Analysis of genetic mutations or alleles on the autosomal chromosomes as possible causes of Multiple Sclerosis

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Summary

Hundreds of studies have been conducted searching for genes that influence susceptibility to MS. To date, no one gene has been found that by itself strongly increases MS risk. The HLA region, in particular the HLA DR2 haplotype, has shown the strongest evidence of association with MS, but even its contribution may be moderate. Scientists studying MS genetics have generally concluded that the genetic component of MS risk is made up of small contributions from a number of genes, and that the alleles that confer risk are likely to be common variants as opposed to rare mutations. If so, locating susceptibility genes for MS will be much more complicated than for single-gene diseases, and will require approaches tailored for the situation.

Note: A spreadsheet compiling relevant details of the published candidate gene studies and genome screens conducted for MS can be found in the file *phase2-genetic-studies.xls* available for download at www.acceleratedcure.org.

Hypothesis

Mutations to or alleles of one or more genes located on the autosomal chromosomes either cause MS or influence susceptibility to MS.

Experimental tests of the hypothesis

Genes influencing susceptibility to MS may be found on the X chromosome, Y chromosome, and/or one or more of the 22 autosomal chromosomes. For the most part, there are few indications that susceptibility genes for MS would or would not be located on any particular autosomal chromosome. The types of chromosomal imbalances that are found on only certain chromosomes (such as Robertsonian translocations) have not been associated with MS. Likewise, there is no evidence that imprinting, another phenomenon specific to certain chromosomes, is implicated in MS. The involvement of inflammation in MS does suggest a role for the human leukocyte antigen (HLA)/major histocompatibility complex (MHC) gene cluster on chromosome 6, and this role has been extensively investigated as described below. Overall, each of the autosomal chromosomes must be considered a plausible candidate for harboring one or more susceptibility genes.

Candidate gene studies: Indications that MS has a genetic basis have motivated hundreds of studies attempting to locate the susceptibility genes for MS. Most of these have analyzed specific genes considered to be candidates due to the function of their encoded proteins. In this type of study, known polymorphisms in or near these genes are evaluated in MS subjects and controls to determine if any significant differences exist between the two groups.

A search of reports listed in the NIH's PubMed database through August 2006 located several hundred candidate gene studies analyzing 312 individual candidate genes or gene regions. (See the file *phase2-genetic-studies.xls* for details of each of these studies.) Of these 312 genes, 241 have been the subjects of only one or two published studies. Only 32 genes have been studied five or more times. The MHC class I and II regions have been the most frequently studied locations, with a combined total of 192 published reports.

Important parameters of these candidate gene studies include:

- The gene and polymorphism under study
- The regional or ethnic population studied: typically European or North American Caucasian, although Japanese, Australian, Chinese, and other populations have also been studied
- Clinical characteristics of the population under study
- Type of analysis: linkage, case-control, transmission disequilibrium, etc.
- Type of controls used: family members, unrelated controls, or previously compiled population data
- Number of participants: typically a few hundred subjects, although several studies involved fewer than a hundred participants, including one study of people with both MS and neurofibromatosis that had only four subjects

Of all the genes studied, the HLA (MHC) class II region has demonstrated the strongest association with MS to date. The DR2/DR15 haplotype (HLA DRB1*1501/DQA1*0102/DQB1*0602) is particularly noteworthy, having shown evidence of association in over 45 studies covering multiple ethnicities and nationalities. Efforts to further explore this association have correlated the presence of DR2 with juvenile onset MS[1] as well as with the development of probable or definite MS in people with acute unilateral optic neuritis[2]. A dose effect for DR2 was also documented in one recent study, which associated homozygosity for this haplotype with increased risk and disease severity[3].

The DR2 association is not universal, however. Certain MS populations have failed to demonstrate a DR2 association and/or have exhibited significant associations with non-DR2 alleles or haplotypes. Japanese subjects with neuromyelitis optica, also known as "Asian-type" or opticospinal MS, which is characterized by predominant optic nerve and/or spinal cord involvement, have not shown an association with DR2, although DPB1*0501 has been positively associated with this type of MS[4-8]. In addition, associations with DR3 and DR4 have been repeatedly found in Sardinians, an ethnically isolated population in which the DR2 haplotype is relatively rare[9-13]. Therefore, while the HLA region appears to influence susceptibility to MS, at this time the relationship appears to be marked by genetic heterogeneity rather than association with a single allele or haplotype. Another complicating feature of HLA associations is that extensive

linkage disequilibrium within this region has hindered the pinpointing of the particular genes or alleles associated with MS risk.

