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Mini review

STRATEGIES FOR THE IDENTIFICATION OF LOCI RESPONSIBLE FOR THE PATHOGENESIS OF MULTIPLE SCLEROSIS

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Abstract: Multiple sclerosis (MS) is a chronic, debilitating disease, which manifests itself by de-myelination of the central nervous system (CNS). MS is predominantly found in Caucasians of European decent and is more prominent in females than males. MS is one of the most prevalent causes of disability of young adults in the world. The exact cause of MS is not known, however genetic susceptibility to MS is linked to the major histocompability complex (MHC). Self reactive CD4+ T cells, specific for CNS antigens, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP), are detectable in MS patients along with pathogenic autoantibodies specific to these CNS antigens produced by B cells. These observations suggest that MS is an autoimmune disease. Epidemiology of MS along with the analysis of sibling pairs and twins suggest that the multiple genetic factors and their interaction with environment contribute to disease susceptibility. Recent developments and advancements in genetic analysis may aid in accurate determination of genetic risk factors for the development of MS.

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Abbreviations used: CNS – central nervous system; EAE – experimental allergic encephalomyelitis; HLA – human leukocyte antigen; MBP – myelin basic protein; MHC – major histocompability complex; MOG – myelin oligodendrocyte glycoprotein; MS – multiple sclerosis; PLP – proteolipid protein; QLT – quantitative trait loci; SNP – single nucleotide polymorphism

We review these developments, advances in technology and discuss recent results in this article.

Key words: Multiple sclerosis, Experimental allergic encephalomyelitis, Single nucleotide polymorphisms, Genetic linkage studies, Genome wide association studies

INTRODUCTION

Multiple sclerosis (MS) is a de-myelinating, disease afflicting up to 0.2 % of the population in high prevalence areas. The pathological hallmark of MS is lesions that accumulate throughout the central nervous system, with a predilection for the optic nerves, brain stem, spinal cord, and peri-ventricular white matter. Although generally considered an autoimmune disorder, the considerable clinical, genetic, and pathological heterogeneity of multiple sclerosis suggests multiple pathogenic mechanisms. MS presents itself in multiple forms. The majority of patients develop isolated attacks that may completely heal however may relapse over time (relapsing-remitting disease). Some patients progress in to secondary progressive disease where disease deteriorates more progressively with fewer relapses. A minority of the patients develop progressive forms of MS that steadily worsens with no clinical remissions. There are a variety of animal models of MS namely Experimental Allergic Encephalomyelitis (EAE) which immunization with an encephalogenic peptides or proteins (MOG, MBP, PLP, myelin extract) is used to induce MS like disease. EAE can be induced in animal species including rats, mice, guinea pigs, rabbits, marmosets, macaques and rhesus monkeys [1-9].

Studies suggest that there are both environmental and genetic risk factors contributing to the pathogenesis of MS [10, 11]. Like other autoimmune diseases, MS is complex, with no pattern of inheritance, and is characterized by a process of demyelination and degeneration of the spinal cord, whose initiation is usually attributed to environmental factors. Among environmental factors suspected of causing MS, the most studied are infectious agents. Molecular mimicry by infectious agents may cause immune attacks to self [12]. Bacterial agents such as Chlamydia pneumonia or viruses such as herpes simplex virus or Epstein Barr virus are suspected to contribute to MS development in susceptible hosts [12-14]. There is a relevant mouse model of EAE that is induced with a murine picornavirus called Theiler's virus [12, 15].

It has been observed, however, that fraternal twins and siblings of MS patients have approximately 150–300 times greater risk and siblings have approximately 20–40 times higher risk to develop the disease, than unrelated individuals in the population [10, 11, 16]. This indicates that there is also a genetic component of the disease. Namely, it is likely that, in addition to the external factors, mutations or polymorphisms in certain genes or loci also contribute to its pathogenesis. The HLA locus on chromosome 6p21 has been consistently implicated as a possible factor to genetic risk in MS [16].

Over the past decade, genetic studies of the susceptibility to MS have been attempted but with limited success. Previous linkage studies involving over of 1500 MS patients have not been able to identify non-HLA genetic risk factors for the disease [17-21]. A risk haplotype that has been identified is HLA DRB1*1501-DQB1*0602 on chromosome 6, which explains between 14% and 50% of the genetic risk [16, 17, 20]. The factors accounting for the significant remaining portion of genetic predisposition for MS remain unknown. For their identification, new techniques must be employed in conjunction with the classic method of linkage analysis. This report presents some of the most intriguing methods that could potentially be used or are already being used for this purpose along with recently identified genes involved in MS.

