starch diet has been shown to be at least moderately effective in decreasing symptoms. Scientists believe this may be due to the involvement of pathogens such as *Klebsiella* bacteria in the inflammation of the bowel and aggravation of the immune system in AS. *Klebsiella* consume monosaccharides and disaccharides, and thus eliminating these from the diet would inhibit any such bacteria (Ebringer & Rashid, 2011). While many patients report improvement of their symptoms with this dietary change, the involvement of bacteria has been questioned, and it is possible that it is a different mechanism or bacteria causing the change (Cederholm et al., 2011). AS can also be improved by exercises that improve spinal mobility and cardiopulmonary fitness. While there is a documented exercise intolerance developed by AS patients due to the progression of the disease, overall health and fitness helps slow and minimize symptoms (Hascelik, Inanici, & Ozdemyr, 2011). As

with many diseases, there are basic lifestyle changes that assist the body as it fights against AS, including dietary change and an increase in exercise.

### **Conclusion**

In summary, Ankylosing Spondylitis affects many different aspects of the body and can differ in presentation whether it is juvenile onset or adult onset, and in a man or in a woman. No matter what the type of presentation, AS can be quantified and qualified using many diagnostic modalities, from a MRI to the most common assessments: the BASFI and BASDI. The BASDI is used to assess the symptoms of the disease, while the BASFI is used to evaluate the clinical severity. While AS was traditionally considered a man's disease, there is increasing documentation of its presence in women. There are unique factors that must be considered when the disease presents in a woman, including the effects of pregnancy and the possibility of hormone replacement therapy as a form of treatment. The manifestation of AS in women has also been linked to a greater genetic load than that of men, and there is an increased possibility of a woman passing AS to her children. While the phenotype presentation of AS may differ from man to woman, the basic genotype is the same.

The genetic basis of AS is a complex puzzle that is constantly evolving as new information is gained over time. In Cell Mediated immunity, the molecule most strongly associated with the AS phenotype is MHC class 1 receptor HLA-B27. HLA-B27 serves to complex with T cell receptors and stimulates inflammatory immune response via cytokines. There are two opposing theories about how the T cells are stimulated. First, some scientists hold that mutation in the HLA-B27 receptor allows it to complex incorrectly and provide continual stimulation of cytotoxic T cells. It has been proposed that in AS it is the chondrocytes that present the aberrant HLA-B27 receptor in the greatest quantity and thus are targeted by cytotoxic



T cells. The second theory holds that inflammation is caused by the unfolded protein response set off in cells by misfolding and accumulation of HLA-B27 in the endoplasmic reticulum. Not only is T cell stimulation associated with AS, but there is increasing evidence for the involvement of humoral immunity activation as seen in the identification of autoantibodies targeting connective tissue and skeletal proteins. The innate arm of the immune system is involved through NK cells via the complex of KIR with mutated HLA-B27. Thus, a wide variety of immune responses are elicited in AS, the key now is to determine why and how each response is caused.

HLA-B27 is not the only associated gene, other genes which are involved in AS include KIR, IL1, ERAP1, TGFB1, HLA-B15 and HLA-DR81. Because of the genetic bases of AS, the disease can be traced through family lines, and the greater the family history the increased risk a person has of developing the disease. There is hope for a normal life for those diagnosed with AS, though, with early diagnosis the prognosis is very good. Treatments available include NSAIDs, DMARDs, and Anti-TNF $\alpha$  medications. While NSAIDs and DMARDs treat the symptoms of AS, monoclonal anti-TNF $\alpha$  antibodies actually stops the progression of the disease, sometimes sending it into remission. However, these drugs do come with side effects, and immunosuppressed individuals must be very careful because of their increased susceptibility to even the smallest virus. The use of these medications has greatly improved the lives of many.

With foundational understanding of AS established, the question now is how each gene associated with AS affects the expression of AS and how the result creates the observed phenotype. As the processes are determined, perhaps it will then be possible to answer definitively questions of inheritance, cause, treatment, and even cure of each etiology.

