Biological significance of associated genes in MS

To explore the biological significance of the genes constituting the significant pathways, we conducted a gene ontology (GO) analysis by using Cytoscape plug-in, clueGO for significant subnetworks identified in both data sets. GO analysis (biological process) identified up to nine significantly enriched categories in all regions except fusiform cortex (Figure 3, Figure S4, Table S6). The category "negative regulation of neuron differentiation" was significantly enriched in inferior parietal (enrichment: 73.5, $P_{corr} = 1.73\times10^{-5}$), paracentral (enrichment: 82.8, $P_{corr} = 1.51\times10^{-6}$), superior parietal (enrichment: 32.3, $P_{corr} = 8.74\times10^{-7}$) and terms related to glutamate biology were identified in banks of superior temporal sulcus ("Glutamate secretion," enrichment: 49.5, $P_{corr} = 5.06\times10^{-5}$) and superior frontal ("Glutamate receptor signaling pathway," enrichment: 21.5, $P_{corr} = 5.18\times10^{-4}$). Terms associated with calcium channel were also identified in two regions (superior frontal, enrichment: 41.9, $P_{corr} = 8.41\times10^{-5}$, supramarginal, enrichment: 40.0, $P_{corr} = 7.84\times10^{-4}$). The most significant GO categories in individual regions were "SMAD protein signal transduction" (inferior parietal, enrichment: 302.8, $P_{corr} = 4.42\times10^{-9}$), "phosphatidylinositol 3 kinase activity" (isthmus of cingulate gyrus, enrichment 350.4, $P_{corr} = 6.81\times10^{-9}$) "fluid transport" (paracentral, enrichment: 71.6, $P_{corr} = 1.97\times10^{-5}$), positive regulation of protein kinase B signaling cascade" (precuneus, enrichment: 30.4, $P_{corr} = 3.49\times10^{-5}$) and "positive regulation of phsphoprotein phosphatase activity" (superior frontal, enrichment: 90.8, $P_{corr} = 1.02\times10^{-4}$).

Discussion

We identified nine cortical regions in which the average thickness of cases and controls was significantly different and conducted a GWAS within MS patients to identify genetic variants that could associate with this phenotype. The thinner regions were distributed mainly along the parietal lobe, with portions of frontal and temporal lobes also involved. This pattern is somewhat different than reported in previous studies, in which the frontal and temporal lobes were predominantly involved (Achiron *et al.*, 2013, Calabrese *et al.*, 2010a, Sailer *et al.*, 2003). The isthmus of the cingulate gyrus and the banks of superior temporal sulcus, were significantly thinner in CIS cases than in controls. When comparing established MS (SP, PP, and PRMS) and controls, eight of the nine regions of interest (except for superior parietal cortex in RRMS) were similarly involved. The finding that these regions were reproducibly identified even in different disease courses underscores the importance of these ROI in MS. Interestingly, cortical thickness in almost all ROI correlated with EDSS, suggesting the relevance of these regions in the development of disability in MS. Intriguingly, 8 out of 9 ROI (except banks of superior temporal sulcus) were previously reported as highly heritable based on twin studies (Joshi *et al.*, 2011, Kremen *et al.*, 2010, Rimol *et al.*, 2010), indicating a genetic effect to cortical thinning in MS.

Possibly due to the limited size of the cohorts analyzed, no marker exceeded a genome-wide significant p value ($p < 9.42\times10^{-8}$), and no replication was observed between the two data sets at the SNP level. However, markers detected in GWAS explain a limited fraction of the heritable component of common diseases and traits and a sizable proportion of risk alleles are still being missed under an assumption that each marker has independent effect (Bodmer & Bonilla, 2008).

Recently, novel approaches to GWAS analysis have been proposed. These strategies focus on the combined effects of several loci, acknowledging that each may make a small contribution to the overall phenotype, and potentially providing valuable insights into the genetic basis of common disease ((Imsgc) *et al.*, 2010, Purcell *et al.*, 2009). By analyzing gene-wise p-values we report replication of more nominally significant genes ($p < 0.05$) than expected by chance in two data sets (IP and FUS) (Table S2). Also, while discrepancies existed between studies (probably due to the limited size of the smaller dataset), the list of nominally significant genes in each cohort contain more shared associations than expected (Table S3). However, when genes arranged in interaction networks were compared between the two cohorts, higher replication rates were observed (Table 3). This strategy produces comparable results to the more established approach extending a fixed genetic distance from the lead SNP to the next recombination hotspot and maximizes the potential of finding bona fide associations((Imsgc), 2013). In fact, proteins encoded by nominally associated genes were more connected in the PIN than what would be expected by chance in most of the regions (Figure 2). Thus, analyzing nominal gene-level significance and studying genes in the context of biological networks seems a reasonable approach for these data sets.