Of the 311 non-HLA genes studied to date, none has yet shown overwhelming evidence of influencing susceptibility to MS. As previously mentioned, most genes have been studied only a few times. Those that have received more attention have produced at best mixed results (see Table 1 below). For example, approximately half of the 21 published reports focusing on the T cell receptor beta chain gene found evidence, sometimes weak, supporting an association with MS, but the other half were unable to confirm this association. Also, 20 studies have been published for the myelin basic protein (MBP) gene; eight of these reported evidence for association but the other 12 did not.

Table 1 Genes most frequently investigated as potential risk factors for MS (with five or more studies)

Gene name	Location	# of studies	Overall results
HLA class I and II	6p21	192	Evidence for association, especially for certain loci/haplotypes
TNF	6p21.3	36	Mixed results
CTLA-4	2q33	25	Mixed results
APOE	19q13.2	24	Mixed results, mostly negative
T-cell receptor beta chain	7q34	21	Mixed results
Myelin basic protein	18q23	20	Mixed results, mostly negative
Immunoglobulin G heavy chain	14q32.33	19	Mixed results
CCR5	3p21	15	Mixed results, mostly negative
Interleukin-1 receptor antagonist	2q14.2	14	Mixed results, mostly negative
Interleukin-10	1q31-q32	12	Mixed results, mostly negative
Interferon gamma	12q14	12	Mixed results, mostly negative
PTPRC	1q31-q32	11	Mixed results, mostly negative
Interleukin-1 beta	2q14	10	All negative
T-cell receptor alpha chain	14q11	9	Mixed results, mostly negative
TGF-beta 1	19q13	9	Mixed results, mostly negative
Interleukin-2	4q26-27	8	Mixed results, mostly negative
Interleukin-4	5q31.1	8	Mixed results, mostly negative
TAP	6p21.3	8	Mixed results, mostly negative
Properdin factor B	6p21.3	8	Mixed results, mostly negative
ICAM-1	19p13.3-p13.2	8	Mixed results, mostly negative
Myelin oligodendrocyte glycoprotein	6p22-21.3	7	Mixed results, mostly negative
Interleukin-6	7p21	7	Consistent results, all negative
Vitamin D receptor	12q12-q14	7	Mixed results
Interleukin-4 receptor	16p12.1-p11.2	7	Mixed results
Vitamin-D binding protein	4q12-13	6	Mixed results, mostly negative
ABO blood group	9q34	6	Mixed results, mostly negative
Fas	10q24.1	6	Mixed results
Myeloperoxidase	17q23.1	6	Mixed results
Osteopontin	4q21-q25	5	Mixed results

Interleukin-12 p40	5q31.1-q33.1	5	Mixed results, mostly negative
Nitric oxide synthase 2A	17q11.2-q12	5	Mixed results

(Contents of this table are derived from data on individual studies as compiled in the spreadsheet *phase2-genetic-studies.xls,* which is available at www.acceleratedcure.org.)

Genome screens: In recent years, a number of genome screens have been conducted to simultaneously analyze hundreds or thousands of genetic markers in people with MS and in controls. The goal of these studies is to find loci with significantly different allele distributions in MS subjects compared with controls, under the premise that these loci may be located in or near a susceptibility gene.

Variable study parameters include those listed above for the candidate gene studies, as well as:

- Number/density of markers used: highest number used to date is 11,555
- Significance threshold for reporting loci of interest: these tend to vary from study to study – for instance, NPL > 2.0, lod > 0.7, lod > 2.0, or $p < 0.05$

A total of 55 screens and follow-up studies have been conducted so far. (See the file *phase2-genetic-studies.xls* for details of each of these studies.) In addition, four meta-analyses have been conducted, three of several MS-specific screens and one of autoimmune disease screens. Grouping the loci identified in these studies into broad regions (for example, grouping 22q13 with 22q13.1 and 22q13.31) results in 262 regions of interest, 31 of which were identified five or more times. The two regions most frequently identified were 6p21 and 19q13 (which met the significance criteria or were otherwise highlighted in 44 and 19 studies, respectively).

Table 2 Most frequently identified regions of interest from 55 MS genome screens and follow-up studies

Locus	# of studies
1p36	5
1p34	5
1p21	5
1q44	5
2p16	6
4p15	5
5p15	6
5q11	5
5q14	8
6p21	44
7p15	5
7q21	8
9p21	5
9q21	5
11p15	8
11q22	6

Locus	# of studies
12q24	7
14q32	7
15q21	5
16p13	10
17p13	7
17q11	5
17q21	7
17q22	7
18p11	11
19p13	7
19q13	19
20p12	5
21q22	6
22q13	8
Xp11	7

(Contents of this table are derived from data on individual studies as compiled in the spreadsheet *phase2-genetic-studies.xls,* which is available at www.acceleratedcure.org.)