## References

- Aggarwal, A., Agrawal, S., Misra, R., Pandya, S., Sharma, B., Srivastava, R. (2008). IL1RN2 allele of IL-1receptor antagonist VNTR polymorphism is associated with susceptibility to Ankylosing Spondylitis in Indian patients. *Clinical Rheumatology*. 27.
- Alacacioglu, A., Bilgir, O., Kebapcilar, L., Kozaci, D., Sari, I., Taylan, A., Yildiz, Y. (2009). Increased levels of asymmetric dimethylarginine (ADMA) in patients with Ankylosing Spondylitis. *Inter Med*. 48 , 1363-1368.
- Appel, J., Braun, H., Heldmann, Listing, M., Rudwaleit, F., Sieper, J., Song. (2009). Major Clinical Response of Rituximab in Active TNF-Blocker-Naive Patients with Ankylosing Spondylitis but Not in TNF-Blocker-Failure Patients- An Open Label Clinical Trial. *Arthritis and Rheumatism*. 16-21, Abstract Supplement.
- Appel, H., Erben, U., John, T., Kuhne, M., Kohler, D., Radbruch, A., Schulze-Tanzil, G., Seiper, J., Wu, P. (2009). HLA-B27-restricted antigen presentation by human chondrocytes to CD8+ T cells. *Arthritis & Rheum*. 60 (6). 1635-1646.
- Appleton, L.H., Brown, M.A., Evans, D.M., Farrar, C., Harvey, D., Karaderi, T., ... Wordsworth, B.P. (2009). Investigating the genetic association between erap1 and Ankylosing Spondylitis. *Human Molecular Genetics*, 18 (21), 4204–4212.
- Arellano, J., Barile-Fabris, L., Jimenez-Balderas, F.J., Fraga, A., Perez-Rodriguez, M.E., Sanchez, M.L., Talavera, J.O., Zonana-Nacah, A. (2003). Maternal age and family history are risk factors for ankylosing spondylitis. *J Rheumatol*. 30(10), 2182-5

- Arends, S., Brouwer, E., Bruyn, G.A., Houtman, P.M., Kallenber, C.G., Leijnsma, M.K., Van der Veer, E. (2011). The relation between bone mineral density, bone turnover markers, and vitamin D status in Ankylosing Spondylitis patients with active disease: A cross-sectional analysis. *Osteoporos Int.* 22(5), 1431-9
- Arends, S., Brouwer, E., Groen, H., Houtman, P., Janse, T., Kallenberg, C., Leijnsma, M., Spoorenberg, A., Van der Veer, E. (2011). Baseline predictors of response and discontinuation of tumor necrosis factor-alpha blocking therapy in Ankylosing Spondylitis: A prospective longitudinal observation cohort study. *Arthritis, Research & Therapy.* 13, R94.
- Armas, J.B., Blanco-Gelaz, M., Couto, A., Gonzalez, S., Lopez-Larrea, C., Lopez-Vazquez, A., Martinez-Borra, J., Suarez-Alvarez, B., Torre-Alonso, J. (2006). Contribution of KIR3DL1/3DS1 to Ankylosing Spondylitis in human leukocyte antigen B-27 Caucasian populations. *Arthritis Research and Therapy.* 8, R101
- Ausayakhun, S., Kunavisarut, P., Leetrakool, N., Pathanapitoon, K., Rothova, Suksomboon, S., Wattanikorn, S. (2006). HLA-B27-associated acute anterior uveitis in the University Referral Centre in North Thailand: Clinical presentation and visual progress. *Br J Ophthalmol.* 90, 1448-1450.
- Avakian, H., Ebringer, A., Entwistle, CC., Welsh, J. (1980). Ankylosing spondylitis, HLA-B27 and Klebsiella. II. Cross-reactivity studies with human tissue typing sera. *Br J Ex Pathol.* 61(1), 92-6

