No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis

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Genome-wide association studies have been successful in identifying common variants that influence the susceptibility to complex diseases. From these studies, it has emerged that there is substantial overlap in susceptibility loci between diseases. In line with those findings, we hypothesized that shared genetic pathways may exist between multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). While both diseases may have inflammatory and neurodegenerative features, epidemiological studies have indicated an increased co-occurrence within individuals and families. To this purpose, we combined genome-wide data from 4088 MS patients, 3762 ALS patients and 12 030 healthy control individuals in whom 5 440 446 single-nucleotide polymorphisms (SNPs) were successfully genotyped or imputed. We tested these SNPs for the excess association shared between MS and ALS and also explored whether polygenic models of SNPs below genome-wide significance could explain some of the observed trait variance between diseases. Genome-wide association meta-analysis of SNPs as well as polygenic analyses fails to provide evidence in favor of an overlap in genetic susceptibility between MS and ALS. Hence, our findings do not support a shared genetic background of common risk variants in MS and ALS.

INTRODUCTION

Multiple sclerosis (MS, OMIM: 126200) is a common disease of the central nervous system characterized by inflammation, demyelination and axonal loss (1). Large extended families with the disease are extremely rare (2), but a genetic component in susceptibility to MS has been clearly demonstrated (1). Currently known risk variants include four classical human leukocyte antigen (HLA) alleles and >50 single-nucleotide polymorphisms (SNPs) outside the HLA region (3,4).

Amyotrophic lateral sclerosis (ALS, OMIM: 105400) is a neurodegenerative condition with devastating impact. Multiple cellular events contribute to the pathobiology, including mitochondrial dysfunction, excitotoxicity, protein aggregation in the cytosol, impaired axonal transport, neuroinflammation and dysregulated RNA signaling (5). About 10–20% of cases are familial, and up to 50% of these can be explained by known mutations in 18 genes including *SOD1*, *FUS*, *TARDBP* and *C9orf72* (6). The majority of patients are isolated cases, however. Not all results from genome-wide association studies (GWAS) have been replicated, but two regions of association have been confirmed in independent studies: a locus on chromosome 9 and variation in the *UNC13A* region (7–11).

One of the lessons learned in the GWAS era is the substantial overlap in susceptibility loci between diseases. This has been demonstrated for immune-related (12,13), metabolic (14) and psychiatric (15) disorders and indicates, sometimes unexpectedly, commonalities and differences between diseases. MS indeed shares several susceptibility loci with other immune-related disorders, including type 1 diabetes and Crohn's disease (3). However, besides the immune component, key features of neurodegeneration, i.e. axonal transection, neuronal cell atrophy and neuronal death, are early pathological events in MS (1). Moreover, the irreversible disability seen in patients correlates stronger with neuronal damage than with inflammatory demyelination (16), although the cause of the neuronal damage remains elusive. On the other hand, for diseases classified as neurodegenerative such as ALS, an inflammatory or immune component has been implicated but is not yet conclusive (17,18). Case reports have described patients affected by both diseases (19–24) and an increased co-occurrence of MS and ALS compared with what is expected has been observed (25,26). Studies also report an increased risk of MS among relatives of patients suffering from ALS and vice versa (27–29), and some but not all studies report geographical correlation in mortality rates of both diseases (30,31).

In order to assess the shared genetic contribution between MS and ALS, possibly through common pathways of neurodegeneration or inflammation, we investigated the overlap of common susceptibility variants using available GWAS data.

RESULTS

We first investigated previously reported (3,4,7–11) susceptibility loci in one disease for evidence of association in the other. None of the reported ALS susceptibility loci show evidence for association with MS (Table 1). Out of 56 established, independent MS susceptibility loci (3,4), 4 (7.1%) show nominal significance for association with ALS, but none survived multiple testing for the number of SNPs investigated (Table 2). As expected because of the overlap between the datasets used here and those used in the original studies of each disease separately, all previously reported risk factors for either MS or ALS show the same direction of effect for the respective disease in this dataset as in the original studies. Regarding the other disease, 4/5 reported ALS risk SNPs show the same direction of effect in MS as in ALS (sign test $P = 0.38$), and among established MS-associated SNPs, 26/56 (46%) SNPs show the same direction of effect in ALS (sign test $P = 0.69$). Four SNPs were previously highlighted for reaching suggestive P-values of $<10^{-5}$ for association with disease course (bout onset versus primary progressive MS) (3). Only one of these shows evidence for association with ALS but in the opposite direction (data not shown).

