

**ABSTRACT:** Sporadic inclusion-body myositis (sIBM) is the most common acquired muscle disease in Caucasians over the age of 50 years. Pathologically it is marked by inflammatory, degenerative, and mitochondrial changes that interact in a yet-unknown way to cause progressive muscle degeneration and weakness. The cause of the disease is unknown, but it is thought to involve a complex interplay between environmental factors, genetic susceptibility, and aging. The strongest evidence for genetic susceptibility comes from studies of the major histocompatibility complex (MHC), where different combinations of alleles have been associated with sIBM in different ethnic groups. The rare occurrence of familial cases of inclusion-body myositis (fIBM) adds additional evidence for genetic susceptibility. Other candidate genes such as those encoding some of the proteins accumulating in muscle fibers have been investigated, with negative results. The increased understanding of related disorders, the hereditary inclusion-body myopathies (hIBM), may also provide clues to the underlying pathogenesis of sIBM, but to date there is no indication that the genes responsible for these conditions are involved in sIBM. This review summarizes current understanding of the contribution of genetic susceptibility factors to the development of sIBM.

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## GENETICS OF INCLUSION-BODY MYOSITIS

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**S**poradic inclusion-body myositis (sIBM) is the most common acquired muscle disease associated with aging but may occasionally also occur in younger individuals. Classically it causes a selective pattern of muscle weakness involving the forearm flexor and quadriceps femoris muscles, often with later involvement of the distal leg, proximal arm, and pharyngeal muscles resulting in dysphagia.<sup>1,23,40,53,101</sup> Pathologi-

cally it is characterized by an intramuscular inflammatory component of variable severity, with a predominance of CD8<sup>+</sup> T-cells which are clonally expanded,<sup>89</sup> and upregulation of major histocompatibility complex (MHC) class I antigen in nonnecrotic muscle fibers.<sup>66</sup> There is also evidence of mitochondrial involvement as evidenced by segmental deficiency of cytochrome *c* oxidase (COX-deficient fibers) and ragged-red fibers (RRF),<sup>97</sup> as well as a degenerative component with rimmed vacuole formation, tubulofilamentous inclusions, and eosinophilic inclusions in muscle fibers.<sup>19</sup> The protein inclusions are made up of a number of Alzheimer-type proteins including  $\beta$ -amyloid and the amyloid precursor protein (APP), phosphorylated tau,  $\alpha$ -1-antichymotrypsin, ( $\alpha$ 1ACT),  $\alpha$ -synuclein, prion protein, and apolipoprotein E (ApoE).<sup>17</sup>

Recent research has highlighted the importance of both the inflammatory and the degenerative processes in the pathogenesis of sIBM, but the manner in which, or indeed whether, these pathological features interact remains uncertain. On the one hand, local expression of proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor alpha

**Abbreviations:** AAA-ATPase, ATPases associated with a variety of activities; AGER, advanced glycosylation end product-specific receptor; AH, ancestral haplotype; AD, autosomal-dominant; APP, amyloid precursor protein; ApoE, apolipoprotein E; AR, autosomal-recessive; COX, cytochrome *c* oxidase; DMRV, distal myopathy with rimmed vacuoles; ER, endoplasmic reticulum; fIBM, familial inclusion-body myositis; hIBM, hereditary inclusion-body myopathy; HLA, human leukocyte antigen; IBMPFD, IBM associated with Paget's disease and frontotemporal dementia; IL-1, interleukin 1; IFN- $\gamma$ , interferon gamma; LAMA2, laminin  $\alpha$ 2; LGMD, limb-girdle muscular dystrophy; MHC, major histocompatibility complex; OPMD, oculopharyngeal muscular dystrophy; PABN, polyadenylate-binding protein; RRF, ragged-red fibers; sIBM, sporadic inclusion-body myositis; TCR, T-cell receptor; TNF- $\alpha$ , tumor necrosis factor alpha; VCP, valosin-containing protein

**Key words:** genes; genetics; inclusion-body myopathy; inclusion-body myositis

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(TNF- $\alpha$ ), and interferon gamma (IFN- $\gamma$ ) could be an early upstream pathogenic event causing both the inflammatory and degenerative components of sIBM, as hypothesized by Dalakas in 2006.<sup>31</sup> Proinflammatory cytokines are very effective inducers of MHC class I expression in human myotubes.<sup>86</sup> It has been suggested that MHC class I expression exerts a stressor effect on the endoplasmic reticulum (ER) causing NF $\kappa$ B upregulation,<sup>92</sup> leading to further enhancement of MHC class I antigen assembly and cell membrane expression,<sup>92</sup> which may in turn lead to a self-sustaining T-cell response.<sup>91</sup> Proinflammatory cytokines (particularly IL-1),<sup>49,55</sup> as well as NF $\kappa$ B<sup>54</sup> have been shown to increase APP transcription, which results in increased  $\beta$ -amyloid production. This could then cascade, causing a cycle of ER stress, proteasome dysfunction, and protein accumulation.

On the other hand, it is possible that  $\beta$ -amyloid accumulation (due to overproduction or abnormalities in processing in APP) is an early upstream event as proposed by Askanas and Engel,<sup>20</sup> causing ER stress, oxidative stress, and a T-cell response to peptides derived from the accumulating proteins. This relationship between the inflammatory and degenerative processes is supported by observations that in sIBM muscles there is a linear relationship between the mRNA level of cytokines and chemokines, and that of APP, tau, and ubiquitin.<sup>111</sup> Genes encoding APP, tau, ApoE, and oxidative stress proteins are overexpressed in sIBM, but as with their respective proteins, these genes are also known to be overexpressed in polymyositis, hereditary inclusion-body myopathy (hIBM), and other myopathies.<sup>52,106</sup> The ultimate cause of the postulated proinflammatory cytokine expression or  $\beta$ -amyloid overproduction is still unknown.