Out of the 194 genes that were nominally significant in both data sets, 53 were observed in more than one region. This finding correlates well with a previous report that both global and regionally specific genetic factors influence cortical surface areas (Eyler *et al.*, 2011). Variation within *NPAS3* was a robust finding in 8 out of the 9 ROI. *NPAS3* encodes a member of the basic helix-loop-helix and PAS domain-containing family of transcription factors and the protein is involved in neurogenesis. Chromosomal abnormalities that affect the coding potential of this gene are associated with schizophrenia and mental retardation (Pickard *et al.*, 2005) and

SNPs within this domain are associated with efficacy of antipsychotic iloperidone in patients with schizophrenia (Lavedan *et al.*, 2009).

Of interest, most of the genes identified in this study do not harbor variants associated with susceptibility, most of which are involved in inflammatory processes ((Imsgc) *et al.*, 2011, Patsopoulos *et al.*, 2011). Thus, our results underscore the need to differentiate genetic variation that affects susceptibility, from that affecting endophenotypes such as neural degeneration (Kutzelnigg *et al.*, 2005, Wegner *et al.*, 2006). In this model, inflammation is a pervasive feature in the pathology of MS, and varying degrees of neurodegeneration ensue thereafter. Results from the work presented here suggest that part of the variability in neurodegeneration might be due to genetic variation. Notable exceptions are *FOXP1* (paracentral), *SDK1* (fusiform, paracentral, precuneus, superior frontal, superior parietal and supramarginal), *SLC2A4RG* (paracentral), and *WWOX* (precuneus and superior parietal). *NXPH1* had nominally significant effect on cortical thickness of inferior parietal cortex in both data sets and *EPHA4* was included in a significant pathway for superior frontal cortex. SNPs proximal to these genes have been recently reported to have a significant effect on GM density of frontal lobe in Alzheimer's disease. Interestingly, GO categories related to glutamate biology were significantly enriched in banks of superior temporal sulcus and superior frontal cortex. We previously identified a module with high relevance to glutamate biology by using GWAS for glutamate concentration in MS and reported that individuals carrying a higher number of associated alleles from genes in the module showed greater decreases in brain volume over 1 year of follow-up (Baranzini *et al.*, 2010). Thus, pathways related to glutamate may influence cortical thickness as well as overall brain volume. The GO categories "glutamate toxicity" and "neuron differentiation" were also involved in more than one region highlighting their potential role in the neurodegenerative

process seen in MS. For example, *PAX6* is a critical gene shown to regulate development of central nervous system and axon guidance (Georgala *et al.*, 2011). It is then plausible that genes involved in neural development would influence brain cortical thickness and disability in MS.

Other significant GO categories were “cell proliferation” and “calcium signaling”. Multiple growth factors, their receptors (*EGFR*, *FGF12*, *IGF1R*, *IGF2*, *KIT*, *PDGFB*, and *PDGFRB*) and their downstream signaling cascade (Phosphatidylinositol 3-kinase cascade) were identified, all of which are related to cell proliferation or survival and overlap with neural development pathways. RYR2 (Ryanodine Receptor 2), which had nominally significant p values in all regions of interest in both data sets, mediates the release of Ca^{2+} from the endoplasmic reticulum (ER) into the cytoplasm. The ryanodine receptor-mediated calcium release from the ER cause intra-axonal calcium overload and results in axonal injury (Stirling & Stys, 2010). In superior frontal cortex, genes controlling re-uptake of cytoplasmic calcium (*CHRM3* and *ADCY2*) were also involved. These findings suggest that dysregulation of intra-axonal calcium level contributes to the cortical thinning.

In summary, here we report variation of genes associated with cortical thinning, and indirectly with disability in MS by GWAS based on two independent data sets. The genes identified were largely independent from those harboring variants associated with MS risk and were involved in glutamate signaling, neural development and an adjustment of intracellular calcium concentration. These results suggest that excitotoxicity and genetic vulnerability for axonal damage can poise the MS brain to initiate the cascade that will result in neurological disability. This study highlights the genetic influence on an aspect of neurodegeneration of MS and may be helpful in the search for therapeutic targets of disability in MS.