We next combined summary results from both MS and ALS datasets in a meta-analysis, looking for modest effects in each dataset that strengthen each other in the combined analysis. The combined analysis of both diseases included a total of 5 440 446 SNPs (Fig. 1). The genomic inflation factor (λ_s) was 1.033 for MS, 0.997 for ALS and 1.005 for the combined MS–ALS meta-analysis. In the meta-analysis, the HLA region reaches genome-wide significance, but this is driven by the MS component (P ALS with same direction of effect ≥ 0.01). One

**Table 1.** Association for reported ALS susceptibility loci with MS

Chromosome	Rsid	Position (hg19)	Gene	Risk allele	P ALS	OR ALS	P MS	OR MS
1	rs6700125	59702797	FGGY (9)	T	0.087	1.06	0.085	1.06
7	rs10260404	154210798	DPP6 (10)	C	0.0049	1.10	0.55	1.02
9	rs3849942	27543281	C9orf72 (7,8)	T	5.8E-06	1.19	0.26	1.04
12	rs2306677	26636386	ITPR2 (11)	A	0.080	1.10	0.60	1.03
19	rs12608932	17752689	UNC13A (7)	C	8.3E-09	1.21	0.39	0.97

Table 2. Association for independent, established MS susceptibility loci with ALS

Chromosome	Rsid	Position (hg19)	Gene	Risk allele	P MS	OR MS	P ALS	OR ALS
1	rs4648356	2709164	MMEL1 (TNFRSF14)	C	0.012	1.09	0.97	1.00
1	rs11810217	93148377	EVI5	T	0.00032	1.14	0.12	0.94
1	rs11581062	101407519	SLC30A7	G	0.032	1.08	0.025	1.08
1	rs1335532	117100957	CD58	A	1.2E-08	1.35	0.97	1.00
1	rs1323292	192541021	RGS1	A	0.0098	1.11	0.53	1.03
1	rs7522462	200881595	C1orf106	G	0.00083	1.13	0.023	0.92
2	Rs6718520[a] (4)	43325570	ZFP36L2 (THADA)	A	1.2E-05	1.16	0.84	1.01
2	rs12466022	43359061	ZFP36L2 (THADA)	C	4.2E-05	1.16	0.76	0.99
2	rs7595037	68647095	PLEK	T	1.6E-05	1.15	0.32	0.97
2	rs17174870	112665201	MERTK	C	0.00012	1.15	0.79	1.01
2	rs10201872	231106724	SP140	T	0.00056	1.15	0.13	1.07
3	rs669607	28071444	intergenic	C	2.5E-05	1.15	0.57	0.98
3	rs2028597	105558837	CBLB	G	0.56	1.03	0.52	1.04
3	rs2293370	119219934	C3orf1	G	0.056	1.08	0.29	0.96
3	rs9282641	121796768	CD86	G	0.0015	1.22	0.52	0.96
3	rs2243123	159709651	IL12A	C	0.17	1.05	0.25	1.04
4	rs228614	103578637	MANBA	G	0.0092	1.18	0.23	0.625
5	rs6897932	35874575	IL7R	C	0.0014	1.12	0.20	0.96
5	rs4613763	40392728	PTGER4	C	0.00014	1.19	0.87	0.99
5	rs2546890	158759900	IL12B	A	3.8E-06	1.16	0.78	1.01
6	rs12212193	90996769	BACH2	G	0.0055	1.09	0.14	1.05
6	rs802734	128278798	PTPRK	A	0.0014	1.12	0.89	1.00
6	rs11154801	135739355	AHI1	A	0.014	1.08	0.49	0.98
6	rs17066096	137452908	IL22RA2	G	0.00096	1.13	0.29	0.96
6	rs1738074	159465977	TAGAP	C	0.00075	1.12	0.45	0.98
7	rs354033	149289464	ZNF767	G	0.00079	1.13	0.26	1.04
8	rs1520333	79401038	PKIA	G	0.11	1.06	0.41	1.03
8	rs4410871	128815029	MYC	C	0.018	1.09	0.54	1.02
9	rs2150702	5893861	MLANA	G	2.5E-05	1.14	0.015	1.08
10	rs3118470	6101713	IL2RA	C	0.00078	1.12	0.76	1.01
10	rs1250550	81060317	ZMIZ1	A	0.0024	1.11	0.66	0.98
10	rs7923837	94481917	HHEX	G	0.015	1.08	0.18	0.96
11	rs650258	60832282	CD5	C	0.00018	1.14	0.097	0.95
11	rs630923	118754353	CXCR5	C	0.033	1.11	0.066	1.08
12	rs1800693	6440009	TNFRSF1A	G	NA[b]	NA	0.67	1.01
12	rs10466829	9876091	CLECL1	A	0.0009	1.11	0.49	0.98
12	rs12368653	58133256	AGAP2	A	0.0018	1.10	0.31	0.97
12	rs949143	123595163	ARL6IP4	G	0.015	1.08	0.57	0.98
14	rs4902647	69254191	ZFP36L1	C	0.00022	1.12	0.72	0.99
14	rs2300603	76005557	BATF	T	0.014	1.10	0.10	0.94
14	rs2119704	88487689	GPR65	C	0.045	1.13	0.23	0.93
16	rs2744148	1073552	SOX8	G	0.023	1.10	0.30	0.95
16	rs7200786	11177801	CLEC16A	A	8.8E-05	1.14	0.58	0.98
16	rs13333054	86011033	IRF8	T	0.063	1.09	0.98	1.00
17	rs9891119	40507980	STAT3	C	0.00016	1.13	0.86	0.99
17	rs180515	58024275	RPS6KB1	G	0.093	1.06	0.74	1.01
18	rs7238078	56384192	MALT1	T	0.00075	1.13	0.99	1.00
19	rs1077667	6668972	TNFSF14	C	0.033	1.10	0.10	0.94
19	rs8112449	10520064	CDC37	G	0.14	1.05	0.83	0.99
19	rs874628	18304700	MPV17L2	A	0.021	1.09	0.65	0.98
19	rs2303759	49869051	DKKL1	G	0.0075	1.11	0.034	1.08
20	rs2425752	44702120	NCOA5	T	0.0001	1.14	0.40	0.97
20	rs2248359	52791518	CYP24A1	C	0.00085	1.12	0.29	1.04
20	rs6062314	62409713	ZBTB46	T	0.047	1.14	0.52	1.04
22	rs2283792	22131125	MAPK1	G	0.00036	1.12	0.23	1.04
22	rs140522	50971266	ODF3B	T	0.0022	1.12	0.72	0.99