Multiple genetic factors may contribute to the development and progression of sIBM. The prevalence of sIBM differs between different ethnic groups, with a seemingly reduced incidence in Korean, Mesoamerican Mestizos, Polish,<sup>114</sup> Middle Eastern, and Southern Mediterranean populations as compared with North American Caucasians, Dutch, and Australians, in whom prevalence figures of between 4.9–9.3 per million have been reported.<sup>21,40,100</sup> These differences in prevalence may be due to differences in genetic makeup of different racial groups, differences in environmental factors, or a combination of both. Particular genetic predisposing factors may determine the effects of different inducing agents, which may vary in their geographical distribution.

When investigating genetic associations with presumed autoimmune diseases, including the inflam-

matory myopathies, the most commonly considered genes are those that encode immunologically relevant proteins, particularly those in the MHC, the T-cell receptor (TCR) genes, and the immunoglobulin genes. So far the search for susceptibility genes in sIBM has been based solely on candidate gene studies, as the rarity of the condition has precluded the use of more robust genetic methods such as twin studies, whole genome screening, and transmission disequilibrium testing. Multiple case reports of two or more siblings being affected in the one family, and rare reports of affected twins<sup>2</sup> clearly suggest a familial predisposition for developing sIBM. So far, MHC associations provide the strongest evidence for a genetic component in sIBM. Unique MHC associations are seen with different racial groups and may reflect different T-cell receptor repertoires or different inducing agents. Identifying the susceptibility gene in the MHC will be extremely important in helping to elucidate the pathogenesis of sIBM. Although the MHC contains many genes that influence immune function, it also contains a range of other genes that encode proteins involved in a number of basic cellular and regulatory functions. It will be of great interest to determine whether this MHC-associated susceptibility gene is involved in determining the immune and inflammatory component of the disease, or is involved in other cellular functions that influence muscle degeneration.

In addition to MHC associations, polymorphisms and mutations in genes encoding the deposited proteins including APP,<sup>115</sup> prion protein,<sup>98</sup> ApoE,<sup>12,48,56</sup> and  $\alpha$ 1ACT,<sup>50</sup> as well as multiple mitochondrial mutations,<sup>62,96</sup> have all been investigated. Clues for further candidate gene studies are arising from the progress that has been made over the last decade in identifying genes associated with hIBM and other vacuolar myopathies that have features similar to sIBM (see below), as well as gene expression profiling studies.<sup>52</sup> The identification of susceptibility genes is fundamental to elucidating the pathogenesis of sIBM and will provide clues to the development of targeted therapies.

#### HEREDITARY INCLUSION-BODY MYOPATHIES

Hereditary IBM is a term introduced in 1993<sup>10</sup> to describe a group of autosomal-dominant (AD) or autosomal-recessive (AR) adult-onset muscle disorders, which have variable clinical phenotypes but have a number of pathological features resembling sIBM, including rimmed vacuoles and intracytoplasmic and intranuclear tubulofilamentous inclusions. Clinically there is an earlier age of onset and the

rarity of inflammatory changes and negative MHC class I staining on the muscle biopsy help distinguish these forms from sIBM.<sup>53,116</sup>

**Autosomal-Recessive hIBM.** Several different types of hIBM have been described under various names in different ethnic groups.<sup>38</sup> The prototypic AR form (IBM2; OMIM 600737) first described in Iranian Jews by Argov and Yarom<sup>3</sup> was characterized in Jews of Persian descent as a quadriceps-sparing myopathy. Genomic linkage was established among the Iranian Jews in 1996,<sup>88</sup> the genomic susceptibility region was subsequently narrowed to 9p13,<sup>36</sup> and finally a UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (*GNE*) founder gene mutation (M712T) was found in Persian Jews.<sup>37</sup> This discovery led to confirmation that the Japanese muscle disease previously called Nonaka myopathy or distal myopathy with rimmed vacuoles (DMRV) is the same disease when a variety of homozygous or compound-heterozygous mutations were described in either the epimerase or kinase domains of the *GNE* gene.<sup>68</sup> Many different *GNE* missense mutations have now been described in various ethnic groups, including people from Italy, the US, Germany, Ireland, Bahamas, Tunisia, and East India.<sup>38</sup> A number of variations in phenotypes have also been described. These include forms in which the quadriceps are involved,<sup>6</sup> as well as pathological differences including the presence of muscle inflammation in rare cases.<sup>6,75,124</sup> It has been hypothesized that these differences are mutation specific.<sup>33</sup>

*GNE* is a bifunctional enzyme that catalyzes the first two steps in the synthesis of N-acetylneuraminic (sialic) acid. *GNE* consists of two functional domains: a UDP-GlcNAc-2-epimerase domain and a ManNAc kinase domain. In hIBM, mutations in both domains have been reported,<sup>38</sup> resulting in reduction in both enzymatic activities<sup>99</sup> that may lead to a disturbance of sialic acid metabolism.<sup>51</sup> Nishino et al.<sup>94</sup> showed that the loss of function in the *GNE* enzyme can be recovered by adding *GNE* metabolites such as ManNAc and NeuAc. Sialic acid is considered to play important roles in several cellular processes including cellular adhesion,<sup>99</sup> formation of or marking recognition sites for toxins and other pathogenic agents, stabilization of glycoprotein structures, signal transduction, and cell-mediated immune responses.<sup>27</sup> Whether hyposialation of glycoproteins in skeletal muscle plays a role in the pathogenesis of hIBM, and why mutations in *GNE* affect predominately the skeletal muscle, remain to be determined.