- Baek, H.J., Choi, H.J., Chung, W.T., Hong, S.J., Hong, Y.S., Jeong, J.C... Suh, C.H. (2010). Clinical and radiographic features of adult-onset Ankylosing Spondylitis in Korean patients: Comparisons between males and females. *Journal of Korean Medical Science*, 25 (4), 532-5.
- Bakland, G., Gran, J.T., Nossent, H.C., (2005). Incidence and prevalence of Ankylosing Spondylitis in Northern Norway. *Arthritis Rheum.*, 53 (6), 850-5.
- Balog, A., Himer, L., Reusz, G., Szebeni, B., Szakál, D.N., Sziksz, E., Tulassay, T., Vannay, A. (2010). Role of Th17 cells in rheumatoid arthritis. *Orv Hetil.* 151,1003-1010.
- Barnardo, M.C., Brown, M.A., Herzberg, I., Jaakkola, E., Kaarela, K., Kauppi, M. ...Wordsworth, B.P. (2006). Finnish HLA studies confirm the increased risk conferred by HLA-B27 homozygosity in Ankylosing Spondylitis. *Annals of the Rheumatic Diseases*, 65 (6), 775-80.
- Biddison, W.E., Carreno, B.M., Hansen, T.H., Taurog, J.D., Winter, C.C. *Int. Immunol* (1993). 5(4), 353-60
- Bitti, P.P., Carcassi, C., D'amato, M., Fiorillo, M.T., Mathieu, A., Sorrentino, R., Tosi, R., Zuccarelli, A. (1995). Relevance of residue 116 of HLA-B27 in determining susceptibility to Ankylosing Spondylitis. *European Journal of Immunology*. 25 (11), 3199-3201.
- Bourne, J., Huskisson, E., Kumar, P., Mageed, R., Unsworth, D., Wojtulewski. (1985). Arthritis and Coeliac Disease. *Annals of the Rheumatic Diseases*. 44, 592-598.
- Bowness, P., Fischer, R., Kessler, B., LaBaer, J., Sibani, S., Trudgian, D., Wright, C. (2010). Detection of Multiple Autoantibodies in Patients with Ankylosing Spondylitis Using Nucleic Acid Programmable Protein Arrays. *MCP Papers in Press*. Manuscript.

- Bradbury, L., Brophy, S., Brown, M.A., Calin, A., Cardon, L.R., Hamersma, J., & van der Horst-Bruinsma, I. (2001). Is disease severity in Ankylosing Spondylitis genetically determined? *Arthritis and Rheumatism*, 44 (6), 1396-400.
- Bradbury, L., Brophy, S., Brown, M.A., Calin, A., Crane, A. M., Herzberg, I., Jaakkola, E., Kaarela, K., Kauppi, M., Laiho, K., Sims, A., Toumilehto, J., Wordsworth, B.P. (2004). The effect of transforming growth factor *b1* gene polymorphisms in Ankylosing Spondylitis. *Rheumatology*. 43, 32–38
- Braun, A., Braun, J., Grifka, J., Saracbasi, E., & Schnitker, J. Identifying patients with axial spondyloarthritis in primary care: How Useful are items Indicative of Inflammatory Back Pain? (2011). *Ann Rheum Dis*. 70 (10) 1782-7.
- Brent, L. H., Kataria, R.K. (2004). Spondyloarthropathies. *Am Fam Physician*. 69 (12) 2853-2860.
- Brown, M.A. (2009). Progress in Spondylarthritis. Progress in studies of the genetics of Ankylosing Spondylitis. *Arthritis Research and Therapy*, 11 (5), 254.
- Brucke, L. J., Cooper, D., Cortinovis, D., Inman, R.D., Stone, M., Warren, R.W. (2005). Juvenile-onset Ankylosing Spondylitis is associated with worse functional outcomes than adult-onset Ankylosing Spondylitis. *Arthritis and Rheumatism*, 53 (3), 445-51.
- Burgos-Vargas, R., Castillo, E., Cardiel, M.H., Granados, J., Hernández-Pacheco, G., Londoño, J.D. ...Vargas-Alarcón, G. (2002). Effect of HLA-B and HLA-DR genes on susceptibility to and severity of spondyloarthropathies in Mexican patients. *Annals of the Rheumatic Diseases*, 61 (8), 714-7.
- Calin, A. (2005). BASDAI: Bath Ankylosing Spondylitis Disease Activity Index. Retrieved from: <http://www.basdai.com>