GENETICAL GENOMICS THROUGH MICROARRAY ANALYSIS

An emerging approach to the task of identifying the complete array of genes contributing to MS pathogenesis seeks to capitalize on the options provided by the recently developed technique of microarray analysis. Microarray-based studies describing expression patterns in MS lesions have been published [22-24]. The common picture that emerges from these studies indicates that the transcriptional activity of genes related to the process of inflammation is elevated in MS patients, particularly in samples taken from the edge of active plaques. Specifically, the most abundant transcript found uniquely in MS plaques was alpha β-crystallin, an inducible heat shock protein localized in the myelin sheath and a putative target for T cells in MS [25]. The next most up-regulated genes in active plaques were those for prostaglandin D synthase, prostatic binding protein, ribosomal protein L17, and osteopontin [26]. Genes with decreased expression included those for several myelin components such as proteolipid protein, myelin associated glycoprotein, and myelin oligodendrocyte glycoprotein. Besides reflecting the catabolic demyelinating process, this last finding may also be interpreted as an indication of ineffective myelin repair.

The capacity to quantify changes in transcription levels through microarray analysis is the key that has opened up the possibility for the new approach of Genetical Genomics. The idea behind this method is to evaluate gene expression levels as potential quantitative trait loci (expression QTL, abbreviated as eQTL) in linkage analyses, using either microsatellite or SNP-based genetic maps [27]. Therefore, the approach involves the combination of information obtained by traditional linkage analysis with the information of gene-expression analysis. Unlike classic linkage analysis in which a single or only a few phenotypic traits are evaluated for statistical correlation against a genomic region, eQTL analysis involves the consideration of a very large number of gene expression values which are treated as traits. As a result, thousands of LOD scores are calculated, extending de facto the definition of the phenotype. Using this approach, a gene expression pattern strongly associated with murine obesity was identified, leading to the identification of four new QTL linked to this complex phenotype [28].

More recently, genetical genomics yielded the identification of regulatory networks associated with stem cell function, blood pressure, and neural phenotypes [29-31]. Since the pathology of MS involves quantitative traits that are as complex as some of the above mentioned phenotypes, the method of eQTL analysis appears ideal for the study of the genetic basis of the disease.

More specifically, in the case of MS, eQTL mapping can be combined with traditional QTL methodology for best results. It would be practically advantageous to apply the approach in mice because of the availability of a mouse model of MS (Experimental Allergic Encephalomyelitis) and of previously identified QTL that have been shown to be linked to the disease. Moreover, the capability to manipulate/selectively mate mice, as well as the ease of genotyping and selecting samples for analysis of expression levels, makes mice a good experimental organism for a first application of this method. A mouse-human synteny mapping approach may then allow the identification of candidate susceptibility loci for MS in humans based on the location of EAE susceptibility loci in mice.

Experimental autoimmune encephalomyelitis (EAE) is the primary genetic animal model for multiple sclerosis (MS). As a result of the experimental implementation of traditional QTL methods in mice with EAE, 16 loci that control susceptibility to the disease, or specific clinical signs associated with it, have been identified so far [32]. These QTL can be used in order to increase the efficiency of eQTL studies, thus obtaining a more complete and accurate picture of the loci linked to EAE. Specifically, samples of brain tissue taken from mice that are homozygous (either +/+ or -/-) for these previously identified QTL, should be subjected to targeted expression analysis after they are challenged for the disease (i.e., after they are exposed to EAE inducing stimuli). By this approach, the power of the proposed/described method for identifying genuine eQTL within the selected targeted regions increases significantly in comparison to the power of random/standard eQTL analysis. In fact, this is an effective way to overcome one of the main disadvantages of standard eQTL mapping: namely, the identification of many loci that are unrelated to the disease along with those genuinely linked to it.

ADMIXTURE MAPPING FOR IMPROVED LINKAGE ANALYSIS

A second way to address the insufficiency of classic linkage analysis for the identification of genes in multigenic diseases such as MS is to focus on the selection of the sample populations used in linkage studies rather than on the radical modification of the method of linkage analysis itself. Based on the idea that an improved linkage study is not the only way to stratify the genome into high- and low-yield regions in terms of disease susceptibility, a method of association mapping called admixture mapping has emerged as a powerful technique with which to identify disease susceptibility loci. The requirement for