To date, more than 40 different mutations in the *GNE* gene have been reported to cause AR hIBM.<sup>99</sup> Interestingly, homozygous missense *GNE* mutations have been reported<sup>6,94</sup> in some clinically unaffected individuals, indicating that other factors play a role in the pathogenesis of disease. Such factors, which are not yet elucidated, seem to be important in the clinical expression of disease in all patients; although the gene defect is present from conception, it may not result in clinically manifest disease until the third or fourth decade. Mutations in *GNE* have not been found in cases of sIBM,<sup>121</sup> but the possibility that other variations in the gene may influence susceptibility to sIBM has not been investigated.

**AR hIBM without *GNE* Mutation.** There have been case reports of families with AR IBM not associated with a *GNE* gene mutation. Cole et al.<sup>28</sup> in 1988 described an AR familial proximal early-onset quadriceps-involving myopathy with histological changes resembling IBM and an asymptomatic periventricular leukoencephalopathy, which was not linked to 9p1-q1.<sup>4</sup> A similar case was reported by Di Blasi et al.<sup>35</sup> These authors described a 29-year-old man with a leukoencephalopathy and a vacuolar myopathy resembling IBM that was due to a partial laminin  $\alpha$ 2 chain deficiency secondary to two novel *LAMA2* gene mutations.

**Autosomal-Dominant Forms.** AD forms of hIBM have also been described (IBM1 and IBM3; OMIM 601419 and 605637). IBM3 was previously known as "AD myopathy with congenital joint contractures, ophthalmoplegia and rimmed vacuoles." It was reported in 1998 by Darin et al.,<sup>32</sup> and subsequently mapped to 17p13,<sup>83</sup> and found to be due to a mutation in the myosin heavy chain IIa (*MHCIIa*) gene<sup>84</sup> (Glu706-Lys). In this condition only minor abnormalities are seen in childhood and adolescence, from 30–50 years of age; however, there is progressive limb-muscle weakness, with atrophy being most prominent in the pectoralis and quadriceps muscles.

Another AD IBM associated with Paget's disease and frontotemporal dementia (IBMPFD) has been described in four American families<sup>74</sup> and in an Austrian family,<sup>58</sup> and has been linked to a mutation in the valosin-containing protein (*VCP*) gene on chromosome 9p13-p12. *VCP* is a member of the "ATPases associated with a variety of activities" (AAA-ATPase) family, a group of enzymatic molecular chaperones that have been associated with a variety of cellular processes such as ubiquitin-proteasome-mediated degradation, membrane fusion, apoptosis, and cell-cycle control.<sup>58</sup> A number of independent

studies have confirmed that disruption of a specific function of VCP leads to inclusion-body formation and ultimately cell death.<sup>123</sup>

There are other AD-inherited vacuolar myopathies that have been put into this category. These include most of the distal myopathies, including Wexler's distal myopathy (OMIM 604454), which is linked to chromosome 2p13, and tibial muscular dystrophy (Udd myopathy; OMIM 600334), which is due to mutations in the titin gene (2q31).

**Other Rimmed Vacuolar Myopathies.** The differential diagnosis when rimmed vacuoles are present in muscle is wide and includes all of the above conditions, as well as oculopharyngeal muscular dystrophy (OPMD, polyadenylate-binding protein nuclear 1 (*PABPN1*) mutations), X-linked Emery–Dreifuss muscular dystrophy<sup>44</sup> (emerin gene mutations), LGMD2G (telethonin mutations), LGMD1A (myotilin mutations), LGMD1G (Chr 4p21), and rigid spine syndrome. In addition, rimmed vacuoles may even be found in chronic neurogenic conditions such as old poliomyelitis<sup>43,113</sup> or chronic spinal muscular atrophy.<sup>45</sup> However, the clinical presentation, as well as the content of the vacuoles, helps to distinguish these conditions. Askanas and Engel<sup>18</sup> reported that the unusual constellation of proteins found in the inclusions of sIBM and hIBM do not occur in either the vacuolated or nonvacuolated fibers of other muscle diseases except OPMD. However, there is extensive overlap, as the intracellular accumulation of amyloid-related proteins, APP, phosphorylated tau, presenilin-1, apolipoprotein-E,  $\gamma$ -tubulin, clusterin,  $\alpha$ -synuclein, gelsolin, oxidative stress proteins, and all the components of the catalytic core of the proteasomes have been found to be equally expressed in sIBM and the myofibrillar myopathies.<sup>41,42</sup> Therefore, different etiologies may lead to a common downstream pathogenic cascade that is responsible for the muscle degeneration, similar to the concept that in Alzheimer's disease different genetic factors and unknown causes of sporadic Alzheimer's disease lead to the same pathological findings.

Thus, the group of diseases encompassed by the term hIBM is heterogeneous, both clinically and genetically, and may or may not provide clues to the underlying etiology and pathogenesis of sIBM.

#### **FAMILIAL INCLUSION-BODY MYOSITIS (fIBM)**

Familial IBM is associated with the typical clinical and histopathological findings that are seen in sIBM, but occurs in siblings in the same generation or, rarely,

with a dominant pattern of transmission.<sup>85,93</sup> It differs from hIBM with respect to the age of onset, the selective pattern of clinical muscle involvement (the quadriceps and long finger flexor muscles as seen in sIBM<sup>117</sup>), and typical histological features.<sup>108</sup> It has been described in several families,<sup>59,108,117,119</sup> and in some families the disease is associated with particular human leukocyte antigen (HLA) alleles. In particular, fIBM has been associated with DR3(DRB1\*0301/0302)<sup>108,117</sup> and DR15(2)/4 (DRB1\*1502/0405).<sup>119</sup> This association with DR3 indicates that fIBM is not only phenotypically and histologically identical to sIBM, but is also associated with similar genetic markers, raising the possibility that the two subsets of IBM share the same inherited determinants of susceptibility to the inflammatory process.<sup>117</sup> The familial occurrence of such a rare disease highlights the importance of genetic predisposition in the etiology and pathogenesis of sIBM.

