

The Current State of Multiple Sclerosis Genetic Research

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Abstract

Introduction: Multiple sclerosis (MS) is the most common genetic disease of the nervous system with onset usually in young adulthood. Four genome-wide searches in different Caucasian populations for MS susceptibility loci have been performed, but none reported any linkage at a level that would be regarded as significant according to current criteria. Significant linkage of MS to allelic variants of the major histocompatibility (MHC) locus on chromosome 6p21 has been established although its overall contribution to MS susceptibility has proven difficult to quantify. The objective of this review is not only to provide the reader with an update of MS genetics research, but also to provide a basic knowledge of the techniques being employed to map MS susceptibility genes. The different methodologies are discussed, and specific studies are reviewed in context. **Methods:** This review is based on findings from original articles, however, the results of recent candidate gene studies are intended to update previous review articles. **Results:** There remains no concrete non-MHC locus for MS, although there are enough findings of sufficient interest to warrant further investigation and optimism. Stratification of genome scan data based on MHC class II suggests that it interacts differentially with non-MHC loci and that it contributes moderately to disease susceptibility. Candidate gene studies have continued to return negative and ambiguous results, and follow-up fine mapping of suggestive linkages from the UK genome scan has proven unsuccessful in identifying significant linkages. Genetic analysis of crosses between mouse strains that are differentially susceptible to experimental allergic encephalomyelitis (EAE) has yielded linkages corresponding to putative MS susceptibility loci. However, recent successes in transgenic mice may provide an alternative to EAE, regarded by some as a poor model of MS. **Conclusion:** The first whole genome search for a common human disease was performed over five years ago, and it is now clear, from the lack success in this field, that the genetic complexity of these traits has been underestimated. The genome-wide searches for MS susceptibility genes have suffered from insufficient statistical power, which has probably been compounded by disease and genetic heterogeneity. Studies in isolated populations and better laboratory and clinical definitions of disease are both steps in the right direction to solving these problems. Notwithstanding the negative effects of genetic heterogeneity, pooling of resources for meta-analyses may provide the increase in statistical power required for detection of loci that exert a moderate or small effect on disease predisposition.

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Multiple Sclerosis—The Disease

It was Charcot (1868) who first made the formal pathological correlation between lesions in the central nervous system (CNS) and a disorder termed, 'la sclérose en plaques'. Today, a number of other immune-mediated, demyelinating CNS diseases have been recognised and hence, classical multiple sclerosis (MS) is now specified as

MS-Charcot variant.¹ MS is characterised, clinically, by episodes of focal disorder of the central nervous system that remit to a variable extent and have a tendency to recur over many years. The variable clinical presentations are determined by the varied location, extent and progression of inflammatory demyelination within the CNS, which is the characteristic lesion. Multiple sclerosis has been

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historically difficult to diagnose with no definitive diagnostic test but there are now established clinical, para-clinical [most notably magnetic resonance imaging (MRI)] and laboratory-based criteria to facilitate accurate diagnosis of the condition. For research purposes, the most commonly used diagnostic criteria are those described by Poser and colleagues.² A characteristic of MS is heterogeneity of clinical presentation, though the spectrum of phenotypic expression of MS is well established. Clinical parameters have been devised to expedite subtype grouping into three major categories: relapsing-remitting disease, in which the disease exhibits a fluctuating course; primary progressive disease, in which the course of the condition is inexorably progressive; and secondary progressive disease, in which the initial course of the disease was relapsing-remitting, but becomes progressive in nature.

Despite intensive investigation, the aetiology of MS remains unknown. It has been a widely held view that MS is an example of autoimmune disease in which there is a pathogenetic role for myelin-reactive T cells possibly activated by exposure to environmental antigens. However, the nature of the responsible environmental factors remains elusive, although it has been suggested that exposure to infectious agents, such as viruses, could be involved.³ This has led to attempts to alter the course of MS by the use of immunomodulatory therapies, such as interferon- β .⁴⁻⁶ To date, however, there remains no cure for MS and the available disease-modifying drug treatment is expensive.