Funding: This study was supported by a grant from the National Institutes of Health (R01NS26799). SEB is a Harry Weaver Neuroscience fellow from the National Multiple Sclerosis Society.

References

(IMSGC), I.M.S.G.C. (2013) Network-based multiple sclerosis pathway analysis with GWAS data from 15,000 cases and 30,000 controls. American journal of human genetics, 92, 854-865.

(IMSGC), I.M.S.G.C., Bush, W.S., Sawcer, S.J., de Jager, P.L., Oksenberg, J.R., McCauley, J.L., Pericak-Vance, M.A. & Haines, J.L. (2010) Evidence for polygenic susceptibility to multiple sclerosis--the shape of things to come. American journal of human genetics, 86, 621-625.

(IMSGC), I.M.S.G.C. & Sawcer, S. & Hellenthal, G. & Pirinen, M. & Spencer, C.C. & Patsopoulos, N.A. & Moutsianas, L. & Dilthey, A. & Su, Z. & Freeman, C. & Hunt, S.E. & Edkins, S. & Gray, E. & Booth, D.R. & Potter, S.C. & Goris, A. & Band, G. & Oturai, A.B. & Strange, A. & Saarela, J. & Bellenguez, C. & Fontaine, B. & Gillman, M. & Hemmer, B. & Gwilliam, R. & Zipp, F. & Jayakumar, A. & Martin, R. & Leslie, S. & Hawkins, S. & Giannoulatou, E. & D'Alfonso, S. & Blackburn, H. & Martinelli Boneschi, F. & Liddle, J. & Harbo, H.F. & Perez, M.L. & Spurkland, A. & Waller, M.J. & Mycko, M.P. & Ricketts, M. & Comabella, M. & Hammond, N. & Kockum, I. & McCann, O.T. & Ban, M. & Whittaker, P. & Kemppinen, A. & Weston, P. & Hawkins, C. & Widaa, S. & Zajicek, J. & Dronov, S. & Robertson, N. & Bumpstead, S.J. & Barcellos, L.F. & Ravindrarajah, R. & Abraham, R. & Alfredsson, L. & Ardlie, K. & Aubin, C. & Baker, A. & Baker, K. & Baranzini, S.E. & Bergamaschi, L. & Bergamaschi, R. & Bernstein, A. & Berthele, A. & Boggild, M. & Bradfield, J.P. & Brassat, D. & Broadley, S.A. & Buck, D. & Butzkueven, H. & Capra, R. & Carroll, W.M. & Cavalla, P. & Celius, E.G. & Cepok, S. & Chiavacci, R. & Clerget-Darpoux, F. & Clysters, K. & Comi, G. & Cossburn, M. &

Cournu-Rebeix, I. & Cox, M.B. & Cozen, W. & Cree, B.A. & Cross, A.H. & Cusi, D. & Daly, M.J. & Davis, E. & de Bakker, P.I. & Debouverie, M. & D'Hooghe M, B. & Dixon, K. & Dobosi, R. & Dubois, B. & Ellinghaus, D. & Elovaara, I., et al. (2011) Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature, 476, 214-219.

Achiron, A., Chapman, J., Tal, S., Bercovich, E. & Gil, H. (2013) Superior temporal gyrus thickness correlates with cognitive performance in multiple sclerosis. Brain Struct Funct, 218, 943-950.

Baranzini, S.E., Galwey, N.W., Wang, J., Khankhanian, P., Lindberg, R., Pelletier, D., Wu, W., Uitdehaag, B.M., Kappos, L., Polman, C.H., Matthews, P.M., Hauser, S.L., Gibson, R.A., Oksenberg, J.R. & Barnes, M.R. (2009a) Pathway and network-based analysis of genome-wide association studies in multiple sclerosis. Hum Mol Genet, 18, 2078-2090.

Baranzini, S.E., Srinivasan, R., Khankhanian, P., Okuda, D.T., Nelson, S.J., Matthews, P.M., Hauser, S.L., Oksenberg, J.R. & Pelletier, D. (2010) Genetic variation influences glutamate concentrations in brains of patients with multiple sclerosis. Brain, 133, 2603-2611.