Source of variants: (3), except where specified: (4).
[a] $r^2 = 0.15$ with adjacent variant rs12466022, [b] No SNP with $r^2 > 0.6$.
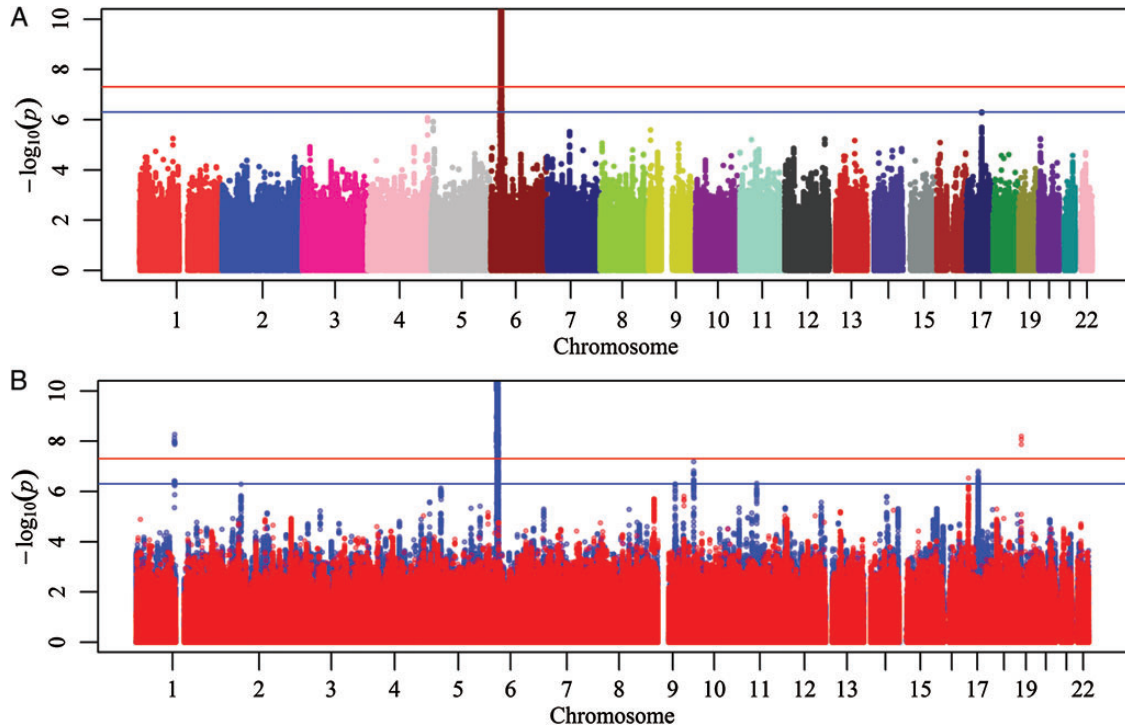
Figure 1. Manhattan plot of (**A**) a combined MS–ALS analysis and (**B**) an overlay of the individual components consisting of both diseases (blue: MS, red: ALS). The *y*-axis has been cut off at -logP = 10. Red and blue horizontal lines indicate genome-wide ($P < 5 \times 10^{-8}$) and suggestive ($P < 5 \times 10^{-7}$) evidence.