The Epidemiology of MS

MS is most common in Caucasians of North European origin where the population prevalence is approximately 1:1000.⁷ As in most diseases with a putative autoimmune aetiology, MS is more prevalent in females than males (2-3:1).^{8,9} Disease onset usually occurs in the third to fourth decade of life although children younger than 6 years of age have been diagnosed with clinically definite MS.¹⁰ A number of studies have proposed that susceptibility to MS is environmentally determined, based on reports of post-World War II epidemics,^{11,12} links to canine distemper¹³ and the often cited correlation between distance from the equator and an increased prevalence of MS.^{9,14} To date, no infectious agent has been unequivocally linked to MS and the relationship of MS to latitude is inconsistent.⁷ Indeed, the geographical distribution of MS, taking into account an individual's genetic/racial origin, seems due to a combination of heterogeneous genetic and environmental factors.¹⁵ From population-based studies it appears that MS may be triggered, in genetically susceptible individuals, by exposure to an environmental agent. Support for this theory is endorsed by prevalence rates of MS in different ethnic groups living in the same location.^{16,17}

Multiple Sclerosis has a Genetic Component

Recurrence risk studies have been carried out extensively in an attempt to assess the heritability of MS. The premise being that if MS susceptibility is governed by genetic factors, then in individuals with a common genetic ancestry, predisposition to disease should be greater than the population risk. However, for family members sharing the same living environment, an infective nature for a disease cannot be ruled out. Ebers and colleagues¹⁸ addressed this issue by showing that familial aggregation of MS is genetically determined. From studying the prevalence of MS in adopted index cases and first-degree non-biological relatives, they found that the prevalence of MS in non-biological relatives of an index case was the same as the population prevalence, which was lower than that for biological relatives of an index case.¹⁸ The relative risk value, λ , is a measure of the genetic component of a disease or trait. It is defined as the prevalence of disease in a "target" group (e.g. sibs of affected individuals) divided by the prevalence of disease in "another" group (usually the general population). These types of analyses have shown that the genetic component of MS is stronger than for type I diabetes mellitus. Twin concordance analysis represents a powerful tool that can be used to quantify the heritability of a trait. Monozygous (MZ) and dizygous (DZ) twins share 100% and 50% of their genomes, respectively, and because twins develop in the same womb (at the same time), environmental factors that may influence foetal predisposition to disease are also shared. Twin concordance figures for MZ ($\lambda_{\text{MZ}} = 250-300$) and DZ ($\lambda_{\text{DZ}} = 30-50$) twins probably provide the most striking evidence that genetics plays a role in susceptibility to MS.¹⁹⁻²¹ As might be expected, the relative risk to a full sibling of an affected individual ($\lambda_s = 20-50$), is essentially the same as for a DZ twin.^{22,23} Ebers and Dyment²⁴ have recently reviewed recurrence risk rates for other relationships.

Familial MS only accounts for 15% to 20% of the disease,^{25,26} with less than 10% of pedigrees having more than 3 affected members.²⁷ It is believed that a family history of the disease corresponds to an increase in genetic load, which has encouraged a number of groups to study multiplex families in an attempt to define the clinical demographics of familial and sporadic MS.^{27,28} No overt phenotypic differences appear to exist between familial and sporadic MS, and no clear model has emerged for its inheritance. However, sibling pair concordance studies hint that certain features of MS, such as age-of-onset,^{29,30} disease severity³¹ and progression,^{23,32} may be under some degree of genetic control. Although there is no strong evidence supporting genetic heterogeneity between clinical subtypes of MS in humans, disease subtype-modifying loci have recently been mapped in the experimental allergic

