New insights into the genetics of multiple sclerosis

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Abstract—Tissue injury in multiple sclerosis (MS) results from an abnormal immune response to one or more myelin antigens that develop in genetically susceptible individuals after exposure to a causal agent that is yet undefined. The genetic component of MS etiology is believed to result from the action of several genes of moderate effect. The incomplete penetrance of MS susceptibility alleles probably reflects interactions with other genes, posttranscriptional regulatory mechanisms, and significant environmental influences. Equally significant is that genetic heterogeneity also likely exists, meaning that specific genes influence susceptibility and pathogenesis in some affecteds but not in others. Some loci may be involved in the initial pathogenic events, while others could influence the development and progression of the disease. The past few years have seen significant progress in the developments of laboratory and analytical approaches to study non-Mendelian complex genetic disorders and to define the pathological basis of demyelination. These developments have set the stage for the final characterization of the genes involved in MS susceptibility and pathogenesis. The identification and characterization of the genes are likely to define the basic etiology of the disease, improve risk assessment, and influence therapeutics.

Key words: chromosome mapping, chromosomes, genetic markers, genetic linkage, major histocompatibility complex, multiple sclerosis, pedigree.

INTRODUCTION

On February 15, 2001, the International Human Genome Sequencing Consortium and the private company Celera simultaneously reported the completion of the first draft of the human genome sequence (1,2). This landmark effort resulted in an extraordinary amount of fundamental information and the promise of advancing our understanding of the underlying genetic basis of complex multifactorial diseases. A complex trait is defined by a genetic component that is not strictly Mendelian (dominant, recessive, or sex-linked) and involves the interaction, either programmed or stochastic, of two or more genes. This category clusters most of the common diseases in children (birth defects, mental retardation) and in adults (cancer, cardiovascular diseases, and autoimmunity, including multiple sclerosis (MS)).

Compelling data indicate that susceptibility to MS can be inherited (Figure 1). Familial aggregation, recognized by Charcot in the late nineteenth century, is well documented with an increased relative risk to siblings ($\lambda_s = 20$ to 40) compared to the general population (3,4). Concordant siblings tend to share age of symptoms onset rather than year of onset, and second- and third-degree relatives of MS patients also have an increased risk for MS, suggesting that inherited factors distinct from a common environmental exposure influence susceptibility. Studies of half-siblings and adoptees support the concept that genetic, and not environmental factors, are primarily responsible for familial aggregation (5,6). Furthermore, twin studies from different populations consistently indicate that a monozygotic twin of an MS patient is at higher risk (25 to 30 percent concordance) for MS.
than is a dizygotic twin (3 to 5 percent) (7,8), providing additional evidence for a significant, but complex, genetic etiology. A strong genetic component in MS pathogenesis is indicated foremost by the relative high-recurrence risk in family members of affected individuals and frequent occurrence in some ethnic populations (particularly those of northern European origin) compared with others (African and Asian groups), irrespective of geographic location (9,10).

A simple Mendelian model of inheritance for all MS is unlikely because it cannot account for the nonlinear decrease in disease risk in families, with increasing genetic distance from the proband (Figure 2). Recurrence risk estimates in families, combined with twin data, predict that the MS-prone genotype results from multiple independent or interacting polymorphic genes, each exerting a small or, at most, moderate effect to the overall risk. Hence, although a Mendelian-like genetic etiology cannot be ruled out for a small subset of multiple-affected pedigrees, overall the data support the long-held view that MS is a polygenic disorder. In addition, beyond the impact of genes that are inherited and act in their germline configuration, a number of postgenomic DNA (deoxyribonucleic acid) changes may influence MS risk. These include genes that rearrange from their position in the germline to encode a vast variety of T cell receptors (TCRs) and immunoglobulins, somatic mutations, post-transcriptional regulatory mechanisms, and incorporation of retroviral sequences. It is also likely that interactions with nutritional, geographical, infectious, and other environmental influences affect susceptibility (11). The final layer of difficulty in the study of MS is encountered when we consider the significant clinical, histopathologic (12), and genetic (13) heterogeneity that characterizes this disease (Figure 3).

NARROWING SEARCH AND IDENTIFYING CANDIDATE GENES

Although genetic components in MS are present, the lack of an obvious and homogeneous mode of transmission has prevented the application of classical genetic epidemiologic techniques. Statistical techniques to identify disease loci have been available since the 1950s; however, only recently have newer techniques been applied to the problem of detecting susceptibility loci (Figure 4). A reasonable approach for gene discovery in complex disorders involves first determining the chromosomal region of the genomic effect by linkage analysis. Establishing genetic linkage requires the collection of family pedigrees with more than one affected member to track the inheritance of discrete chromosomal segments that deviate from independent segregation and cosegregate with the disease. Once these regions have been identified and confirmed, a narrow and well-defined list of candidate genes can be compiled for analysis, even in the absence of a unifying model of pathogenesis (Figure 5). The early success of this approach with complex traits, such as the discovery of the role of
APOE (apolipoprotein E) in late-onset Alzheimer’s disease (14) and the availability of detailed maps of highly polymorphic markers (i.e., microsatellites) for all chromosomes, powered the rationale for the wide application of this method in non-Mendelian disorders.