Baranzini, S.E., Wang, J., Gibson, R.A., Galwey, N., Naegelin, Y., Barkhof, F., Radue, E.W., Lindberg, R.L., Uitdehaag, B.M., Johnson, M.R., Angelakopoulou, A., Hall, L., Richardson, J.C., Prinjha, R.K., Gass, A., Geurts, J.J., Kragt, J., Sombekke, M., Vrenken, H., Qualley, P., Lincoln, R.R., Gomez, R., Caillier, S.J., George, M.F., Mousavi, H., Guerrero, R., Okuda, D.T., Cree, B.A., Green, A.J., Waubant, E., Goodin, D.S., Pelletier, D., Matthews, P.M., Hauser, S.L., Kappos, L., Polman, C.H. & Oksenberg, J.R. (2009b) Genome-wide association analysis

of susceptibility and clinical phenotype in multiple sclerosis. Human molecular genetics, 18, 767-778.

Beecham, A.H. & Patsopoulos, N.A. & Xifara, D.K. & Davis, M.F. & Kemppinen, A. & Cotsapas, C. & Shah, T.S. & Spencer, C. & Booth, D. & Goris, A. & Oturai, A. & Saarela, J. & Fontaine, B. & Hemmer, B. & Martin, C. & Zipp, F. & D'Alfonso, S. & Martinelli-Boneschi, F. & Taylor, B. & Harbo, H.F. & Kockum, I. & Hillert, J. & Olsson, T. & Ban, M. & Oksenberg, J.R. & Hintzen, R. & Barcellos, L.F. & Agliardi, C. & Alfredsson, L. & Alizadeh, M. & Anderson, C. & Andrews, R. & Sondergaard, H.B. & Baker, A. & Band, G. & Baranzini, S.E. & Barizzone, N. & Barrett, J. & Bellenguez, C. & Bergamaschi, L. & Bernardinelli, L. & Berthele, A. & Biberacher, V. & Binder, T.M. & Blackburn, H. & Bomfim, I.L. & Brambilla, P. & Broadley, S. & Brochet, B. & Brundin, L. & Buck, D. & Butzkueven, H. & Caillier, S.J. & Camu, W. & Carpentier, W. & Cavalla, P. & Celius, E.G. & Coman, I. & Comi, G. & Corrado, L. & Cosemans, L. & Cournu-Rebeix, I. & Cree, B.A. & Cusi, D. & Damotte, V. & Defer, G. & Delgado, S.R. & Deloukas, P. & di Sapio, A. & Dilthey, A.T. & Donnelly, P. & Dubois, B. & Duddy, M. & Edkins, S. & Elovaara, I. & Esposito, F. & Evangelou, N. & Fiddes, B. & Field, J. & Franke, A. & Freeman, C. & Frohlich, I.Y. & Galimberti, D. & Gieger, C. & Gourraud, P.A. & Graetz, C. & Graham, A. & Grummel, V. & Guaschino, C. & Hadjixenofontos, A. & Hakonarson, H. & Halfpenny, C. & Hall, G. & Hall, P. & Hamsten, A. & Harley, J. & Harrower, T. & Hawkins, C. & Hellenthal, G. & Hillier, C., et al. (2013) Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nature genetics, 45, 1353-1360.

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W.H., Pages, F., Trajanoski, Z. & Galon, J. (2009) ClueGO: a

Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics, 25, 1091-1093.

Blum, D., Yonelinas, A.P., Luks, T., Newitt, D., Oh, J., Lu, Y., Nelson, S., Goodkin, D. & Pelletier, D. (2002) Dissociating perceptual and conceptual implicit memory in multiple sclerosis patients. Brain Cogn, 50, 51-61.

Bo, L., Vedeler, C.A., Nyland, H.I., Trapp, B.D. & Mork, S.J. (2003) Subpial demyelination in the cerebral cortex of multiple sclerosis patients. Journal of neuropathology and experimental neurology, 62, 723-732.

Bodmer, W. & Bonilla, C. (2008) Common and rare variants in multifactorial susceptibility to common diseases. Nature genetics, 40, 695-701.

Calabrese, M., De Stefano, N., Atzori, M., Bernardi, V., Mattisi, I., Barachino, L., Morra, A., Rinaldi, L., Romualdi, C., Perini, P., Battistin, L. & Gallo, P. (2007) Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. Archives of neurology, 64, 1416-1422.

Calabrese, M., Rinaldi, F., Mattisi, I., Bernardi, V., Favaretto, A., Perini, P. & Gallo, P. (2011) The predictive value of gray matter atrophy in clinically isolated syndromes. Neurology, 77, 257-263.

Calabrese, M., Rinaldi, F., Mattisi, I., Grossi, P., Favaretto, A., Atzori, M., Bernardi, V., Barachino, L., Romualdi, C., Rinaldi, L., Perini, P. & Gallo, P. (2010a) Widespread cortical thinning characterizes patients with MS with mild cognitive impairment. Neurology, 74, 321-328.