#### **MAJOR HISTOCOMPATIBILITY COMPLEX**

The MHC is densely packed with immunologically important genes. The extended MHC has been associated with over 100 diseases, most of which are immune-mediated. This is not surprising, given that 28% of the 252 transcripts encoded by genes in the extended MHC are immune-related.<sup>61</sup> The association of polymorphic variants of genes within the MHC with autoimmune disease often relates directly to the important role that MHC molecules play in regulating the types and degree of immune responses to environmental agents, T-cell receptor repertoire development, and peripheral tolerance to self antigens.<sup>95</sup> It has been speculated that dysregulation of MHC-mediated immunological recognition events contributes to a breakdown of self-tolerance and the resultant development of autoimmune pathology. However, the MHC also contains many genes whose function is not related to the immune system, and altered function of these genes may lead to disease by nonimmunological mechanisms (e.g., 21-hydroxylase deficiency<sup>82</sup>). Detailed intra-MHC mapping is often required to identify the causative gene once an association with the MHC has been described.

A hallmark of the MHC is the existence of strong linkage disequilibrium, which is manifest in the concurrence of certain alleles at a number of loci within the MHC more frequently than expected by chance. This strong linkage can facilitate the identification of indirect disease associations but sometimes confounds the identification of causative genes. This concurrence of certain combinations of alleles on

**Table 1.** Frequency of DR3 (DRB1\*0301) and HLA-B8 alleles in sIBM.

Reference no.	Frequency of DR3 in sIBM (control population)	Frequency of HLA-B8 in sIBM (Control population)	Country of study
80	52% (not stated)	Not reported	USA
47	93% (15%)	75% (28%)	Australia
71	77% (15%)	Not reported	USA
78	27% (10%)	21% (9%)	Germany
22	79% (25%)	69% (23%)	Netherlands
104	74% (28%)	67% (29%)	Australia

specific haplotypes allows the definition of a series of ancestral haplotypes (AH) that have been conserved during evolution and occur commonly throughout the population.<sup>34</sup> The concept of the AH predicts that haplotypes from unrelated people who share certain marker alleles (at loci such as HLA-B and HLA-DR) will be identical for the alleles at loci between those markers.<sup>72</sup> Although AHs are highly conserved, recombination between different AHs has occurred during evolution so that the presence of varying lengths of individual AH on unrelated haplotypes is not uncommon. These recombinant AHs can be used to localize the gene or genes in the MHC associated with a particular disease.<sup>103</sup>

Diseases that have been demonstrated to have MHC associations include type 1 diabetes, ankylosing spondylitis, Graves' disease, Addison's disease, and myasthenia gravis. In some cases the MHC association is strongest with an individual allele (such as HLA-B27 in ankylosing spondylitis); in other cases the associations are with alleles or combinations of alleles that form part of a conserved AH. In particular, the AH characterized by HLA-A\*01, -B\*0801, -DRB1\*0301, -DQB1\*0201, -DQA1\*05 (referred to as the 8.1 AH), is considered the "autoimmune haplotype" in Caucasians because it has been associated with many different autoimmune diseases.<sup>103</sup> The 8.1 AH is common in Caucasian populations, particularly those of Northern European origin. This haplotype has been associated with a number of variations in immunological function<sup>103</sup> including an alteration in the cytokines produced, as well as differences in the early stages of cellular activation.

#### MAJOR HISTOCOMPATIBILITY COMPLEX AND SPORADIC INCLUSION-BODY MYOSITIS

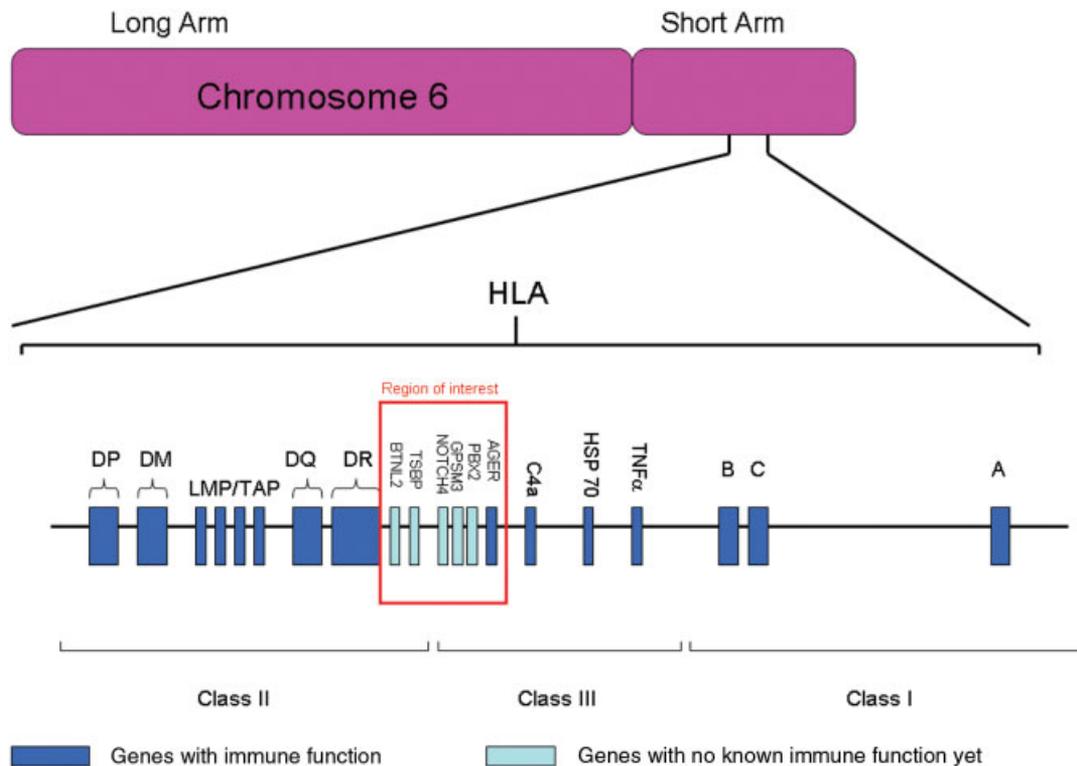
The strong association of HLA-DR3 and the extended 8.1 AH with sIBM was first reported in 1994 by Garlepp et al.<sup>47</sup> This association between sIBM and alleles characteristic of 8.1 AH has subsequently been confirmed in a number of studies<sup>22,71,78,104</sup> (Table 1). Identification of the critical MHC genes is