- Calin, A., Gaisford, P., Garret, S., Jenkinson, T., Kennedy, L.G., & Whitelock, H. (1994). A new approach to defining disease status in Ankylosing Spondylitis: The Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol.* 21:2286-2291.
- Cao, S., Gu, J., Huang, J., Huang, F., Li, C., Li, T. et al. (2009). Association of chromosome 2q36.1-36.3 and autosomal dominant transmission in Ankylosing Spondylitis: Results of genetic studies across generations of Han Chinese families. *Journal of Medical Genetics*, 46 (10), 657-62.
- Cauli, A., Fiorillo, M.T., Lapadula, G., Mathieu, A., Paladini, F., Passiu, G., Punzi, L., Scarpa, R., Sorrentino, R., Taccari, E. (2005). Distribution of HLA-B27 subtypes in Sardinia and continental Italy and their association with spondylarthropathies. *Arthritis Rheum.* 52 (10). 3319-21
- Cauli, A., Fiorillo, M.T., Magnacca, A., Mathieu, A., Nurzia, E., Paladini, F., Panimolle, F., Sorrentino, R. (2010). CD8+ T-cell mediated self-reactivity in HLA-B27 context as a consequence of dual peptide conformation. *Clinical Immunology.* 135, 476-482.
- Cederholm, T., Johansson, G., Kokkonen, H., Sundstrom, B., Wallberg-Johsson, S. (2011). Plasma phospholipid Fatty acid content is related to disease activity in Ankylosing Spondylitis. *J Rheumatol.* 39 (2), 327-33.
- Chen, D., Chen, H., Chen, T., Chen, Y., Ying-Ming, C. (2011). Gender differences in Ankylosing Spondylitis-associated cumulative healthcare utilization: A population-based cohort study. *Clinics.* 66(2), 251-254.
- Chiu, B., Haroon, R., Inman, N., Tsui, F., Tsui, H. (2010). Serum cytokine receptors in Ankylosing Spondylitis: Relationship to inflammatory markers and endoplasmic reticulum aminopeptidase polymorphisms. *J Rheumatol.* 37:1907-1910.

- Colbert, R.A., DeLay, M.L., Klenk, E.I., Layh-Schmitt, G. (2010). From HLA-B27 to spondyloarthritis: A journey through the ER. *Immunological Reviews*, 233 (1), 181-202.
- Colbert, R.A., DeLay, M.L., Layh-Schmitt, G., Sowders, D.P. (2009). HLA-B27 Misfolding and spondyloarthropathies. *Prion*, 3 (1), 15-26.
- Cruyssen, H.P., Elewaut, D., De Vos, M., Georges, M., Laukens, D., Libiouille, C., Mni, M., Peeters, H., Sandorz, C. (2010). Evidence for Significant Overlap between Common Risk Variants for Crohn's Disease and Ankylosing Spondylitis. *PLoS ONE* 5(11). Epublished. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2970560/pdf/pone.0013795.pdf>
- Davis, J.C. Jr, Learch, T.J., Lee, W., Reveille, J.D., Ward, M.M., Weisman, M.H. (2007). Are there gender differences in severity of Ankylosing Spondylitis? Results from the PSOAS cohort. *Annals of the Rheumatic Diseases*, 66 (5), 633-8.
- De Vries, M., Dijkmans, B., Hazenberg, B., Huizer, E., Nurmohamed, M., Peters, M., Van Eijk, I., Van de Stadt, R., Van der Horst-Bruinsma, E. (2009). Improvement of lipid profile is accompanied by atheroprotective alterations in high-density lipoprotein composition upon tumor necrosis factor blockade. *Arthritis & Rheum.* 60 (5) 1324-1330.
- Dougados, M., Gossec, L., Rostom, S. (2010). New tools for diagnosing spondyloarthropathy. *Joint Bone Spine.* 77:108-114.
- Drapé, J.L. (n.d.) MRI of Spondylarthropathies: Protocols and Pitfalls. University Paris Descartes, Sorbonne Paris Cité. Department of Radiology B, Hospital Cochin, Paris, France. <http://wjweiser.com/secure/iss/meetings/2011/2011%20CD/Presentation%20Abstracts/MUSCULOSKETEAL%20DAY%202/Drape.pdf>