Calabrese, M., Rocca, M.A., Atzori, M., Mattisi, I., Favaretto, A., Perini, P., Gallo, P. & Filippi, M. (2010b) A 3-year magnetic resonance imaging study of cortical lesions in relapse-onset multiple sclerosis. Annals of neurology, 67, 376-383.

Calabrese, M., Seppi, D., Cocco, E., Poretto, V., Rinaldi, F., Perini, P. & Gallo, P. (2012) Cortical Pathology in RRMS: Taking a Cue from Four Sisters. Mult Scler Int, 2012, 760254.

Chard, D.T., Griffin, C.M., Parker, G.J., Kapoor, R., Thompson, A.J. & Miller, D.H. (2002) Brain atrophy in clinically early relapsing-remitting multiple sclerosis. Brain : a journal of neurology, 125, 327-337.

Chen, J.T., Narayanan, S., Collins, D.L., Smith, S.M., Matthews, P.M. & Arnold, D.L. (2004) Relating neocortical pathology to disability progression in multiple sclerosis using MRI. Neuroimage, 23, 1168-1175.

Dale, A.M., Fischl, B. & Sereno, M.I. (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage, 9, 179-194.

Dalton, C.M., Chard, D.T., Davies, G.R., Miszkiel, K.A., Altmann, D.R., Fernando, K., Plant, G.T., Thompson, A.J. & Miller, D.H. (2004) Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. Brain : a journal of neurology, 127, 1101-1107.

De Stefano, N., Matthews, P.M., Filippi, M., Agosta, F., De Luca, M., Bartolozzi, M.L., Guidi, L., Ghezzi, A., Montanari, E., Cifelli, A., Federico, A. & Smith, S.M. (2003) Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. Neurology, 60, 1157-1162.

Desikan, R.S., Segonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S. & Killiany, R.J. (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage, 31, 968-980.

Eyler, L.T., Chen, C.H., Panizzon, M.S., Fennema-Notestine, C., Neale, M.C., Jak, A., Jernigan, T.L., Fischl, B., Franz, C.E., Lyons, M.J., Grant, M., Prom-Wormley, E., Seidman, L.J., Tsuang, M.T., Fiecas, M.J., Dale, A.M. & Kremen, W.S. (2012) A comparison of heritability maps of cortical surface area and thickness and the influence of adjustment for whole brain measures: a magnetic resonance imaging twin study. Twin Res Hum Genet, 15, 304-314.

Eyler, L.T., Prom-Wormley, E., Panizzon, M.S., Kaup, A.R., Fennema-Notestine, C., Neale, M.C., Jernigan, T.L., Fischl, B., Franz, C.E., Lyons, M.J., Grant, M., Stevens, A., Pacheco, J., Perry, M.E., Schmitt, J.E., Seidman, L.J., Thermenos, H.W., Tsuang, M.T., Chen, C.H., Thompson, W.K., Jak, A., Dale, A.M. & Kremen, W.S. (2011) Genetic and environmental contributions to regional cortical surface area in humans: a magnetic resonance imaging twin study. Cerebral cortex, 21, 2313-2321.

Filippi, M., Rocca, M.A., Calabrese, M., Sormani, M.P., Rinaldi, F., Perini, P., Comi, G. & Gallo, P. (2010) Intracortical lesions: relevance for new MRI diagnostic criteria for multiple sclerosis. Neurology, 75, 1988-1994.

Fischl, B., Sereno, M.I. & Dale, A.M. (1999) Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. Neuroimage, 9, 195-207.

Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Segonne, F., Salat, D.H., Busa, E., Seidman, L.J., Goldstein, J., Kennedy, D., Caviness, V., Makris, N., Rosen, B. & Dale, A.M. (2004) Automatically parcellating the human cerebral cortex. Cerebral cortex, 14, 11-22.

Fisniku, L.K., Brex, P.A., Altmann, D.R., Miszkiel, K.A., Benton, C.E., Lanyon, R., Thompson, A.J. & Miller, D.H. (2008) Disability and T2 MRI lesions: a 20-year

follow-up of patients with relapse onset of multiple sclerosis. Brain : a journal of neurology, 131, 808-817.

Georgala, P.A., Carr, C.B. & Price, D.J. (2011) The role of Pax6 in forebrain development. Developmental neurobiology, 71, 690-709.