hampered by very strong linkage disequilibrium in the 8.1 AH, which extends relatively stably over a distance of at least 3 Mb.

In considering the HLA data available, it became clear that within the MHC the association with sIBM was stronger with HLA-DR3 than with HLA-B8, so that the region of interest was considered to be closer to HLA-DR than to HLA-B (Garlepp et al., unpubl.). Further refinement by mapping of recombinant AH in patients with sIBM using the HSP70 and TNF markers narrowed the region to between HLA-DR and complement C4<sup>72</sup> (Fig. 1). Subsequent work by the same group enabled the exclusion of genes centromeric of HLA-DR and DQ, at least in Caucasians.<sup>103</sup> Fine mapping using microsatellites and SNPs has narrowed the susceptibility region to between PBX2 and HLA-DRB1.<sup>104</sup>

In considering the identification of an MHC-encoded susceptibility gene for sIBM, it is important to consider the possible disease mechanisms. As indicated above, pathological features of the disease include accumulation of a range of proteins, particularly  $\beta$ -amyloid, vacuolar degeneration, and an inflammatory infiltrate, characterized by a predominance of CD8<sup>+</sup> T-cells, which are often intimately associated with muscle fiber membranes. CD8<sup>+</sup> T-cells interact with class I MHC antigens on target cells, and immunohistochemical staining of biopsies from patients with sIBM often reveals upregulation of MHC class I antigen expression on muscle cells. MHC class II overexpression on muscle cells is not a commonly reported feature of sIBM, so that a direct role for MHC class II as a target for T-cell damage is unlikely. However, an indirect role via antigen presentation by antigen-presenting cells cannot be excluded.

It is not clear whether the inflammation seen in sIBM is causative of the muscle degeneration or is a response to the degeneration and abnormal accumulation of the proteins characteristic of the disease. Indeed, some early data suggested that the degenerative process can continue despite control of



**FIGURE 1.** Schematic representation of the MHC showing susceptibility region in sIBM.

the inflammatory response, a possible explanation for the reported lack of efficacy of corticosteroids in the treatment of sIBM.<sup>24</sup> The association with the 8.1 AH may reflect the influence of this haplotype on the inflammatory response but, given the range of genes in the MHC that control nonimmunological functions and the detailed mapping that has focused interest in the 8.1 AH to a region telomeric of DRB1, it is appropriate to consider genes without an obvious immunological function that are located in this region.

A few genes within this region have been suggested as candidates for further study, including *BTNL2* (butyrophilin-like MHC class II-associated gene, which is expressed in skeletal muscle), *TSBP* (testis-specific basic protein), *NOTCH4* (a transmembrane receptor that regulates cell fate decisions), *GPSM3* (a predicted gene with an unknown function previously known as G18), and *AGER* (advanced glycosylation end product-specific receptor, previously known as *RAGE*).<sup>72,104</sup>

*AGER* is located approximately 150 kb centromeric to the *C4* gene (Fig. 1) and is a member of the immunoglobulin superfamily of cell-surface molecules. It acts as a binding site for advanced glycation end products (which are formed as a consequence of irreversible glycation of protein or lipids) as well as

the  $\beta$ -amyloid fragment of APP. Increased expression of *AGER* has been demonstrated in the brains of patients with Alzheimer's disease, and in vitro binding of  $\beta$ -amyloid to *AGER* expressed in neurons has been shown to be neurotoxic.<sup>125</sup> *AGER*-mediated activation of NF $\kappa$ B has also been observed in various cell types, particularly endothelial cells and mononuclear leukocytes.<sup>57</sup> *AGER* protein is upregulated in regenerating muscle fibers and mononuclear cells in polymyositis and dermatomyositis<sup>57</sup> but has not been reported in sIBM.

In addition to the 8.1 AH, other AH and alleles have been associated with sIBM. In the US, Love et al.<sup>80</sup> reported an association of sIBM with HLA-DR52, which probably reflected the association with the 8.1 AH, although DR52 is also part of a number of other AHs. Price et al.<sup>104</sup> found that the 35.2 AH (defined by DR1, *BTNL2*(E6)\*2, *PBX2*\*T, *AGER*\*T, and B35) was increased in sIBM and suggested that this haplotype may also confer susceptibility to sIBM in Caucasians. In Japanese sIBM patients, Scott et al.<sup>112</sup> reported an association with HLA-B\*5201 and HLA-DRB1\*1502, which are found together as part of the conserved 52.1 AH (defined as HLA-A\*2402, Cw\*1202, B\*5201, DRB1\*1502, DQA1\*0103, DQB1\*0601). In the Japanese population, the 52.1 AH is also associated with susceptibility to Takayasu's

arteritis<sup>69,70</sup> and ulcerative colitis.<sup>118</sup> Association with the 52.1 AH was also found in an Ashkenazi Jewish patient with sIBM.<sup>85</sup> The 8.1, 35.2, and 52.1 AHs do not share any common alleles, which suggests that the genes responsible for disease etiology are not HLA genes per se, but reside elsewhere in the MHC.<sup>112</sup> Alternatively, of course, this observation may reflect differences in inducing agents or background genes at other loci that interact with different MHC AHs.