- Ebringer, A., & Rashid, T. (2011). Gut-mediated and HLA-B27 associated arthritis: An emphasis on Ankylosing Spondylitis and Crohn's disease with a proposal for the use of new treatment. *Discovery Med.* 12(64), 187-94
- Elewaut, D., Matucci-Cerinic, M. (2009). Treatment of Ankylosing Spondylitis and extra-articular manifestations in everyday rheumatology practice. *Rheumatology.* 48(9), 1029-35
- Feltelius, N., Hedenstrom, H., Hillerdal, G., Hallgren, R. (1986). Pulmonary involvement in Ankylosing Spondylitis. *Annals of the Rheumatic Disease.* 45, 736-740
- Forger, F., Ostensen, M., Villiger, P.M. (2009). Pregnancy in patients with Ankylosing Spondylitis: Do regulatory T Cells play a role? *Arthritis Rheum.* 61 (2) 279-283.
- Fu-cheng, Z., Jie-ruo, G., Li, L., Qiu-jing, W., Qu, L., Shuang-yan, C., Tian-wang, L., Ze-tao, L., Shi-ming, L. (2009). Value of the peripheral blood B-cells subsets in patients with Ankylosing Spondylitis . *Chin Med J.* 122 (15), 1784-1789.
- Galocha, B., Lopez de Castro, J.A. (2010). Mutational analysis reveals a complex interplay of peptide binding and multiple biological features of HLA-B27. *J Biol Chem.* 285(50), 39180–39190.
- Geirsson, A.J., Gudbjornsson, B., Kristjansson, K. (2010). A strong familiarity of Ankylosing Spondylitis through several generations. *Annals of the Rheumatic Diseases,* 69 (7), 1346-8.
- Ghaffarpassand, F., Habibagahi, Z., Heiran, H.R., Nazarinia, M.A. (2009). Pattern of Ankylosing Spondylitis in an Iranian population of 98 patients. *Modern Rheumatology,* 19 (3), 309-15.



Gladman, D., Greenwood, C., Melay, W., Peddle, L., Rahman, P., Snelgrove, T., Sun, S. (2006).

Association between the interleukin-1 family gene cluster and psoriatic arthritis. *Arthritis Rheum.* 54(7), 2321-5.

Gladman, D.D., Inman, R.D., Maksymowych, W.P., Pope, A., Rahman, P., Reeve, J.P.

(2009). Association of a specific erap1/arts1 haplotype with disease susceptibility in Ankylosing Spondylitis. *Arthritis and Rheumatism*, 60 (5), 1317-23.

Goldsby, R.A., Kindt, T.J., Osborne, B. 2007. *Kuby Immunology* 6<sup>th</sup> Edition. NY, NY: W.H. Freeman and Co..

Gotlieb, D. Cox 1 and 2: The Cyclooxygenase System. <http://www.arthritis.co.za/cox.html>

Greenwood, C.M., Hu, P., Inman, R.D., Kim, T.J., Kim, T.J., Lee, H.J., Peddle, L., Rahman, P.

(2008). Interleukin 1 polymorphisms in patients with Ankylosing Spondylitis in Korea. *J. Rheumatol*, 35(8), 1603-8

Gu, J., Wu, Z. (2007). A meta-analysis on interleukin-1 gene cluster polymorphism and genetic susceptibility for Ankylosing Spondylitis. *Zhonghua Yi Xue Za Zhi.* 87.

Guzman, F. (May 2009). Mechanism of Action, Indications and Adverse Effects of: Etanercept, Infliximab and Adalimumab. Pharmacology Corner.