Conclusions

Despite the hundreds of studies searching for MS genes, no candidate gene or region aside from the HLA region has yet been consistently linked with increased risk in MS. Even in the HLA region, linkage disequilibrium makes it difficult to tell which gene or genes may be involved.

There are several reasons why MS susceptibility genes have proven so elusive. It seems likely that MS is a multifactorial disease (see our separate analyses on Mendelian vs. incompletely penetrant genetic disorders). It is also quite possible that MS is a genetically heterogeneous disease, with different sets of genes influencing risk in different populations or even within populations. For instance, one of the MBP gene association studies, conducted in several Finnish populations, found an association only in families from Southern Ostrobothnia in western Finland, but not from other parts of Finland[14].

These two factors greatly complicate the search for genetic influences, creating the need for large scale investigations and studies of multiple populations. However, most candidate genes have been the focus of only one or two published studies. It may be that the limited progress to date in MS gene identification is due in part to not focusing on the right genes. Those genes that have been studied more frequently have typically shown either consistently negative or mixed results. One recent meta-analysis of association studies[15] concluded that underpowered studies producing false negatives may contribute to inconsistencies in association studies of genes with modest effect, and perhaps this is the case with some of the MS investigations. Differences in the ability of various types of analyses to detect relationships, as well as the possible existence of genetic heterogeneity or phenocopies in MS, may also account for discrepancies between studies.

Genome screens have also generally suffered from small numbers of participants. In addition, the use of different markers and significance thresholds across studies limits the ability to compile and analyze data from multiple studies. Collaborative efforts such as the European GAMES consortium (http://www-gene.cimr.cam.ac.uk/MSgenetics/GAMES) have recognized this difficulty, however, and have used standardized markers and reporting to enable meta-analysis of individual study results. New technologies that are reducing the cost of genotyping will also assist in enabling larger studies than have previously been possible. Furthermore, advancements such as high-density SNP (single nucleotide polymorphism) maps continue to increase the speed and accuracy of genotyping[16, 17].

References

Note: The spreadsheet *phase2-genetic-studies.xls* contains the PubMed ID of all candidate gene and genome screen studies which were included in this analysis.

1 A. N. Boiko, E. I. Gusev, M. A. Sudomoina, A. D. Alekseenkov, O. G. Kulakova, O. V. Bikova, O. I. Maslova, M. R. Guseva, S. Y. Boiko, M. E. Guseva and O. O. Favorova, *Association and linkage of juvenile MS with HLA-DR2(15) in Russians*. Neurology, 2002. **58**(4): p. 658-60. PubMed ID: 11865153.
An association was found for DR2(15) in Russians with juvenile onset MS (onset occurring at 15 years of age or younger). Similar frequencies of DRB1 alleles were found in children with MS and subjects with age of onset at 16 years or more.

2 S. L. Hauser, J. R. Oksenberg, R. Lincoln, J. Garovoy, R. W. Beck, S. R. Cole, P. S. Moke, K. E. Kip, R. L. Gal and D. T. Long, *Interaction between HLA-DR2 and abnormal brain MRI in optic neuritis and early MS. Optic Neuritis Study Group*. Neurology, 2000. **54**(9): p. 1859-61. PubMed ID: 10802800.
The HLA haplotype DR2 was present in 85 (48%) of 178 subjects enrolled in the Optic Neuritis Treatment Trial. This haplotype was associated with higher odds of conversion to probable or definite MS at five years, especially in subjects with brain MRI signal abnormalities.

3 L. F. Barcellos, J. R. Oksenberg, A. B. Begovich, E. R. Martin, S. Schmidt, E. Vittinghoff, D. S. Goodin, D. Pelletier, R. R. Lincoln, P. Bucher, A. Swerdlin, M. A. Pericak-Vance, J. L. Haines and S. L. Hauser, *HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course*. Am J Hum Genet, 2003. **72**(3): p. 710-6. PubMed ID: 12557126.
An investigation of the dose effect for HLA-DR2 in MS found that two copies of this haplotype increases disease risk as well as clinical severity.