In addition, a study by O'Hanlon et al.<sup>95</sup> of 571 North American Caucasian myositis patients, including 53 sIBM cases, confirmed the association of sIBM with multiple alleles including DRB1\*0101, DQA1\*0101, HLA-A\*03, B\*35, and Cw\*04. They also reported that DQA1\*0201 allele and the associated peptide-binding motif (<sup>47</sup>KLPLFHRL<sup>54</sup>) and the DRB1\*04-DQA1\*03 haplotype were protective factors for sIBM. Badrising et al.<sup>22</sup> in a study of 52 Dutch IBM patients also reported that HLA-DR53 may be protective. HLA-DR53 is the alternative allele to DR52 at the DRB3 locus and so the decrease in its frequency may reflect the increase in DR52 in sIBM. It is sometimes difficult to ascribe a protective role for HLA alleles in the presence of a very high frequency of particular alleles in disease.

Thus, to date, at least three AHs have been associated with sIBM, with the 8.1 AH being the predominant one in Caucasians. Identification of the actual genes involved will be important in helping to elucidate the pathogenesis of sIBM. Sporadic IBM is a complex disease, and although the MHC contains important genetic determinants, there are clearly other susceptibility genes and environmental factors that are likely to play a role in disease development.

#### **GENES ENCODING PROTEINS CHARACTERISTIC OF SPORADIC INCLUSION-BODY MYOSITIS**

In general, the aggregation of proteins in any tissue may be a consequence of increased transcription, impaired breakdown, inappropriate protein assembly, aberrant modification, or environmental stress.<sup>67</sup> Askanas and Engel<sup>20</sup> proposed in a recent review that the formation of the characteristic ubiquitinated multiprotein inclusions in sIBM muscle is due primarily to protein unfolding and misfolding related to  $\beta$ -amyloid accumulation, rather than increased transcription of the various proteins. Unfolded or misfolded proteins are thought to aggregate due to the interaction of partly unfolded or misfolded polypeptides by virtue of inappropriately exposed hydrophobic surfaces.<sup>39</sup> Correctly folded proteins are soluble or remain localized to

intracellular structures or associated with cell membranes. Amino acid sequence variation as a consequence of gene mutation has the potential to trigger such events; therefore, candidate susceptibility genes for sIBM include those encoding the deposited proteins.

More than 20 proteins that have a propensity to unfold, misfold, and form  $\beta$ -pleated sheet amyloid have been found to accumulate in muscle fibers in sIBM. Thus far, a number of genes related to the various "Alzheimer-type" proteins as well as some that have been identified as potential susceptibility genes for Alzheimer's disease have been studied as candidate genes for sIBM. Although these studies have not yet revealed any definite susceptibility genes, the existing data are summarized here.

The expression of a number of nonimmune genes is reportedly increased in sIBM muscle based on differential expression at the protein level, mRNA level, or both. These include  $\beta$ -amyloid precursor protein,<sup>109</sup> phosphorylated tau,<sup>11</sup> ubiquitins,<sup>7</sup> prion protein,<sup>110</sup> alpha 1 antichymotrypsin ( $\alpha$ 1ACT),<sup>26</sup> ApoE,<sup>87</sup> copper zinc superoxide dismutase (*SOD1*),<sup>13</sup> and manganese superoxide dismutase (*SOD2*),<sup>120</sup> apoptotic regulators Bcl-2, Bcl-x, and BAX,<sup>102</sup> and lipoprotein receptors.<sup>63</sup> Microarray studies, such as that done by Greenberg et al.,<sup>52</sup> confirmed the increased expression, at the mRNA level, of  $\beta$ -amyloid, ApoE, SOD2, BAX, low-density lipoprotein receptors, and low-density lipoprotein receptor-related protein, but also found all of these genes overexpressed in other inflammatory myopathies, some at much higher-fold ratios than in sIBM. Furthermore, they found tau expression to be increased 6.8-fold in dermatomyositis and  $\alpha$ 1ACT 6.8-fold in polymyositis compared to healthy muscle. Furthermore, a recent study<sup>107</sup> showed that the genes encoding most of the proteins that aberrantly accumulate in the muscle fibers in desmin myopathy are not upregulated or altered compared to hIBM. To the contrary, genes encoding  $\alpha$ -actin and gelsolin are more upregulated in hIBM than desmin myopathy muscle. These findings are consistent with the hypothesis that many of the nonimmune changes at the level of mRNA expression seen in IBM are non-specific and may also be seen in other myopathies, suggesting that abnormal accumulation of these proteins is due to posttranscriptional events. Microarray technology measuring levels of mRNA allows identification of genes that are upregulated and downregulated, but provides no information regarding cause or effect. In addition, it is limited by available knowledge of gene function and sequence and the accuracy of gene databases. However, these tech-

niques can be useful in identifying further candidate genes for study.

### **$\beta$ -AMYLOID**

It has been established that  $\beta$ -amyloid, the C and N-terminal regions of APP, and APP mRNA accumulate in both sIBM and hIBM,<sup>8</sup> with the  $\beta$ -amyloid42 isoform being more common than  $\beta$ -amyloid40.<sup>20</sup>  $\beta$ -Amyloid42 is more hydrophobic, more prone to self association and oligomer formation to generate beta-pleated sheet amyloid, and more cytotoxic than  $\beta$ -amyloid40,<sup>29</sup> and has been correlated with apoptotic cell death.<sup>105</sup> The accumulation of APP and its fragments is often stated to precede other abnormalities in IBM muscle fibers, including congophilia,<sup>16</sup> suggesting that it plays an important early role in disease. Moreover, both cell culture<sup>14</sup> and transgenic mouse models overexpressing APP<sup>46,64</sup> display some of the key features of IBM. The mechanism of overproduction is not yet fully elucidated, but some of the genes responsible for early-onset Alzheimer's disease have been investigated.