The potential of genetic mapping for gene identification in complex diseases was highlighted in a study of type 2 diabetes (15). The investigators followed original linkage data that implicated the distal long arm of chromosome 2 and identified a disease-associated intronic polymorphism in calpain-10, a ubiquitously expressed member of the calpain-like family of cysteine proteases. The identification in 1996 of a locus linked to Crohn’s disease on chromosome 16 resulted in the recent identification of a frameshift mutation in NOD2, a member of the Apaf-1/Ced-4 superfamily of apoptosis regulators, associated with disease susceptibility (16,17).

Genetic studies in MS in the previous decade were influenced by three large multistage whole genome screens performed in multiple-affected families ascertained in the United States, United Kingdom, and Canada (18–20). A fourth study concentrated on a genetically isolated region of Finland but was based on a small number of families (21). Follow-up screenings in confirmatory and additional data sets have been completed as well. The studies identified about 60 genomic regions with a potential involvement in MS, but total or even predominant overlapping between the different screens was absent. This was partly because of the strategy of reporting all “hits” suggestive of linkage, which recognized that false positives will be generated. It is also possible that the study design in each case underestimated the confounding influence of disease heterogeneity and the limitations of parametric methods of statistical analysis. It should be noted, however, that because each study used a somewhat overlapping but different set of genetic markers and different clinical inclusion criteria, the direct comparison of results is not straightforward.

Nevertheless, the careful analysis of the composite published data identifies 13 common regions of interest between the four genomic scans (22). In addition, a formal meta-analysis of the published data singled out discrete overlapping MS-susceptibility regions at chromosomes 5, 6, 17, and 19 (23). Recently, raw genotyping data from the genome screens were pooled to conduct a global meta-analysis (24). Eight regions had cumulative positive but
modest scores, including the 17q11 and 6p21 segments. A second type of meta-analysis attempted to cluster autoimmune-susceptibility loci from a comparison of the linkage results from 23 human and experimental immune-mediated diseases, including MS and the animal model, experimental autoimmune encephalitis (EAE) (25). Overlapping of susceptibility loci was detected, suggesting that in some cases, part of the pathophysiology of clinically distinct autoimmune disorders may be controlled by a common set of genes.

Although further work is necessary to better define the complete roster of MS loci, these studies represent real progress in mapping the full set of MS-associated genes.

The next step is to systematically explore the degree of variability, primarily in coding but also in regulatory and intronic regions, in genes mapped to the candidate regions for direct association with disease (Figure 5). Single-nucleotide polymorphisms (SNPs) are the most frequently found DNA sequence variation in the human genome (on average 1 per 1,000 or 2,000 bases). SNPs are thought to represent old and stable mutations evenly distributed throughout the entire genome. These characteristics make them good markers for genetic studies (26,27). In addition, although most SNPs are most likely neutral, some may contribute to disease susceptibility and/or resistance and may directly identify the “causative” sequence difference.
Studies will require large collections of multiplex and/or nuclear families and/or well-matched cases and control groups. Key to the success of the proposed studies will be the availability of rapid reliable nonlabor-intensive methods for high-throughput polymorphism screening. In all likelihood, the use of phenotypic (clinical and paraclinical), epidemiological, and demographic variables will assume increasing importance as stratifying elements so as to address the fundamental question of genotype-phenotype correlation in autoimmune demyelination. These studies will be linked necessarily to the development of novel mathematical formulations designed to identify modest genetic effects, as well as interactions between multiple genes, and interactions between genetic, clinical, and environmental factors.

The HLA-DR2 haplotype (DRB1*1501 DQB1*0602) within the major histocompatibility complex (MHC) on chromosome 6 is the strongest genetic effect identified in MS and has consistently demonstrated both linkage and association in family and case-control studies (28,29). MHC class I and class II molecules are polymorphic cell-surface glycoproteins, whose primary role in an immune response is to display short antigenic peptide fragments to peptide/MHC-specific CD4+ and CD8+ T cells. These cells can then become activated by a second stimulatory signal and initiate an immune response. In addition, MHC

Figure 5. Methods for genetic analysis.
molecules present on stromal cells on the thymus during development help determine the specificity of the mature T cell repertoire. The human MHC (the Human Leukocyte Antigen (HLA) system) consists of linked gene clusters located on the short arm of chromosome 6 at 6p21.3, spanning almost 4 million base pairs. Many of the HLA genes are highly polymorphic, resulting in the generation of enormously diverse numbers of different genotypic combinations or haplotypes. The polymorphic residues that define an HLA allele are clustered in the antigen-peptide-binding groove of the molecule. Hence, the ability of an individual to respond to an antigen, whether foreign or self, and the nature of that response are largely determined by the unique amino acid sequences of HLA alleles. This observation provided the rationale for focusing on associations between HLA genotypes and susceptibility to autoimmune disease (30).