encephalomyelitis (EAE) mouse model of MS.³³

Experimental Allergic Encephalomyelitis (EAE) as a Model for Multiple Sclerosis

EAE is regarded as the primary animal model of MS. Remlinger³⁴ in 1905, hypothesised that CNS tissue contaminating the Pasteur rabies vaccine was responsible for the acute disseminated encephalomyelitis (ADE) observed in 0.1% of individuals vaccinated. The origins of EAE can be traced back to then, however, it was not until the middle of the 20th century before murine EAE was established as a model for CNS demyelinating diseases, such as MS.³⁵ EAE is typically induced by injection or administration in susceptible mouse strains with a putative autoantigen, such as peptides corresponding to: myelin basic protein (MBP),³⁶ myelin oligodendrocyte glycoprotein (MOG), proteolipoprotein (PLP)^{37,38} or a spinal cord homogenate.³⁹ The autoimmune response in EAE is thought to be initiated by activated CD4⁺ T-cells that infiltrate the CNS, and recruit additional members of the immune repertoire across the blood-brain barrier, which take part in the inflammatory process leading to demyelination.³⁵ However, the laboratory-derived EAE model and MS share fundamental differences in aetiology; firstly, EAE is highly penetrant, which is not the case in MS; secondly, EAE must be induced with autoantigen, whereas MS is spontaneous. It is possible that while some of the pathogenetic mechanisms of the EAE phenotype will be relevant to MS, there are also likely to be “EAE-specific genes” that are not involved in susceptibility to MS and vice versa.⁴⁰

A number of studies have carried out genetic crosses between resistant and susceptible mouse strains leading to the identification of at least 13 *eae* loci.^{33,36,41–43} Some of these loci have been shown to overlap with regions of the human genome that have shown suggestive linkage to MS; *Eae 2* and chromosome 5p12-14;^{44–47} *eae1*³⁶ and *eae5*,^{43,48} are syntenic with the HLA region on human chromosome 6p21; and *eae7*,⁴³ corresponds to human chromosome 17q11-23.^{46,47} Recently, Encinas and colleagues⁴⁹ mapped a quantitative trait locus (QTL) influencing autoimmune diabetes and EAE to the same 0.15 cM region on mouse chromosome 3 containing the fibroblast growth factor 2 (*Fgf2*) and interleukin 2 (*il2*) genes. The locus was refined by generating NOD congenic lines carrying a B6-derived interval encompassing the *Idd3* locus, which represents a precise definition of this region.

The problems associated with EAE as a model for MS have been partially addressed by groups that have exploited transgenic technology to dissect the autoimmune response in mice.^{50–53} Madsen et al⁵⁴ recently generated mice that expressed three human components involved in T-cell

recognition of a peptide corresponding to the putative MS autoantigen, myelin basic protein (MBP). The MHC class II candidate gene found in Caucasians of North European origin, DRA*0101/DRB*1501 (HLA-DR2); a MS-patient-derived T-cell receptor (specific for the HLA-DR2/MBP 84-102 peptide complex); and the human CD4 co-receptor, were expressed in the same mice simultaneously. The most significant outcome of this work was that a small proportion of these mice developed MS spontaneously and that HLA-DR2 was able to mediate both induced and spontaneous disease. It is clear that transgenic studies will play a crucial role in the genetic dissection of MS.

Candidate Gene Studies

The investigation of specific candidate genes directly for polymorphisms or mutations that are associated with disease has been performed exhaustively for MS (Table I). It is generally assumed by researchers that an educated guess based on functional knowledge in selecting a gene for analysis is better than selecting from the genome at random. This makes sense except when we consider that our knowledge of the pathogenesis of MS is incomplete. Due to the lack of success of the candidate gene approach, it is obvious that a better knowledge of MS pathophysiology is required before a higher level of confidence in this approach can be achieved.

Linkage Analysis

In linkage analysis, one seeks to establish the co-inheritance of a disease or trait with genetic marker loci in

TABLE I: A SUMMARY OF CANDIDATE LOCI TESTED FOR ASSOCIATION WITH MS*

Further investigation:	References
Myelin basic protein (MBP)	55
Monocyte chemotactic protein-3 (MCP-3)	56
IL-1 β and IL-1 receptor agonist	57
Cytotoxic T lymphocyte antigen 4 (CTLA4)	58
IL4	59
Myeloperoxidase (MPO)	60
Spinocerebellar ataxia 2 (SCA2) gene/(CAG) ₂₂ trinucleotide repeat	61
Negative association:	
2', 3'-cyclic nucleotide—3' phosphodiesterase (CNPase)	62,63
Nitric oxide synthase (NOS1)	64
23 distinct oligodendrocyte growth factors	65
Complement factors C6 and C7	66
Transforming growth factor (TGF) - β 1 and β 2	63,67
Chemokine receptor 5 deletion (ccr5 Δ 32)	68,69