Mutations in exons 16 and 17 of the APP gene on chromosome 21 have been identified in patients with early-onset familial Alzheimer's disease. Similar studies in sIBM and hIBM did not find mutations in these exons<sup>115</sup> (Garlepp et al., unpublished data), although this does not completely exclude the possibility that a mutation could be present elsewhere in the gene. It is of interest, however, that individuals with trisomy 21 who have an extra copy of the APP gene and universally develop Alzheimer's disease by the age of 40 years, and sometimes as young as 20 years,<sup>122</sup> are not reported to have a higher incidence of sIBM.

Mutations in the presenilin 1 (*PS1*) and presenilin 2 (*PS2*) genes are also responsible for familial early-onset Alzheimer's disease. Mutations in these genes lead to selective increase in the levels of  $\beta$ -amyloid42 species.<sup>122</sup> Although PS1 accumulates in the brain in both sporadic and familial Alzheimer's disease, missense mutations are not found in the sporadic form of the disease. In sIBM, although PS1 protein has been demonstrated immunohistochemically in muscle fibers in both hIBM and sIBM,<sup>15</sup> mutations in the *PS1* or *PS2* genes have not yet been investigated in either hIBM or sIBM.

### **APOLIPOPROTEIN E**

ApoE protein but not mRNA has been found in both hIBM and sIBM inclusions.<sup>87</sup> The *apoE*  $\epsilon 4$  allele is known to be a risk factor for developing late-onset

sporadic Alzheimer's disease. Although one study found an increased frequency of the  $\epsilon 4$  allele in sIBM,<sup>48</sup> four other studies have not replicated this finding.<sup>12,50,56,81</sup> The differences between these studies may reflect genetic heterogeneity in this disease, and the fact that genetic predisposition to the development of IBM is probably multifactorial. If *ApoE*  $\epsilon 4$  is a predisposing factor to the development or progression of sIBM, then it is clearly not the dominant factor and may have its effect via interaction with other genetic and environmental influences. The elucidation of whether *ApoE* alleles actually influence the development of sIBM is difficult, largely because of difficulties in obtaining a control group who have all the risk factors for developing sIBM but do not manifest the disease.<sup>25</sup> However, it remains that significant numbers of patients are negative for the *ApoE*  $\epsilon 4$  allele, emphasizing the likelihood that other factors contribute more strongly to disease pathogenesis.

### **$\alpha$ -1-ANTICHYMOTRYPSIN**

$\alpha$ 1ACT is considered an acute-phase protein that belongs to the serpin superfamily and inhibits serine proteases. Like ApoE, it binds to  $\beta$ -amyloid peptide with high affinity and is a strong stimulatory factor in the polymerization of  $\beta$ -amyloid peptide into filaments. Abnormal  $\alpha$ 1ACT accumulations are found in sIBM muscle and it colocalizes with  $\beta$ -amyloid,<sup>26</sup> as is the case in the brains of patients with Alzheimer's disease. In Alzheimer's disease a common polymorphism of the  $\alpha$ 1ACT gene (A/A) has been shown to modify the risk of developing disease conferred by *ApoE*  $\epsilon 4$ . The combination of  $\alpha$ 1ACT (A/A) and *ApoE*  $\epsilon 4$  was found to occur more frequently in Alzheimer's disease than in the general population,<sup>65</sup> and increased the risk of developing disease 2–3-fold beyond the risk associated with possession of *ApoE*  $\epsilon 4$  alone.<sup>60,65</sup> Nacmias et al.<sup>90</sup> found that this association was most important in the subset of late-onset familial Alzheimer's disease, but the  $\alpha$ 1ACT genotype did not represent an additional risk factor in families that carried mutations in the *APP*, *PS-1* or *PS-2* genes. The  $\alpha$ 1ACT polymorphisms by themselves do not seem to be an independent risk factor for Alzheimer's disease. Gossrau et al.<sup>50</sup> analyzed *ApoE* and  $\alpha$ 1ACT gene polymorphisms in 35 sIBM patients and could not identify any statistically significant correlation between the distinct *ApoE* and  $\alpha$ 1ACT genotypes and the risk of developing sIBM. However, the numbers in this study were small, with only seven patients having the  $\alpha$ 1ACT (A/A) polymorphism, no patients being homozygous for *ApoE*  $\epsilon 4/\epsilon 4$ , and only 8

of 35 patients being *ApoE3/ε4* heterozygous, so that definite conclusions are not possible, and larger studies need to be performed.

### PRION PROTEIN

Prion protein and prion mRNA are also accumulated in sIBM and hIBM<sup>9,110</sup> (as well as other inflammatory myopathies), although only the normal isoform PrP<sup>c</sup>, not PrP<sup>sc</sup> (the protease-resistant insoluble isoform) has been detected.<sup>126</sup> A study by Orth et al.<sup>98</sup> analyzing 41 sIBM patients for codon 129 amino acid status found that 21 patients were homozygous for methionine (Met), one patient was homozygous for valine, and 19 were heterozygous. This was not significantly different from the normal population, suggesting that sIBM is not linked to the Met/Met polymorphism at codon 129 of the prion protein gene. These data are consistent with data from our own group (Garlepp et al., unpublished data), but contrasts with the original report by Lampe et al.,<sup>76</sup> who reported that 64% of their 22 sIBM patients were homozygous for Met/Met polymorphism at codon 129. They subsequently revised this<sup>77</sup> after studying a further 16 patients, when they reported a reduced frequency of 55%.