Geurts, J.J., Pouwels, P.J., Uitdehaag, B.M., Polman, C.H., Barkhof, F. & Castelijns, J.A. (2005) Intracortical lesions in multiple sclerosis: improved detection with 3D double inversion-recovery MR imaging. Radiology, 236, 254-260.

Giorgio, A., Stromillo, M.L., Rossi, F., Battaglini, M., Hakiki, B., Portaccio, E., Federico, A., Amato, M.P. & De Stefano, N. (2011) Cortical lesions in radiologically isolated syndrome. Neurology, 77, 1896-1899.

Hauser, S.L. & Oksenberg, J.R. (2006) The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron, 52, 61-76.

Henry, R.G., Shieh, M., Okuda, D.T., Evangelista, A., Gorno-Tempini, M.L. & Pelletier, D. (2008) Regional grey matter atrophy in clinically isolated syndromes at presentation. Journal of neurology, neurosurgery, and psychiatry, 79, 1236-1244.

Ideker, T., Ozier, O., Schwikowski, B. & Siegel, A.F. (2002) Discovering regulatory and signalling circuits in molecular interaction networks. Bioinformatics, 18 Suppl 1, S233-240.

Joshi, A.A., Lepore, N., Joshi, S.H., Lee, A.D., Barysheva, M., Stein, J.L., McMahon, K.L., Johnson, K., de Zubicaray, G.I., Martin, N.G., Wright, M.J., Toga, A.W. & Thompson, P.M. (2011) The contribution of genes to cortical thickness and volume. Neuroreport, 22, 101-105.

Kochunov, P., Glahn, D.C., Lancaster, J., Thompson, P.M., Kochunov, V., Rogers, B., Fox, P., Blangero, J. & Williamson, D.E. (2011) Fractional anisotropy of

cerebral white matter and thickness of cortical gray matter across the lifespan. Neuroimage, 58, 41-49.

Kremen, W.S., Prom-Wormley, E., Panizzon, M.S., Eyler, L.T., Fischl, B., Neale, M.C., Franz, C.E., Lyons, M.J., Pacheco, J., Perry, M.E., Stevens, A., Schmitt, J.E., Grant, M.D., Seidman, L.J., Thermenos, H.W., Tsuang, M.T., Eisen, S.A., Dale, A.M. & Fennema-Notestine, C. (2010) Genetic and environmental influences on the size of specific brain regions in midlife: the VETSA MRI study. Neuroimage, 49, 1213-1223.

Kurtzke, J.F. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology, 33, 1444-1452.

Kutzelnigg, A., Lucchinetti, C.F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., Schmidbauer, M., Parisi, J.E. & Lassmann, H. (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain : a journal of neurology, 128, 2705-2712.

Lavedan, C., Licamele, L., Volpi, S., Hamilton, J., Heaton, C., Mack, K., Lannan, R., Thompson, A., Wolfgang, C.D. & Polymeropoulos, M.H. (2009) Association of the NPAS3 gene and five other loci with response to the antipsychotic iloperidone identified in a whole genome association study. Molecular psychiatry, 14, 804-819.

Liu, J.Z., McRae, A.F., Nyholt, D.R., Medland, S.E., Wray, N.R., Brown, K.M., Hayward, N.K., Montgomery, G.W., Visscher, P.M., Martin, N.G. & Macgregor, S. (2010) A versatile gene-based test for genome-wide association studies. American journal of human genetics, 87, 139-145.

Nakamura, K. & Fisher, E. (2009) Segmentation of brain magnetic resonance images for measurement of gray matter atrophy in multiple sclerosis patients. Neuroimage, 44, 769-776.

Noseworthy, J.H., Lucchinetti, C., Rodriguez, M. & Weinshenker, B.G. (2000) Multiple sclerosis. The New England journal of medicine, 343, 938-952.

Okuda, D.T., Srinivasan, R., Oksenberg, J.R., Goodin, D.S., Baranzini, S.E., Beheshtian, A., Waubant, E., Zamvil, S.S., Leppert, D., Qualley, P., Lincoln, R., Gomez, R., Caillier, S., George, M., Wang, J., Nelson, S.J., Cree, B.A., Hauser, S.L. & Pelletier, D. (2009) Genotype-Phenotype correlations in multiple sclerosis: HLA genes influence disease severity inferred by 1HMR spectroscopy and MRI measures. Brain, 132, 250-259.

Patenaude, B., Smith, S.M., Kennedy, D.N. & Jenkinson, M. (2011) A Bayesian model of shape and appearance for subcortical brain segmentation. Neuroimage, 56, 907-922.

