Devorak, J., Mokry, L. E., Morris, J. A., Forgetta, V., Davey Smith, G., Sawcer, S. J., & Richards, J. B. (2017). Large differences in adiponectin levels have no clear effect on multiple sclerosis risk: A Mendelian randomization study. *Multiple Sclerosis Journal*, 23(11), 1461-1468. <https://doi.org/10.1177/1352458516681196>

Peer reviewed version

Link to published version (if available):
[10.1177/1352458516681196](https://doi.org/10.1177/1352458516681196)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the final published version of the article (version of record). It first appeared online via Sage at <http://journals.sagepub.com/doi/10.1177/1352458516681196>. Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Large Differences in Adiponectin Levels Have No Clear Effect on Multiple Sclerosis Risk: A Mendelian Randomization Study

Julia Devorak¹, Lauren E. Mokry², John A. Morris³, Vincenzo Forgetta¹, George Davey Smith⁴, Stephen Sawcer⁵, J. Brent Richards^{1,2,3,6,7}

¹Centre for Clinical Epidemiology, Department of Epidemiology, Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, QC, Canada

²Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, QC, Canada

³Department of Human Genetics, McGill University, Montréal, QC, Canada

⁴MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom

⁵Department of Clinical Neurosciences, Cambridge Biomedical Campus, University of Cambridge, Cambridge, UK

⁶Department of Medicine, McGill University, Montréal, QC, Canada

⁷Department of Twin Research and Genetic Epidemiology, King's College London, United Kingdom

Keywords

multiple sclerosis, adiponectin, Mendelian randomization analysis, genetic epidemiology

Corresponding Author:

J. Brent Richards, Lady Davis Institute for Medical Research, Jewish General Hospital, H413.1-3755 chemin de la Côte-Ste-Catherine, Montréal, QC, H3T 1E2, Canada; Email: brent.richards@mcgill.ca; Telephone: 1-514-340-8222 ext. 4362; Fax: 1-514-340-7502.

Abstract

Background: Mendelian randomization (MR) studies have demonstrated strong support for an association between genetically increased body mass index and risk of MS. The adipokine adiponectin may be a potential mechanism linking body mass to risk of MS.

Objective: To evaluate whether genetically-increased adiponectin levels influence risk of MS. Methods: Using genome-wide significant single nucleotide polymorphisms (SNPs) for adiponectin, we undertook an MR study to estimate the effect of adiponectin on MS. This method prevents bias due to reverse causation and minimizes bias due to confounding. Sensitivity analyses were performed to evaluate the assumptions of MR.

Results: MR analyses did not support a role for genetically-elevated adiponectin in risk of MS (OR = 0.93 per unit increase in natural-log-transformed adiponectin, equivalent to a two-standard deviation increase in adiponectin on the absolute scale; 95% CI: 0.66-1.33; $p = 0.61$). Further MR analysis suggested that genetic variation at the adiponectin gene, which influences adiponectin level, does not impact MS risk. Sensitivity analyses, including MR-Egger regression, suggested no bias due to pleiotropy. Conclusion: Lifelong genetically-increased adiponectin levels in humans have no clear effect on risk of MS. Other biological factors driving the association between body mass and MS should be investigated.

Introduction

Multiple sclerosis is the most common chronic inflammatory disease of the central nervous system (1), affecting an estimated 2.3 million people worldwide (2). MS is

1 thought to be initiated by T-cells which target self-antigens in the CNS, resulting in
2 demyelination and progressive neuroaxonal injury and degeneration (1). Disease onset
3 usually occurs in early adulthood, and prognosis is variable; however, the disease is often
4 progressively debilitating (3).

5
6 Both genetic and environmental factors have been implicated in the etiology of MS (4).
7 Genetic risk profiles in individuals with MS are often complex, and many non-genetic
8 factors have been associated with the disease (4), including body weight. High BMI
9 during childhood and early adolescence has been associated with a 1.15-1.18-fold
10 increased risk of MS in adulthood (5), and overweight and obesity in late adolescence
11 and early adulthood have been associated with an approximate two-fold increased risk of
12 MS in adulthood (6,7). Furthermore, childhood overweight and obesity have been
13 associated with an approximate 1.5- to 3.75-fold increased odds of pediatric-onset MS,
14 depending on the extent of overweight or obesity (8). In addition, recent Mendelian
15 randomization (MR) analyses have demonstrated support for a causal association
16 between body mass index (BMI) and MS, whereby an increase in BMI by approximately
17 5 kg/m² increased the odds of MS by 40% (9). However, the underlying biological
18 mechanisms linking BMI to MS are unclear.