Although examination of the genes encoding the proteins accumulated in sIBM has not yet identified any of them as susceptibility genes, their accumulation remains an important, undisputed feature of the disease. The genetic analysis to date has been somewhat targeted, and in most cases analysis of the complete gene has not been conducted, cues having been taken from data accumulated for Alzheimer's disease. The control of expression of these proteins and their metabolism is often complex, so that complete exclusion of genetic variation in these processes in sIBM will require more intensive analysis. Understanding why these proteins accumulate and how they contribute to the etiology, pathogenesis, and progression of sIBM remains a key issue in sIBM.

### MITOCHONDRIAL DNA (mtDNA) ABNORMALITIES

Another important feature of sIBM pathology, and one that may lead to the identification of further susceptibility genes, is the mitochondrial changes seen in cytochrome-oxidase (COX)-deficient muscle fibers and ragged-red fibers (RRF). These are more prevalent in sIBM compared with normal aging, polymyositis, or dermatomyositis. A recent review by Oldfors et al.<sup>97</sup> summarized the mitochondrial changes seen in sIBM, and highlighted new tech-

niques that have enabled many discoveries in the last decade.

The application of in-situ hybridization allowed the demonstration that many COX-deficient RRF had accumulated large-scale deletions in mtDNA. Extended studies, using different DNA probes on serial sections, found different deletions in separate muscle-fiber segments, implying that clones of mtDNA with large-scale deletions had expanded focally causing segmental COX-deficiency.<sup>97</sup> A study by Horvath et al.<sup>62</sup> found by detailed in-situ hybridization a patient with a large number of affected fibers that large-scale single mtDNA deletions were the most common mtDNA abnormalities observed in COX-deficient fibers. Oldfors et al.,<sup>96</sup> using sensitive polymerase chain reaction (PCR) analysis, demonstrated that multiple mtDNA deletions were found in most sIBM patients, whereas PCR analysis of isolated, single muscle fibers showed the presence of mtDNA with only one type of deletion, and the deficiency of wildtype mtDNA in each COX-deficient muscle fiber. This finding was supported by results from in-situ hybridization using different mtDNA probes on consecutive sections. The etiology of multiple mtDNA deletions and COX-deficient fibers in sIBM is not clear, and they may be a nonspecific finding, as clonal expansion of somatic multiple large-scale mtDNA deletions has been demonstrated in other conditions including late-onset mitochondrial myopathy, autosomal-dominant chronic progressive external ophthalmoplegia, and to a lesser degree in normal aging. Oldfors et al.<sup>97</sup> investigated the *POLG1*, *ANT1* and *C10orf2* mitochondrially encoded genes, as variants of these genes have previously been associated with multiple mtDNA mutations, and did not find any mutations in these genes in five sIBM patients.

MtDNA variants have been shown to be associated with many diseases. Mutations at mtDNA nucleotide positions 3192, 3196, 3397, and 4336 have been described in association with late-onset Alzheimer's disease. These were analyzed in sIBM patients by Kok et al.,<sup>73</sup> and in this study the 4336G variant was not significantly increased in patients with either sIBM or Alzheimer's disease compared with controls. None of the patients with sIBM carried mutations at nucleotide positions 3192, 3196, or 3397. Phylogenetic analysis of patients with sIBM, Alzheimer's disease, and controls based on D loop sequence showed that individuals with 4336G and 4580A variants clustered together in their respective group, as would be predicted. A group of patients with IBM also clustered together on a separate branch of the phylogenetic tree, independent of these polymorphisms.

Closer investigation of this group revealed a common polymorphism at nucleotide position 16311. The frequency of the 16311C variant was higher in sIBM than in Alzheimer's disease and controls, although when only Caucasian patients were considered the increased frequency was not statistically significant. Further studies will be required to determine whether this variant plays a role in the pathogenesis of sIBM.<sup>73</sup>

The significance of the mtDNA deletions in sIBM is unclear, particularly given that magnetic resonance spectroscopy (MRS) studies using 31P MRS has not shown any impairment of oxidative metabolism in sIBM patients.<sup>5,79</sup> However, in normal aging mtDNA mutations have been associated with muscle fiber atrophy, and they may be an important factor in the sarcopenia associated with aging. By the same token, mtDNA mutations in sIBM muscle may contribute to the muscle atrophy and weakness. It has been proposed that the mitochondrial abnormalities are a consequence of muscle damage or other factors involved in the disease process such as the *APP* gene (as cultured muscle cells overexpressing APP also show evidence of mitochondrial abnormalities) or the proinflammatory cytokine IL-1 $\beta$  (as myotubes treated with IL-1 have abnormal mitochondria<sup>30</sup>). The overwhelming opinion appears to be that these mitochondrial changes are a secondary phenomenon to the cellular abnormalities occurring in sIBM, rather than being a primary pathogenic abnormality.

## CONCLUSIONS

The pathogenesis of sIBM is slowly being elucidated, and is likely to involve a complex interaction between environmental triggers (possibly viral) and genetic susceptibility. In-depth histopathological studies have identified three important features: the immune changes, degenerative changes of protein accumulation, and mitochondrial changes. Research into candidate genes in all of these areas over the last decade has shown that genes located within the MHC are most strongly associated with sIBM. Genes encoded within mtDNA, genes associated with reactive oxygen species production and breakdown, as well as genes controlling proteasomal protein breakdown and the immune system are areas warranting future studies. The identification of the genetic factors that influence the development and progression of this currently difficult to control disease will not only be a tremendous step forward in understanding the process of the disease but should allow the development of targeted therapies.

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