<http://pharmacologycorner.com/mechanism-of-action-indications-and-adverse-effects-of-etanercept-infliximab-and-adalimumab/>

Hascelik, Z., Inanici, F., & Ozdemir, O. (2011) Reduced vital capacity leads to exercise

intolerance in patients with Ankylosing Spondylitis. *Eur J Phys Rehabil Med.* 47, 391-397.

Hemminki, K., Li, X., Sundquist, J., Sundquist, K. (2009). Shared familial aggregation of

susceptibility to autoimmune diseases. *Arthritis and Rheumatism*, 60 (9), 2845-7.

- Hickling, P., Parkin, A., Robinson, P.J. (1982). Regional lung ventilation in Ankylosing Spondylitis. *Br J Radiol.* 55(659), 833-6
- Husby, G., & Gran, J.T. (1990). Ankylosing Spondylitis in women. *Seminars in Arthritis and Rheumatism*, 19 (5), 303-312.
- Husby, G., Ostensen, M., & Romberg, O. (1982). Ankylosing Spondylitis and Motherhood. *Arthritis Rheum.* 25(2), 140-3.
- Ishigame, H., & Iwakura, Y. (2006). The IL-23/IL-17 axis in inflammation. *J Clin Invest.* 116(5), 1218–1222.
- Jamil, K.M., & Salim I. K. (2011). KIR/HLA Interactions and Pathogen Immunity. *J. Biomed Biotechnol.* Published online.  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3100571/?tool=pubmed>
- Jessamine, A. M. (1968). Upper Lung Fibrosis in Ankylosing Spondylitis. *Canad Med Assoc J.* 98.
- Jimenez-Balderas, F.J., Madero-Cervera, J.I., Mintz, G., Murrieta, S., Tapia-Serrano, R. (1990). Ovarian function studies in active Ankylosing Spondylitis in women. Clinical response to estrogen therapy. *J Rheumatol.* 17(4), 497-502
- Kimball, J.W. (2011) Histocompatibility Molecules. Kimball's Biology Pages. Accessed [http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/H/HLA.html#Class\\_I\\_Histocompatibility\\_Molecules](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/H/HLA.html#Class_I_Histocompatibility_Molecules)
- Koncz, A., Nagy, G., & Perl, A. (2003). T Cell Activation-Induced Mitochondrial Hyperpolarization Is Mediated by  $Ca^{2+}$ - and Redox-Dependent Production of Nitric Oxide. *The Journal of Immunology.* 171, 5188-5197

- Lee, W., Reveille, J.D., & Weisman, M.H. (2008). Women with Ankylosing Spondylitis: A Review. *Arthritis & Rheum.* 59 (3), 449-454.
- Matsumoto, A.K., Bathon, J.B., & Clifton, O.B. (n.d.). Rheumatoid Arthritis treatment. The Johns Hopkins Arthritis Center. [http://www.hopkins-arthritis.org/arthritis-info/rheumatoid-arthritis/rheum\\_treat.html](http://www.hopkins-arthritis.org/arthritis-info/rheumatoid-arthritis/rheum_treat.html)
- Momeni, M., Taylor, N., & Tehrani, M. (2011). Cardiopulmonary Manifestations of Ankylosing Spondylitis. *Int J Rheumatol.* Epublished.  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3087354/?tool=pubmed>
- Oldstone, M.B.A., Schwimmbeck, P.L., & Yu, D.T. (1987). Autoantibodies to HLA B27 in the sera of HLA B27 patients with Ankylosing Spondylitis and Reiter's syndrome. *J Exp Med.* 166, 173-181.
- Rosenbaum, J.T., & Smith, J.R. (2002). Anti-TNF therapy for eye involvement in spondyloarthropathy. *Clin Ex Rheumatol.* 20(6 Suppl 28):S143-5.
- Roussou, E., & Sultana, S. (2010). Spondyloarthritis in women: differences in disease onset, clinical presentation, and Bath Ankylosing Spondylitis Disease Activity and Functional indices (BASDAI and BASFI) between men and women with spondyloarthritides. *Clinical Rheumatology.* Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/20882310>
- Sheehan, N.J. (2004). The Ramifications of HLA-B27. *Journal of the Royal Society of Medicine.* 97.