19
20 Obesity is associated with chronic, mild inflammation characterized by abnormal
21 cytokine production and increased pro-inflammatory signaling. Adipose tissue is known
22 to produce cytokines, known as adipokines; however, the relative contribution of
23 adipocyte-derived cytokines to the inflammatory state in obesity is unknown (10).

1 Interestingly, adiponectin, an adipokine with known anti-inflammatory properties in both
2 the innate and adaptive arms of the immune system (10), is reduced in overweight and
3 obese individuals (11,12), and is negatively correlated with BMI (11).

4
5 In light of these findings, animal and human studies alike have been undertaken to better
6 understand adiponectin's role in MS etiology and treatment. Results from studies using
7 experimental autoimmune encephalomyelitis models of MS are suggestive of a protective
8 role for adiponectin in rodents (13,14). However, findings from studies in clinical
9 populations are diverse. One study showed reduced levels of this adipokine in peripheral
10 blood of MS patients following acute relapse (15), while others demonstrate elevated
11 adiponectin in peripheral blood and CSF of patients in remission (16,17), or unaltered
12 adiponectin in newly diagnosed MS patients (18).

13
14 Observational studies, such as those described above, represent an important step in the
15 identification of risk factors in disease. Randomized control trials (RCTs) and/or MR
16 studies can help to clarify the roles of identified risk factors in disease outcome, as
17 findings of observational studies may be biased due to residual confounding and/or
18 reverse causation. Indeed, numerous RCTs and MR studies have provided strong
19 evidence for the presence of bias in previously reported observational associations (many
20 examples reviewed in 19). However, these types of studies can also validate
21 observational associations through demonstration of causality (also reviewed in 19). One
22 such MR study (9) suggested that previously reported observational associations between
23 body weight and MS (e.g. 5-8) are not likely biased due to confounding or reverse

1 causation. Nonetheless, no study to date has provided such evidence for the reported
2 observational association between adiponectin level and MS. Confounding due to reverse
3 causation is of particular concern in epidemiological studies of MS, as timing of disease
4 onset is unknown. Therefore the nature of adiponectin's role in MS etiology therefore
5 merits further investigation.

6
7 In the absence of experimental studies investigating adiponectin's role in MS clinical
8 populations, MR studies can be conducted to evaluate adiponectin's role in disease
9 outcome in a manner that allows for causal inference. This approach is conceptually
10 similar to a randomized control trial, where instead of randomization to a pharmaceutical
11 intervention, individuals in the population are naturally randomized at conception to
12 varying levels of an exposure (e.g. adiponectin level) due to genetic variation.

13
14 MR is a technique which uses genetic variants strongly associated with an exposure (e.g.
15 adiponectin level) to estimate the exposure's effect on disease risk (e.g. MS) (20). Since
16 genetic variants are randomly allocated at meiosis, they are not influenced by
17 confounding factors that may bias observational associations, except confounding by
18 ancestry. Further, reverse causation is overcome since allelic randomization always
19 precedes MS onset.

20 To better understand whether adiponectin levels may influence risk of MS, we undertook
21 an MR study of adiponectin on MS risk using a two-sample MR design, deriving the
22 effects of single nucleotide polymorphisms (SNPs) on adiponectin and MS risk from the
23 largest adiponectin and MS samples available to date: the ADIPOGen Consortium (N =

1 45,891) (21), the International Multiple Sclerosis Genetics Consortium (IMSGC, $n_{\text{cases}} =$
2 14,498 / $n_{\text{controls}} = 24,091$) (22), and the IMSGC/Wellcome Trust Case Control
3 Consortium 2 (IMSGC/WTCCC2, $n_{\text{cases}} = 9,772$ / $n_{\text{controls}} = 17,376$) (23).