The mechanism(s) underlying the genetic association of HLA-DRB1*1501-DQA1*0102-DQB1*0602 with MS are not yet fully understood. These MHC molecules may fail to negatively select (delete) autoreactive T cells within the embryonic thymic microenvironment. Alternatively, HLA-DRA1*0101-DRB1*1501 and/or DQA1*0102-DQB1*0602 genes may encode class II recognition molecules with a propensity to bind peptide antigens of myelin and stimulate encephalitogenic T cells. The HLA-DRα0101-DRβ1501 heterodimer binds with high affinity to the myelin basic protein (MBP) 89–55 peptide. X-ray crystallography of the DR-MBP peptide complex reveals a DRβ1501 structure different from other DRβ molecules in that aromatic residues are preferred in the P4 pocket of the peptide binding domain (Figure 6) (31,32). In addition, two peptide side chains of the p85–99 MBP immunodominant peptide, Val89 and Phe92, were found to be the primary anchors and account for the high-affinity binding of the MBP peptide to HLA-Drα0101/DRβ1501. The structural analysis also revealed that only two primary TCR contact residues of MBP p85–99 had to be conserved to properly stimulate antigen-specific clones (33). The data suggest that microbial peptides with only limited sequence identity with a self-peptide may activate autoreactive T cells.

**SUSCEPTIBILITY GENES VERSUS MODIFIERS**

As summarized above, the MHC locus has consistently demonstrated both association and linkage with MS in case-control and family studies; however, the role of a gene within this region in determining clinical features or subtypes of MS is unclear. HLA-DR2 has been reported to be associated with lower age at onset, gender, severe, relapsing-remitting, and mild MS courses, or to have no influence (34–40). In the EAE disease model, MHC genes appear to influence primarily susceptibility and penetrance, whereas other loci modulate specific phenotypes, such as location in brain or spinal cord, demyelination, and severity of inflammation (41,42). By analogy, it will be of interest to identify which loci are involved in the initial pathogenic events or influence the development and progression of the disease. Here, genes that are logical possibilities to play a role in a disease (candidate genes) are considered; for MS, candidate genes might encode cytokines, immune-receptors, myelin components, and proteins involved in viral clearance (43). Several studies examining the influence of such group of genes (IL-1R, TNF, APOE, CTLA4, and CCR5 among others) on disease course and severity in MS have been reported and await confirmation (44–49).

**FUNCTIONAL GENOMICS**

During the process of lesion formation, lymphocyte activation and recruitment, extravasation, and effector
functions involve several cellular phenotypic changes triggered by the pathways of specific gene expression. Cytokines, adhesion molecules, growth factors, and other molecules (such as free radicals, proteases, and vasoactive amines) induce and regulate numerous critical cell functions. The comprehensive analysis of these cellular transcriptional programs, the “transcriptome,” both in the central nervous system (CNS) and the periphery, should provide the molecular fingerprint of the demyelinating process and help identify the complete array of MS disease-genes (50,51). The field of functional genomics involves the use of high-throughput methods to analyze the expression of hundreds or thousands of genes simultaneously. These large-scale explorations of gene expression have become virtually routine over the past few years. However, the statistical and mathematical treatment of the extraordinary large resulting data sets is largely an emerging discipline (52,53). The careful and methodic mining of expression data could lead to the identification of coregulated genes and characterization of networks that underlie specific cellular process. This complex organization is what ultimately defines the function and, therefore, the phenotype. Mathematical models of gene interaction in a rational scenario of network operation can now be tested, and accordingly, new hypotheses can be generated. Emerging advances in protein analysis (mass spectroscopy, NMR (nuclear magnetic resonance) spectroscopy, X-ray crystallography, yeast two- or three-hybrid systems) (54) will facilitate the transition from gene identification to gene function.

Genes play a primary role in determining who is at risk for developing MS, how the disease progresses, and how someone responds to therapy. With the aid of high-capacity technologies, the combined analysis of genomic and transcriptional information, together with the modeling of genetic networks, will define a useful conceptual model of pathogenesis. The combination will also determine a framework for understanding the mechanisms of action of existing therapies for this disorder, as well as the rationale for novel curative strategies.

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REFERENCES