Table 3. Polygenic score based on MS data in ALS

Model	*P*-value	Number of SNPs	Nagelkerke r^2 corrected for baseline[a]
<5E−8	0.820	75	5.4E−06
<5E−7	0.963	90	2.0E−07
<5E−6	0.987	114	0.0E+00
<5E−5	0.827	184	5.0E−06
<5E−4	0.880	633	2.4E−06
<5E−3	0.414	3454	6.9E−05
<0.05	0.775	22284	8.5E−06
<0.1	0.848	38861	3.8E−06
<0.2	0.986	66276	1.0E−07
<0.3	0.743	89109	1.1E−05
<0.4	0.459	108626	5.7E−05
<0.5	0.412	125558	7.0E−05

[a]Baseline: PC1-3, dummy-coded cohorts.

Table 4. Polygenic score based on ALS in MS

Model	*P*-value	Number of SNPs	Nagelkerke r^2 corrected for baseline[a]
<5E−8	0.843	3	4.5E−06
<5E−7	0.785	4	8.4E−06
<5E−6	0.500	7	5.2E−05
<5E−5	0.452	49	6.4E−05
<5E−4	0.928	389	9.3E−07
<5E−3	0.306	3075	1.2E−04
<0.05	0.032	22315	5.2E−04
<0.1	0.050	38922	4.4E−04
<0.2	0.040	66738	4.8E−04
<0.3	0.057	89592	4.1E−04
<0.4	0.048	108839	4.4E−04
<0.5	0.074	125337	3.6E−04

[a]Baseline: PC1-5, dummy-coded cohorts.

region, near *NPEPPS* on chromosome 17 (rs2935183), reaches suggestive association levels of $P < 5 \times 10^{-7}$ but is once again driven by MS [P (MS) = 6.5×10^{-7}; P (ALS) = 0.41].

Lastly, we investigated the possibility of an overlap of small susceptibility effects (polygenic score or 'en masse' effect). Therefore, we tested collectively SNPs that reached certain thresholds in the MS or ALS GWASs for association with ALS and MS, respectively. After correction for multiple testing, none of the models were significantly associated with disease (Tables 3 and 4), with the best model for each disease explaining only 0.05% of the phenotypic variance. To test whether the lack of association may have been affected by

association results in the HLA region (which is known to be strongly associated with MS, but not with ALS), we repeated the polygenic analysis excluding SNPs in the HLA region (removing all SNPs on chromosome 6 between 29 and 33 Mb). This did not influence the results (Supplementary Material, Table S1).

DISCUSSION

In this study, we have applied several statistical approaches to the investigation of shared susceptibility loci between the

neurological diseases MS and ALS, which are both thought to involve inflammatory and neurodegenerative components (1,17,18) and for which case reports and epidemiological studies have reported co-occurrence within individuals or families (19–29). The strength of the study is that different statistical approaches are consistent in demonstrating that the number of regions in the genome with evidence for an overlap in susceptibility between the two diseases is not more than expected by chance. Among 65 loci having previously been implicated in one disease or disease subgroup, only 5 show nominally significant association with the other disease and none survive correction for multiple testing. There was no significant enrichment for the same direction of effect in both diseases. In a combined analysis of both diseases, no region outside of the HLA reaches genome-wide significance and only one reaches suggestive association levels of $P < 5 \times 10^{-7}$. Moreover, for both these regions with evidence for association in both diseases, results appear driven by strong evidence in MS, despite sample sizes of similar magnitude for both diseases. Furthermore, the polygenic analysis demonstrates that it is unlikely that many common variants with effect sizes that are beyond the detection threshold for association are shared between the two diseases. This contrasts with other diseases where a polygenic risk score calculated for one disease is associated with related diseases, as in the example of schizophrenia and bipolar disorder (15).