4 **Methods**

5 **SNP Selection, Effect Sizes, and Data Sources**

6 Genome-wide significant ($p < 5 \times 10^{-8}$) genetic variants associated with adiponectin
7 levels were obtained from ADIPOGen (21). For this study, we limited our selection of
8 SNPs and summary statistics to those that achieved genome-wide significance in the
9 European sex-combined discovery phase analyses or joint analyses ($30,708 \leq n \leq$
10 $38,276$). The effect of each SNP on natural-log-transformed adiponectin levels was
11 adjusted for age, sex, BMI, the principle components of ancestry, study site (where
12 appropriate), and family structure in cohorts with family members (21). Corresponding
13 effect estimates of the adiponectin-associated SNPs on risk of MS were obtained first
14 from the IMSGC Immunochip study, the largest genetic association study for MS (14,498
15 cases and 24,091 controls) (22), and then from the second largest study, the
16 IMSGC/WTCCC2 (9,772 cases and 17,376 controls) (23), if an adiponectin-associated
17 SNP was not ascertained in the IMSGC Immunochip study. We have previously used
18 these datasets to explore the effects of BMI and vitamin D on risk of MS (9, 24). If
19 summary statistics were not available for an index SNP in either study, a highly
20 correlated proxy ($r^2 > 0.8$) was selected first from the Immunochip study and then from
21 the IMSGC/WTCCC2 study, if the former was unavailable. Linkage disequilibrium (LD)
22 for proxies was measured using UK10K samples ($n = 3,781$) (25).

1

2 **SNP Validation**

3 *Linkage disequilibrium assessment.* MR studies require that the SNPs not be in LD, since
4 strong correlations between selected SNPs may bias results (20). To ensure that the
5 adiponectin-associated SNPs met this requirement, LD was measured between all
6 selected SNPs using European samples from the UK10K project using PLINK software
7 version 1.90 (25). SNPs were excluded from analyses if their measured LD was $r^2 > 0.05$.

8 *Pleiotropy assessment.* MR analyses assume that the SNPs influence the outcome (MS)
9 solely through the exposure of interest (adiponectin). To assess for the presence of
10 pleiotropy, MR-Egger regression was performed as previously described (27). This
11 approach is based on Egger regression, which has been used to examine publication bias
12 in the meta-analysis literature (28). In brief, the SNP's effect upon the exposure variable
13 is plotted against its effect upon the outcome, where an intercept distinct from the origin
14 provides evidence for pleiotropic effects. Funnel plots can also be used for visual
15 inspection of symmetry. In addition, a systematic PubMed literature search was
16 conducted to investigate possible pleiotropic mechanisms of the selected SNPs on MS,
17 using a previously described method (24) (**S1 Methods**). Last, pleiotropy was assessed
18 by examining only the SNP at *ADIPOQ*, which encodes adiponectin. Pleiotropy is less
19 likely to influence results at this locus, since it is likely that genetic variation at *ADIPOQ*
20 influences adiponectin levels directly (29).

21 *Population Stratification.* To reduce this potential source of bias, selected SNPs and
22 summary statistics for both adiponectin and MS were obtained from analyses involving
23 individuals of European descent only. In addition, a literature search was conducted to

investigate potential residual population stratification that may exist among European subgroups with respect to adiponectin levels (30). To the best of our knowledge, no epidemiological studies have investigated adiponectin levels across European subgroups; therefore, mean adiponectin serum concentrations from the ADIPOGen European cohorts were compared to investigate potential differences in population adiponectin levels across Europe. A Shapiro-Wilks test was used to assess normality of mean adiponectin concentration for the following countries: United Kingdom, United States, Netherlands, Germany, Italy, and Finland. Analysis of variance (ANOVA) was then performed to investigate potential differences in adiponectin concentrations across these countries. Shapiro-Wilks test and ANOVA were performed using Graphpad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA).

Mendelian Randomization Estimates

In this previously described two-sample MR study design (24,31) where independent SNPs evaluate the association of exposure to genetically altered adiponectin levels with MS risk, MR estimates were obtained by weighting each of the adiponectin-associated SNPs by the magnitude of its effect upon natural-log-transformed adiponectin level. The individual estimates were then meta-analysed using a fixed-effects model to obtain a summary measure for the effect of genetically increased adiponectin on risk of MS.