4 J. Kira, T. Kanai, Y. Nishimura, K. Yamasaki, S. Matsushita, Y. Kawano, K. Hasuo, S. Tobimatsu and T. Kobayashi, *Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders*. Ann Neurol, 1996. **40**(4): p. 569-74. PubMed ID: 8871575.
In a study of Japanese MS, the DR2 alleles DRB1*1501 and DRB5*0101 were associated with Western-type MS (34 subjects) but not with Asian-type MS (23 subjects) compared with healthy controls.

5 H. Ito, K. Yamasaki, Y. Kawano, I. Horiuchi, C. Yun, Y. Nishimura and J. Kira, *HLA-DP-associated susceptibility to the optico-spinal form of multiple sclerosis in the Japanese*. Tissue Antigens, 1998. **52**(2): p. 179-82. PubMed ID: 9756407.
Frequencies of DPA1*0202 and DPB1*0501 were significantly increased in Japanese subjects with Asian-type MS compared with controls, but not in those with Western-type MS.

6 J. J. Ma, M. Nishimura, H. Mine, H. Saji, M. Ohta, K. Saida, K. Ozawa, H. Kawakami, T. Saida and T. Uchiyama, *HLA-DRB1 and tumor necrosis factor gene polymorphisms in Japanese patients with multiple sclerosis*. J Neuroimmunol, 1998. **92**(1-2): p. 109-12. PubMed ID: 9916885.

An association with HLA-DRB1*1501 was found in 42 Japanese subjects with Western-type MS compared with controls, but not in 38 subjects with Asian-type MS.

7 K. Yamasaki, I. Horiuchi, M. Minohara, Y. Kawano, Y. Ohyagi, T. Yamada, F. Mihara, H. Ito, Y. Nishimura and J. Kira, *HLA-DPB1*0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies*. Brain, 1999. **122**(Pt 9): p. 1689-96. PubMed ID: 10468508.

Frequency of HLA-DPB1*0501 was significantly greater in Japanese subjects with opticospinal MS than in healthy controls, but not in Western type MS or spinal MS.

8 T. Fukazawa, S. Kikuchi, H. Sasaki, I. Yabe, R. Miyagishi, T. Hamada and K. Tashiro, *Genomic HLA profiles of MS in Hokkaido, Japan: important role of DPB1*0501 allele*. J Neurol, 2000. **247**(3): p. 175-8. PubMed ID: 10787110.

The DPB1*0501 allele was confirmed to be positively associated with opticospinal MS in subjects from Hokkaido, Japan. This allele was also higher in subjects with conventional MS than in controls, although this association was not statistically significant.

9 D. G. Haegert, F. Muntoni, M. R. Murru, G. Costa, G. S. Francis and M. G. Marrosu, *HLA-DQA1 and -DQB1 associations with multiple sclerosis in Sardinia and French Canada: evidence for immunogenetically distinct patient groups*. Neurology, 1993. **43**(3 Pt 1): p. 548-52. PubMed ID: 8450999.

A study of 116 Sardinian and 75 French-Canadian MS subjects identified associations with DQA1*0102 and DQB1*0602 in French-Canadians, but no association with these alleles in Sardinians. Alleles associated with Sardinian MS included DQB1*0302 and *0201 and DQA1*0301; these were not associated with French-Canadian MS. DQA1*0102 was found to be protective against MS in Sardinians.

10 M. G. Marrosu, M. R. Murru, G. Costa, F. Cucca, S. Sotgiu, G. Rosati and F. Muntoni, *Multiple sclerosis in Sardinia is associated and in linkage disequilibrium with HLA-DR3 and -DR4 alleles*. Am J Hum Genet, 1997. **61**(2): p. 454-7. PubMed ID: 9311753.

The DR4 association in Sardinian MS subjects was confirmed, and a new association with DR3 (DRB1*0301) was identified through use of the transmission disequilibrium test.

11 M. G. Marrosu, M. R. Murru, G. Costa, R. Murru, F. Muntoni and F. Cucca, *DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population*. Hum Mol Genet, 1998. **7**(8): p. 1235-7. PubMed ID: 9668164.

DR3 and DR4 haplotypes were found to be associated with MS in a study of Sardinian subjects. No individual allele could explain MS susceptibility. Comparison of these two haplotypes with the DR2 haplotype failed to identify any shared epitopes in the DQ or DR molecules that would influence MS risk.

12 P. P. Bitti, B. S. Murgia, A. Ticca, R. Ferrai, L. Musu, M. L. Piras, E. Puledda, S. Campo, S. Durando, C. Montomoli, D. G. Clayton, A. P. Mander and L.