* To avoid unnecessary duplication, this list of candidate gene studies is intended to complement and update a review of the candidate gene approach by Dyment et al.⁷⁰

affected individuals who are related. Paths of descent (segregation) for these marker loci are established for members of the pedigree and a likelihood for a given inheritance profile is calculated using marker and phenotypic information. “Parametric” linkage analysis (PL) requires a model for segregation of the trait and marker/ trait data, whereas for “non-parametric” linkage analysis (NPL) no model needs to be specified. NPL gives a measure of the excess clustering of DNA segments, from marker data, inherited identical-by-descent (IBD) from common ancestors among affected individuals. An increased amount of sharing of the same segment of DNA IBD in affecteds over that seen in unaffecteds indicates that this region may harbour a disease gene. For multigenic or complex traits, it is virtually impossible to specify a genetic model of segregation with any degree of certainty. Hence, NPL is generally the method of choice for the genetic mapping of complex trait loci in small or extended pedigrees. Affected sib-pair analysis (ASP) has been employed extensively for the genetic analysis of complex traits. Mendelian segregation stipulates that full siblings will share both alleles inherited IBD from their parents 25% of the time, one allele 50% of the time, and neither allele 25% of the time, if they are inherited on a random basis. A deviation in allele-sharing away from these expected ratios in a cohort of affected sibling pairs indicates linkage. The addition of parents to the analysis can increase the power of ASP as this can help to establish IBD status of the sibs more definitively, and help identify genotyping errors.

Association

Association studies generally rely on linkage disequilibrium between the marker and disease locus. It is hypothesised that the number of ancestral mutations that give rise to the disease today are limited and that marker loci closely linked to the disease locus have “hitched-a-ride” on the same segment of DNA over time. The closer the marker and disease locus are together, the more generations (meioses) it will require to separate them; therefore, association tests work better for recent mutations in genetically isolated populations. Tests of association are non-parametric and require only marker and trait data on an affected and a carefully matched control group. Such tests simply assess the increase or decrease in frequency of particular marker alleles in affected individuals when compared with unaffected (control) individuals. The extent of the difference of the marker allele frequencies between these two groups defines the strength of the association. Thus, the rigorous pursuit of relevant controls is critical to this type of analysis; false positive associations are easily generated when the disease and control groups differ. For this reason, family-based controls, which form the basis of transmission disequilibrium testing (TDT), are regarded

by some as the best source of control marker data.⁷¹ TDT tests for linkage in the presence of association, by tracking transmission of parental alleles to affected offspring is at a given marker locus. Deviation from the expected transmission rate (50:50) of alleles from a heterozygous parent is indicative of linkage to a disease susceptibility locus.

The Human Leukocyte Antigen Complex (HLA)

The most consistent finding in MS genetic research, which crosses racial/ethnic boundaries, is the association of MS with allelic variants of HLA class II genes.^{27,72-76} In Caucasians of North European origin, the HLA haplotype, DRB1*1501-DQA1*0102-DQB1*0602,⁷³ is most frequently associated with MS, whereas the most frequently associated haplotypes in Caucasians of Mediterranean origin are DRB1*0405-DQA1*0501-DQB1*0301 and DRB1*0301-DQA1*0501-DQB1*0201.⁷⁶ Unfortunately, the high level of linkage disequilibrium that extends some 4 cM across the HLA locus represents a major obstacle to further resolution of the region and, therefore, identification of susceptibility factor(s).⁷⁷ To address this issue, Coraddu et al pursued a finding made in the UK genome scan in an attempt to determine whether the observed excess transmission of the 121 base pair (bp) allele of TNF α was independent of the established HLA association, or whether, it occurred because this gene formed part of the same extended haplotype.⁷⁸ They found that the TNF α 121bp allele was in linkage disequilibrium with the DRB*1501-DQB1*0602 haplotype (121-1501-0602) and that the individual alleles alone, when components of other haplotypes, did not display transmission distortion. The UK genome scan data was subsequently re-analysed after excluding data for sib-pairs sharing the extended haplotype (121-1501-0602). Interestingly, some of the suggestive linkages discovered in the original genome scan appeared to segregate into families sharing (1p, 2p and 17q) and not sharing (5cen, 7p and Xq) the extended HLA haplotype. These and other findings provide further evidence for genetic complexity in MS, which is exemplified by epistatic interactions between the HLA locus and non-HLA susceptibility genes.⁷⁸⁻⁸⁰ Recent family-based association and linkage analyses performed using microsatellite markers, corresponding to peaks of putative linkage,^{60,81,82} and within and flanking candidate myelin⁸³ and cytokine⁸⁰ genes, show no further significant associations.