Sensitivity Analyses

If a given SNP violated any of the underlying assumptions of MR, MR estimates were recalculated excluding that SNP. Further sensitivity analyses were undertaken using (1)

1 only the lead SNP from ADIPOGen, located near the adiponectin-encoding gene
2 *ADIPOQ*, to reduce potential bias from pleiotropy (29); and (2) only the SNPs genotyped
3 in both ADIPOGen and either of the MS studies, to reduce potential bias from random
4 error introduced by use of proxy SNPs

5
6 All statistical analyses were performed using R version 3.2.2 software (32) unless
7 otherwise noted.

9 Results

10 SNP Selection

11 ADIPOGen identified 12 SNPs as genome-wide significant ($p < 5 \times 10^{-8}$) for adiponectin
12 level in European populations (21). Of these, none were genotyped directly in the
13 Immunochip study; however four were found in the IMSGC/WTCCC2 GWAS:
14 rs1108842 (within *GNL3*), rs12922394 (within *CDH13*), rs1597466 (near *TSC22D2*), and
15 rs2925979 (within *CMIP*) (**Table 1**). Proxies ($r^2 > 0.80$) were identified for 6 of the 8
16 remaining SNPs: one from the IMSGC Immunochip study (rs6810075, near the
17 adiponectin-encoding gene *ADIPOQ*), and five from the IMSGC/WTCCC2 GWAS
18 (rs2980879, near *TRIB1*; rs601339, near *GPR109A*; rs7133378, within *DNAH10*;
19 rs7955516, near *PDE3A*; and rs3001032, near *LYPLALI*) (**Table 1**). Therefore, 10 of the
20 12 ADIPOGen SNPs were selected for this MR study. None of the ten adiponectin
21 increasing alleles were significantly associated with MS risk, accounting for multiple
22 testing (all $p > 0.05/10 = 0.005$, **Table 1** and **Figure 1**).

23

1 **SNP Validation**

2 *Linkage disequilibrium.* None of the 10 adiponectin-associated SNPs were found to be in
3 LD (all pairwise $r^2 < 0.05$) in the UK10K European samples (25).

4 *Pleiotropy.* MR-Egger regression analyses suggested that pleiotropy did not greatly
5 influence the results of the MR analyses (Egger intercept $p = 0.21$; 95% CI: -0.015-
6 0.058). Additionally, a literature review failed to unearth pleiotropic mechanisms for any
7 of the investigated SNPs, with the exception of rs12922394. This SNP is located within
8 an intron of the *CDH13* gene, which encodes T-cadherin, a protein known to bind both
9 high molecular weight (HMW) adiponectin and low-density lipoprotein (LDL). It is
10 thought that T-cadherin might function as a receptor for both these ligands (33).

11 Numerous epidemiological studies have demonstrated associations between elevated
12 serum LDL and MS disease progression, as well as adverse clinical and MRI outcomes
13 (34). Based on these findings, the possibility that CDH13 functions independently of
14 adiponectin to produce MS phenotypes could not be eliminated; therefore, sensitivity
15 analyses were undertaken to exclude rs12922394 from MR analyses.

16 *Population Stratification.* A one-way ANOVA revealed that serum log-transformed
17 adiponectin concentrations did not differ across the European subpopulations interrogated
18 in ADIPOGen ($F_{(5,17)} = 1.27$, $p = 0.32$).

19

20 **Mendelian Randomization Estimates**

21 Employing a fixed-effects model including all ten adiponectin-altering alleles revealed
22 that a one-unit increase in natural-log-transformed adiponectin, which corresponds to a 2-
23 standard deviation (SD) change on the absolute scale, was not associated with a clear

1 effect on the odds of MS (OR = 0.93; 95% CI: 0.66-1.33; $p = 0.61$) (**Figure 1**). The I^2
2 estimate of heterogeneity was 0%, suggesting no heterogeneity of effect. Sensitivity
3 analyses excluding rs12922394 (*CDH13*) for possible pleiotropic effects did not
4 influence these results (OR = 0.93; 95% CI: 0.64-1.37; $p = 0.72$). Analysis of the
5 *ADIPOQ* variant rs6810075 alone revealed that a one-unit increase of natural-log-
6 transformed adiponectin did not alter the odds of MS (OR: 0.60; 95% CI: 0.34-1.07; $p =$
7 0.08). Analysis of the pooled non-proxy SNPs rs1108842, rs12922394, rs1597466 and,
8 rs2925979, revealed no evidence of an association with MS risk (OR = 1.06; 95% CI =
9 0.60, 1.88).