Bernardinelli, *Association between the ancestral haplotype HLA A30B18DR3 and multiple sclerosis in central Sardinia*. Genet Epidemiol, 2001. **20**(2): p. 271-83. PubMed ID: 11180452.
A case-control study involving MS subjects from the Nuoro population of Sardinia revealed an association with the ancestral haplotype A30B18DR3. However, DR3 allele was found to be conditionally independent of disease status given the A30B18 haplotype.

13 M. G. Marrosu, R. Murru, M. R. Murru, G. Costa, P. Zavattari, M. Whalen, E. Cocco, C. Mancosu, L. Schirru, E. Solla, E. Fadda, C. Melis, I. Porru, M. Rolesu and F. Cucca, *Dissection of the HLA association with multiple sclerosis in the founder isolated population of Sardinia*. Hum Mol Genet, 2001. **10**(25): p. 2907-16. PubMed ID: 11741834.
A systematic search for HLA associations in sporadic and familial MS subjects from Sardinia revealed five DQB1-DRB1 haplotypes, including those primarily found associated with MS in previous studies. Included in the set are the DRB1*1501–DQB1*0602 haplotype associated with MS in numerous populations, two DR4 haplotypes (DRB1*0405–DQB1*0301 and -DQB1*0302), one DR3 haplotype (DRB1*0301–DQB1*0201) and one DR13 haplotype (DRB1*1303–DQB1*0301).

14 H. Pihlaja, T. Rantamaki, J. Wikstrom, M. L. Sumelahti, M. Laaksonen, J. Ilonen, J. Ruutiainen, T. Pirttila, I. Elovaara, M. Reunanen, S. Kuokkanen, L. Peltonen, K. Koivisto and P. J. Tienari, *Linkage disequilibrium between the MBP tetranucleotide repeat and multiple sclerosis is restricted to a geographically defined subpopulation in Finland*. Genes Immun, 2003. **4**(2): p. 138-46. PubMed ID: 12618862.
A family association study confirmed previously detected associations with MS for the 1.27 kb allele of the MBP short repeat and the 1.27-B10 haplotype in families from the Southern Ostrobothnia region in western Finland. Not even a trend toward association was found in families from southern, eastern or northern Finland.

15 K. E. Lohmueller, C. L. Pearce, M. Pike, E. S. Lander and J. N. Hirschhorn, *Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease*. Nat Genet, 2003. **33**(2): p. 177-82. PubMed ID: 12524541.
An analysis of 301 association studies investigating 25 different possible gene/disease associations revealed that inconsistencies among the studies were not due to publication bias, but may be due to false negative, underpowered studies. The authors recommend adopting more stringent criteria for interpreting association studies; encouraging studies on the order of thousands of case-control pairs when investigating the effects of common variants; and confirming previously reported associations using large samples.

16 S. Sawcer, M. Ban, M. Maranian, T. W. Yeo, A. Compston, A. Kirby, M. J. Daly, P. L. De Jager, E. Walsh, E. S. Lander, J. D. Rioux, D. A. Hafler, A. Ivinson, J. Rimmler, S. G. Gregory, S. Schmidt, M. A. Pericak-Vance, E. Akesson, J. Hillert, P. Datta, A. Oturai, L. P. Ryder, H. F. Harbo, A. Spurkland, K. M. Myhr, M.

Laaksonen, D. Booth, R. Heard, G. Stewart, R. Lincoln, L. F. Barcellos, S. L. Hauser, J. R. Oksenberg, S. J. Kenealy and J. L. Haines, *A high-density screen for linkage in multiple sclerosis*. Am J Hum Genet., 2005. **77**(3): p. 454-67. Epub 2005 Jul 29. PubMed ID: 16080120.

A SNP-based microarray whole genome screen (4,506 markers in 2,692 individuals) revealed highly significant linkage with MS for the major histocompatibility complex on chromosome 6p21 and suggestive linkage on chromosomes 17q23 and 5q33.

17 R. Godde, K. Rohde, C. Becker, M. R. Toliat, P. Entz, A. Suk, N. Muller, E. Sindern, M. Haupts, S. Schimrigk, P. Nurnberg and J. T. Epplen, *Association of the HLA region with multiple sclerosis as confirmed by a genome screen using >10,000 SNPs on DNA chips*. J Mol Med., 2005. **83**(6): p. 486-94. Epub 2005 Mar 16. PubMed ID: 15770496.

A genome screen for association with MS was performed using DNA chips containing 11,555 SNPs. A marker on 6p21.32 yielded the highest significance level.

Terms searched in conjunction with "multiple sclerosis":

imprinting
translocation
gene