The Genome Scans

Four genome-wide searches for MS susceptibility genes in different Caucasian populations (British, American/ French, Canadian and Finnish) provide strong evidence for complexity in MS genetics.^{45-47,84} Unfortunately, the four genome scans were conducted independently using different

TABLE II: A SUMMARY OF THE TWO-STAGE APPROACH CARRIED OUT IN THE FOUR GENOME SCANS FOR MS

Study	Family type	Stage 1 ^a	Stage 2 ^b	Total	Statistical analysis ^c	Chromosomal regions of potential linkage followed up in stage 2
American/French ⁸⁴	Sib-pairs	81 (443/7.9)	45	126	ASP, ARP, PL	6p21
British ⁴⁶	Sib-pairs	143 (311/11.3)	108	251	ASP, TDT, EM	1cen, 5cen, 6p21, 7p, 12p, 14q, 17q22, 19q, 22q
Canadian ⁴⁵	Sib-pairs	100 (261/13.4)	122	222	ASP, TDT, EM	5p, 6p21
Finnish ⁴⁷	Multiplex families	16 (328/10.7)	21	21	ASP, NPL, PL	2q, 3q, 4cen, 10q, 11tel, 17q, 18tel, 19tel

a The number of sib-pairs or families and (genetic markers used in stage 1 of the genome scan/ and the sex-averaged marker density in centimorgans (cM), which is calculated from an average genetic length of the human genome of 3500 cM taking into account the sex bias of the patient sample).

b The number of additional sib-pairs or families genotyped in stage 2.

c Methods of statistical analysis of data: Affected sib-pair analysis (ASP), affected relative pair analysis (ARP), parametric linkage analysis (PL), transmission disequilibrium testing (TDT), exclusion mapping (EM), non-parametric linkage analysis in pedigrees (NPL).

microsatellite marker sets on relatively small samples numbers (Table II), resulting in a lack of power and difficulty in cross-referencing linkages between the different populations. The two-stage approach for whole genome scanning has been employed extensively in the mapping of genes for complex traits.^{85,86} Stage 1 involves genotyping a group of families with usually 250 to 400 genetic markers spaced at 8 to 13 cM intervals (assuming a sex-averaged genetic length for the human genome of 3300 cM). Stage 2 is designed to replicate linkages, with linked markers from stage 1, in another set of families. Using this approach, the effort that would be required to genotype all families for the full set of genome scanning markers is significantly reduced. Linkage was found by all four groups to the human leukocyte antigen (HLA) region on chromosome 6p21 after stage 2 was completed, however, there were no other regions of the genome that appeared linked in all the studies. There are a number factors that may have contributed to the relatively disappointing results reported by the genome scans (below).

Locus or Non-allelic Heterogeneity

A confounding variable to genetic mapping studies, often encountered in diverse populations, is locus or non-allelic genetic heterogeneity. This occurs when the same disease phenotype is caused by two or more genes. It is probable that different combinations of MS susceptibility loci can give rise to clinically indistinguishable MS phenotypes.

Phenotypic Classification

In a genetic mapping study, one would like to assume that all patients are segregating the same disease and hence, the same disease locus or loci. MS is a highly variable phenotype for which current diagnostic criteria recognise three distinct clinical subtypes; relapsing-remitting, secondary

progressive and primary progressive disease.² While all four groups were careful to include only MS patients that conformed to the well-established clinical criteria, it was not feasible to collect enough samples to permit a subtype-specific analysis, hence, disease heterogeneity cannot be ruled out.

Genetic Complexity

Evidence from twin studies (above) suggests that MS represents a good example of a multifactorial trait. In a multifactorial trait, genes and the environment interact variably to modulate the disease phenotype. In MS, gene-gene interactions (epistasis) are also thought to play a role in susceptibility to disease.^{45,46,70,78,79}

Incomplete Penetrance

It is possible that incomplete penetrance exists in MS. The penetrance of a particular genotype at a disease locus is the probability that individuals possessing that genotype will develop the disease trait. Incomplete (partial or reduced) penetrance occurs when not all individuals possessing the disease genotype develop the corresponding phenotype. For instance, a paucity of MS pedigrees with more than 3 affected individuals could be due to recessively acting disease loci or incomplete penetrance, or a combination of both. Discordance between MZ twins may also be due to reduced penetrance; an advantage of affected sib-pair analysis (ASP) is that reduced penetrance is irrelevant.