MS is a clinically heterogeneous disease, with the majority of patients (∼80%) suffering from a bout onset form of the disease with relapses and remissions, possibly followed by secondary progression, and the remaining 20% being characterized by progression from onset (1). It has been speculated that both forms represent a continuous spectrum of disease phenotypes with risk factors driving the balance between inflammation and neurodegeneration (32). Genetic association studies have so far not provided evidence for a different pathogenesis of the two forms (3). On the contrary, *HLA-DRB1*1501*, the strongest risk factor in MS and especially immunological in nature, is shared between both bout onset and primary progressive MS. In this study, there was no evidence for shared loci with the same direction of effect between ALS and primary progressive MS.

A total of >50 common risk variants for MS have now been identified (3,4). There is a highly significant enrichment for immune system genes in this list, with only few variants having a potential neurological function (3). GWAS studies in ALS have seen limited success (8). This discrepancy in the number of common risk variants identified between immunological and other diseases has been suggested to reflect a history of selection and adaptation of variants influencing the immune system (33,34). Mutations in several genes cause familial forms of ALS, and it has been thought that less common (1–5%) or rare (<1%) variants play a role in sporadic forms of the disease as well (35). Similarly, first reports of less common and rare variants in MS are emerging (36,37). This category of variants, which are not well captured by current genome-wide association studies, may explain part of the heritability in MS and ALS that remains unaccounted for by common variants ('missing heritability'), and potentially the shared neurodegenerative component. Next-generation sequencing offers a technology suited to address this hypothesis.

It has recently been demonstrated that a large proportion of ALS is related to a GGGGCC hexanucleotide repeat expansion in intron 1 of *C9orf72* (38,39), located in a region on chromosome 9p previously highlighted in GWAS studies of ALS (7,8). We did not observe any association of the *C9orf72* region with MS. This is in line with the fact that no repeat expansions were observed in a cohort of 215 MS patients (25). Hence, *C9orf72* variation does not appear to be a risk factor for MS. It has been suggested that MS can act as a modifier that increases the likelihood of *C9orf72* expansions becoming penetrant and causing concurrent ALS (25), although further investigation is required (40).

In summary, the overlap of common variants between MS and other autoimmune disorders is not matched by a similar overlap between MS and other neurological disorders, such as ALS in this study. Whether less common or rare variants explain some of the shared neurodegenerative or neuroinflammatory aspects of both diseases cannot be addressed with the currently available datasets and remains to be examined with emerging technologies.

MATERIALS AND METHODS

We used data from 6 datasets totaling 4088 MS patients and 7144 controls from a recent meta-analysis of MS genome-wide association studies (4). Imputation was performed using Beagle v3.1 and the 1000 Genomes Project (1000G) Phase I (a) reference panel (2010/11 data freeze, 2011/6 haplotypes), and analysis was performed as described previously using the post-imputation probabilities and the first five principal components (PC) as covariates (4), leading to association results for a total of 6 948 682 SNPs with INFO of >0.10 and a minor allele frequency of >0.01 in all 6 datasets.

The ALS study population consists of 3 762 patients and 4 886 controls over 11 cohorts, for which details have been described previously (7,41). Imputation was performed using Beagle v.3.1.1. software with the 1000G CEU Aug 2010 reference panel. Analysis on dosage data including 3 PC led to association results for 12 249 385 SNPs.

A/T and C/G SNPs were removed, and results from both datasets on 5 440 446 overlapping SNPs were combined using an inverse variance fixed-effects model as implemented in the PLINK software package (42). Power was >99% for OR of ≥1.2 and >80% for OR of ≥1.15 at a typical risk allele frequency of 30% and genome-wide significance ($P < 5 \times 10^{-8}$).

Polygenic risk scores were calculated per individual to test the collective impact of SNPs that are associated with ALS on MS and vice versa. For each trait (MS and ALS), we first pruned the association results of the GWAS by linkage disequilibrium ($r^2 = 0.1$), preferentially keeping SNPs with lower *P*-values. We selected twelve sets of SNPs (models) based on their GWAS *P*-values ($<5 \times 10^{-8}$, $<5 \times 10^{-7}$, $<5 \times 10^{-6}$, $<5 \times 10^{-5}$, $<5 \times 10^{-4}$, $<5 \times 10^{-3}$, 0.05, <0.1, <0.2, <0.3, <0.4 and <0.5). The smallest model contains three SNPs, whereas the models of $P < 0.5$ contain >125 000 SNPs (Table 3). Next, we calculated a polygenic risk score in all individuals of the other GWAS by summing up the dosages of the risk alleles in each model, multiplied by the log-odds. We then tested the association between the risk score and the phenotype using logistic regression with the same number of PCs as used in the original analysis of each trait (ALS: PC1-3, MS: PC1-5)

and dummy-coded cohorts as covariates. Nagelkerke r^2 was calculated to test the variance explained by each model (43).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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