applying this strategy is the existence of population that is the result of admixture between two parent populations with different prevalence rates for the same disease. In the case of MS, populations exhibiting significantly different prevalence rates for the disease do exist. In particular, it has been determined that among African-Americans the prevalence rate for MS is only 40% that of European-Americans or basically 60% smaller than the prevalence rate of European-Americans. Moreover, Africans are thought to have a dramatically lower prevalence rate for the disease, corresponding to approximately 1% that of European-Americans [33]. These data suggest that a number of MS susceptibility loci exist in the European genome but are absent from the African genome. Therefore, researchers can examine a population of African-Americans with MS and look for genetic regions that have a high proportion of alleles of European ancestry or origin. According to estimations, a project aiming at the identification of chromosomal segments of European ancestry associated with risk of MS will likely involve loci of large sizes (5–10 Mb) [34]. Therefore, in order to carry out the requisite dissection of the implicated region, it is necessary to complement the admixture scan with a haplotype-based approach.

UTILITY OF SNP ANALYSIS IN MS GENOMIC SCREENS

The idea of performing admixture studies is almost 20 years old, but only with recent advances in technology and the increased availability of SNP's did it become possible to actualize it [34-36]. The first disease to be studied using admixture mapping was MS. In 2005, Reich *et al.* published the results of an admixture scan, for which a sample of 605 African-American MS patients and 1,043 African-American control individuals was used. The initial genome-wide scan of 484 cases and 1,043 controls revealed a significant association with MS risk around the chromosome 1 centromere (lod = 4.9) [37]. This position was also shown to exhibit a 5.9% rise in European ancestry compared with the genome-wide average [37]. The increased association of this particular region to European ancestry was determined by comparing the general African-American population to European populations. After a secondary scan, in which 121 additional cases were included and 84 new markers were used, the association of this centromeric locus of chromosome 1 to MS grew stronger (lod = 5.2), reinforcing the initial observations.

Genome wide association studies utilizing SNP's may be further analyzed using available gene sequence information from other species. Conserved sequence clusters from multiple species would suggest that the sequences are of functional importance and may allow focusing genomic screen even if the underlying function of the region is not known [38].

Current SNP arrays can screen an average of 1.2 million SNP's on one chip. A recent genome wide association study conducted by the International Multiple Sclerosis genetics consortium utilizing SNP-arrays on a large cohort of patients and their families resulted on the identification of two SNP's on IL-2RA and one

SNP on IL-7RA that were related to MS. IL-2 and IL-7 are important cytokines that influence T cell development, differentiation, and immune responses. SNP on these receptors may affect the soluble and membrane bound isoforms of the receptors and influence signal transduction. This may, in turn, be significant in the triggering of MS disease in combination with environmental factors [39, 40].

CONCLUSIONS

Significant progress has been made in the study of the multiple genetic factors contributing to the pathogenesis of diseases such as MS thanks to methods such as eQTL analysis, admixture scans, and improved SNP array technologies. Nevertheless, further development of the approaches that are currently available, or of altogether new approaches, is necessary for accomplishing the task of more fully describing the mechanisms and of identifying the causes of pathogenesis in such diseases. With respect to MS, the results obtained so far are only preliminary: because of limitations in eQTL analysis and admixture studies as currently performed, we are still far from the conclusive identification of the majority of the genetic loci that are associated with the disease. In a review of the methods available for the genetic dissection of the immune response, De Koning *et al*. note that a problem with the Genetical Genomics approach of eQTL analysis is that, in its current application, it is only capable of detecting the largest of genuine eQTL effects and suffers by a high rate of false positives [41]. The limited power of handling large amounts of data, which also prevents the performance of extensive experiments in terms of size and number of loci considered, is blamed for these shortcomings of the approach. Nevertheless, as mentioned above and was also suggested by De Koning *et al*., a combination of traditional QTL mapping with eQTL analysis can help increase the power of the latter.

Similar factors also seem to account for the limits of admixture studies. Namely, the size of the sample that can be studied and the number of markers that can be used under currently available computational and other technical means, are not sufficient for the establishment of highly statistically significant associations between well-defined, and small enough to be useful, genetic loci and MS. However, there is undeniable potential for improvement in these areas. The power of genotyping and the level of understanding of the complex structure encoded within the human genome are rapidly increasing. Technological advances in gene chip design improve the quantities of SNP’s tested and allow for more accurate and detailed genome wide association studies. Due to fast technical development the amount of SNP’s identified by a single chip will improve in line with Moore's law and surpass over 1.2 million SNP's. Accordingly, the new tools for exploiting the maximum potential of approaches such as eQTL analysis and admixture studies for the determination of the genetic basis of multigenic diseases such as MS, are expected to be available soon.

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