Power Limitations and Replication of Linkage

The statistical power of a genetic study relates to its ability to reject the null hypothesis, testing at a given false positive rate. Hence, power clearly depends on effect size and available genetic resources (nature and size of sample). Relative risk statistics (λ_s , λ_{DZ} , λ_{MZ}) are measures of the size of the genetic contribution relevant to the resources. For

instance, in stage 1 of a genome scan (Table II), 100 sibling pairs will provide substantial statistical power to detect a locus with a moderate relative risk ($\lambda_s = 3.0$).⁸⁷ Assuming an additive genetic model for MS, where five loci influence susceptibility to disease: the number of families required to replicate linkage will be $n-1$ times the amount of families that were used to establish linkage initially in stage 1 (where n = the number of loci involved).⁸⁸ Hence, for the UK study more than 500 families would be required to replicate a finding for a gene of moderate effect under this model.⁴⁶ Without such a replication set of families, true linkages established in stage 1 may not have been replicated in stage 2. Thus, if linkage to a region is not replicated, it cannot be ruled out as harbouring a disease gene.

Incorrect Model Predictions

The complexity of MS genetics is evident. Despite this, in the American/French⁸⁴ and the Finnish studies,⁴⁷ traditional parametric linkage analysis was performed. In the American/French study, two-point linkage analysis was carried out using dominant and recessive models with different disease allele frequencies ($P = 0.05$ and $P = 0.2$, respectively), incorporating age-adjusted penetrance estimates for unaffected individuals.⁸⁴ In the Finnish study, four modes of inheritance were tested; dominant and recessive models with penetrance estimates ($f = 0.05$ and $f = 0.76$), and different disease allele frequencies (dominant, $P = 0.01$ and $P = 0.006$; recessive, $P = 0.014$ and $P = 0.035$). Five age-adjusted penetrance classes and a phenocopy rate of 0.001 were also incorporated into their genetic model of MS.⁴⁷ These models assume that MS is governed by a single disease locus. The complex nature of MS genetics suggests that these models are unlikely to reflect the truth, leading to a significant loss of power in the linkage analysis due to incorrect specification of the disease model.⁸⁹

The Post-Genome Scan Phase

Following stage 2 of a genome scan (Table II), additional genetic markers, spaced at greater density across the linked region, are genotyped to increase information extraction by improving the accuracy of IBD-sharing estimates. To date, only the UK families have been followed past stage 2 (Table II).⁷⁹ Additional families and markers were genotyped in “interesting” regions of potential linkage identified in the genome scan,⁴⁶ and in regions of the genome where gaps in the original marker sets existed, which were mostly telomere-derived. A chromosome-by-chromosome summary of the results was presented.⁷⁹ Only loci on chromosomes 6p (HLA), 17q and 14q showing increased evidence of linkage, while linkage to some did not change (1p, 3p/cen, 5cen and 6q), and others displayed reduced evidence for involvement in MS (2cen and 4q). Based on exclusion mapping, the authors: (a) excluded

chromosomes 8, 9, 10, 15, 18, 20 and 22 from harbouring genes of biological significance to MS susceptibility (no λ values ≥ 2 were observed); and (b) surmised that genes of large ($\lambda = 5$) and moderate ($\lambda = 2$) effect can be excluded from 95% and 65% of the genome respectively, but that a gene of small effect ($\lambda = 1.2$) cannot be excluded from any chromosome. Exclusion mapping is based on models that predict the behaviour of a single disease locus at given points throughout the genome. Hence, it is likely that these estimates for exclusion of MS disease loci represent an overly pessimistic view of the situation. Further evidence for epistatic interactions between HLA and non-HLA loci were reported after stratification of data based on DR15-sharing and non-sharing status. The authors speculated that disease heterogeneity and differential epistatic effects appeared to be the most likely reasons for the failure of the genome scanning approach to define the genetic aetiology of MS.

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