Patsopoulos, N.A., Esposito, F., Reischl, J., Lehr, S., Bauer, D., Heubach, J., Sandbrink, R., Pohl, C., Edan, G., Kappos, L., Miller, D., Montalban, J., Polman, C.H., Freedman, M.S., Hartung, H.P., Arnason, B.G., Comi, G., Cook, S., Filippi, M., Goodin, D.S., Jeffery, D., O'Connor, P., Ebers, G.C., Langdon, D., Reder, A.T., Traboulsee, A., Zipp, F., Schimrigk, S., Hillert, J., Bahlo, M., Booth, D.R., Broadley, S., Brown, M.A., Browning, B.L., Browning, S.R., Butzkueven, H., Carroll, W.M., Chapman, C., Foote, S.J., Griffiths, L., Kermode, A.G., Kilpatrick, T.J., Lechner-Scott, J., Marriott, M., Mason, D., Moscato, P., Heard, R.N., Pender, M.P., Perreau, V.M., Perera, D., Rubio, J.P., Scott, R.J., Slee, M., Stankovich, J., Stewart, G.J., Taylor, B.V., Tubridy, N., Willoughby, E., Wiley, J., Matthews, P., Boneschi, F.M., Compston, A., Haines, J., Hauser, S.L., McCauley,

J., Ivinson, A., Oksenberg, J.R., Pericak-Vance, M., Sawcer, S.J., De Jager, P.L., Hafler, D.A. & de Bakker, P.I. (2011) Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. Annals of neurology, 70, 897-912.

Pickard, B.S., Malloy, M.P., Porteous, D.J., Blackwood, D.H. & Muir, W.J. (2005) Disruption of a brain transcription factor, NPAS3, is associated with schizophrenia and learning disability. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 136B, 26-32.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., Lublin, F.D., Metz, L.M., McFarland, H.F., O'Connor, P.W., Sandberg-Wollheim, M., Thompson, A.J., Weinshenker, B.G. & Wolinsky, J.S. (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Annals of neurology, 58, 840-846.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. & Sham, P.C. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 81, 559-575.

Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F. & Sklar, P. (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature, 460, 748-752.

Razick, S., Magklaras, G. & Donaldson, I.M. (2008) iRefIndex: a consolidated protein interaction database with provenance. BMC Bioinformatics, 9, 405.

Rimol, L.M., Panizzon, M.S., Fennema-Notestine, C., Eyler, L.T., Fischl, B., Franz, C.E., Hagler, D.J., Lyons, M.J., Neale, M.C., Pacheco, J., Perry, M.E., Schmitt, J.E., Grant, M.D., Seidman, L.J., Thermenos, H.W., Tsuang, M.T., Eisen, S.A.,

Kremen, W.S. & Dale, A.M. (2010) Cortical thickness is influenced by regionally specific genetic factors. Biological psychiatry, 67, 493-499.

Sailer, M., Fischl, B., Salat, D., Tempelmann, C., Schonfeld, M.A., Busa, E., Bodammer, N., Heinze, H.J. & Dale, A. (2003) Focal thinning of the cerebral cortex in multiple sclerosis. Brain : a journal of neurology, 126, 1734-1744.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B. & Ideker, T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research, 13, 2498-2504.

Stirling, D.P. & Stys, P.K. (2010) Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends Mol Med, 16, 160-170.

Tiberio, M., Chard, D.T., Altmann, D.R., Davies, G., Griffin, C.M., Rashid, W., Sastre-Garriga, J., Thompson, A.J. & Miller, D.H. (2005) Gray and white matter volume changes in early RRMS: a 2-year longitudinal study. Neurology, 64, 1001-1007.

Trapp, B.D. & Nave, K.A. (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci, 31, 247-269.

Tur, C., Ramagopalan, S., Altmann, D.R., Bodini, B., Cercignani, M., Khaleeli, Z., Miller, D.H., Thompson, A.J. & Ciccarelli, O. (2014) HLA-DRB1*15 influences the development of brain tissue damage in early PPMS. Neurology, 83, 1712-1718.

Wegner, C., Esiri, M.M., Chance, S.A., Palace, J. & Matthews, P.M. (2006) Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. Neurology, 67, 960-967.

Yates, R.L., Esiri, M.M., Palace, J., Mittal, A. & DeLuca, G.C. (2014) The influence of HLA-DRB1*15 on motor cortical pathology in multiple sclerosis. Neuropathol Appl Neurobiol.

Acknowledgements:

We thank S. Caillier, R. Guerrero, H. Mousavi, and A. Santaniello for sample and database management. We thank the UCSF EPIC Study team and MS patients and controls that participated in the study. None of the authors declared any conflict of interest.

Figure Legends

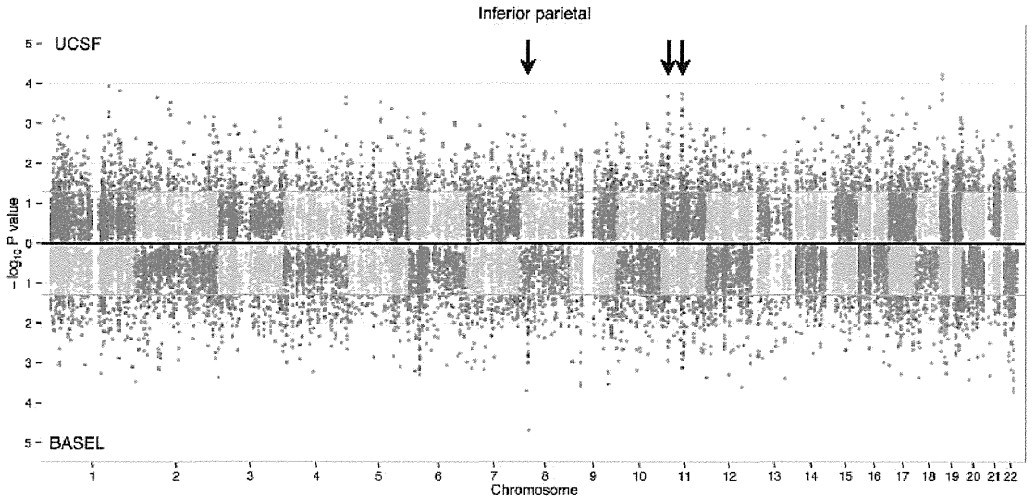

Figure 1. A Manhattan plot showing the gene-level p values of both GWASs for thickness of inferior parietal cortex. Gene-level p values from the GWAS in UCSF data set are displayed at the top, and those corresponding to the GWAS in Basel data set are at the bottom. Genes that are not nominally significant ($p > 0.05$) are displayed as gray points and nominally significant genes are displayed as blue points. Genes that are nominally significant in both two data sets are displayed as red points.

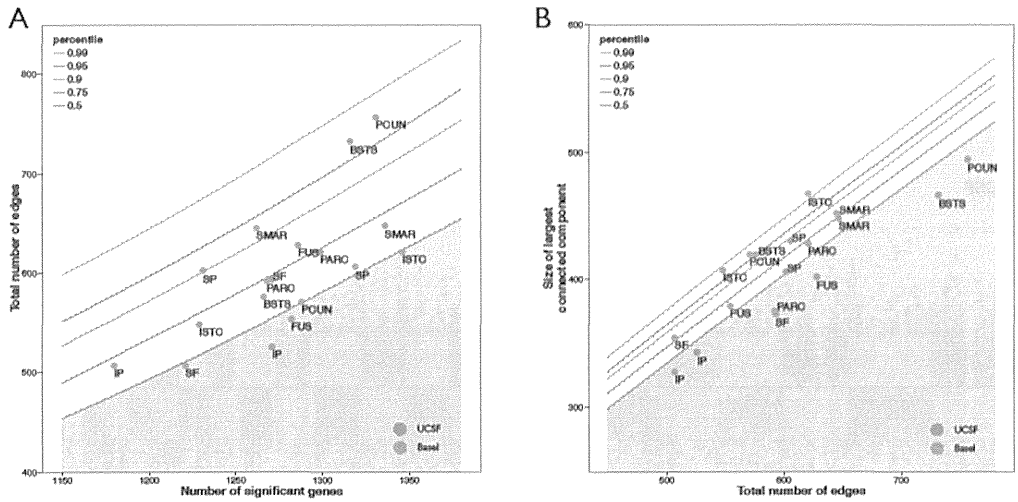

Figure 2. The number of connections among significant genes is evaluated in the background of 1,000 random simulations. (**A**) The total number of edges is plotted as a function of the number of significant genes for each region. (**B**) The size of the largest connected component is plotted as a function of the total number of edges. The colored lines represent the 50th (red), 75th (blue), 90th (green), 95th (purple), and 99th (orange) percentiles obtained through simulations with random gene sets of similar size.