11 Discussion

12 In this MR study investigating the role of adiponectin level upon MS risk, we have
13 demonstrated that a large (2-SD), lifelong genetic increase in adiponectin level was not
14 associated with a clinically-relevant change in the odds of MS. This finding does not
15 support a substantial role for adiponectin in the causal pathway of MS; however, given
16 the wide confidence interval, a small protective or detrimental effect of adiponectin in
17 MS cannot be definitively ruled out, and further studies will be necessary to more clearly
18 ascertain adiponectin's role. Notwithstanding, the present study suggests that a
19 substantial, lifelong alteration in adiponectin levels would be necessary to influence the
20 risk of disease, if adiponectin indeed plays a causal role therein.

21
22 Observational studies aiming to shed light on the clinical relevance of adiponectin levels
23 in MS have yielded variable results (15-18,35). Observational studies such as these are

1 susceptible to bias due to residual confounding, in addition to a number of other factors
2 that may bias observational studies. While the potentially confounding effects of BMI
3 were accounted for in all of these studies, there are several related, physiological effects
4 which were not likely not controlled for through the use of BMI as a measure of obesity,
5 and which could have influenced the reported associations. For example, differences in
6 adipose tissue amount and location can influence adiponectin concentrations, as
7 production of adiponectin is differentially regulated in visceral and subcutaneous
8 adipocytes (36). These differences in adipose distribution are not accounted for in BMI
9 calculations. Differential clearance through the liver could also influence measurements
10 of adiponectin in such studies (36). One strength of the present study is that it utilizes a
11 method of analysis which largely overcomes confounding, due to the random assortment
12 of alleles at conception.

13
14 As the present study assessed the association between lifelong genetically-increased
15 adiponectin levels and the odds of development of MS, the findings reported here suggest
16 that adiponectin is not an ideal preventative treatment target for MS. Adiponectin's
17 therapeutic role in MS following disease onset, on the other hand, cannot be ascertained
18 based on the present findings. Interestingly, two of the adiponectin-modulating SNPs
19 investigated in this study (rs601339 and rs7955516) are located near genes implicated in
20 the both the preventative and therapeutic treatment of MS (*GPR109A* (37) and *PDE3A*
21 (38), respectively). In addition, adiponectin treatment following EAE induction in rodents
22 has been shown to attenuate the clinical course of EAE, findings suggestive of a potential
23 therapeutic role of adiponectin in MS following disease onset (13).

1
2 Observational studies (5-8) and MR analyses (9) have indicated that increased body
3 weight and BMI render individuals more susceptible to MS. As an adipokine with anti-
4 inflammatory properties and which is negatively correlated with BMI, adiponectin is a
5 biological candidate of interest in the investigation of the underlying causal pathway of
6 MS. While the present findings cannot rule out the possibility of a protective or
7 detrimental role for adiponectin in MS etiology, they suggest that adiponectin's role in
8 the causal pathway of this disease is likely to be small. Further studies will be necessary
9 to ascertain which biological factors drive the causal association between BMI and MS.
10
11 The present study has important limitations. The possibility of residual pleiotropy biasing
12 our estimates remains, despite the sensitivity analyses conducted. MR-Egger results can
13 be biased when the effect on pleiotropic pathways is proportional to its effect on
14 adiponectin level. Interestingly, genetic variation at adiponectin-encoding gene *ADIPOQ*
15 was marginally associated with risk of MS ($p = 0.08$), and variation at this locus is less
16 likely to influence MS risk independently of adiponectin than other the SNPs
17 investigated. In addition, it is impossible, using current methods, to directly assess the
18 extent to which canalization, or developmental compensation, may have influenced our
19 results. While variation in adiponectin level explained by ADIPOGen SNPs is relatively
20 high (~5%), MR relies upon the assumption of linear dose-response effects, which may
21 not be suitable. It is also possible that subtle population stratification of adiponectin
22 levels across Europe biased our results. Yet, no differences in adiponectin level across
23 European populations in ADIPOGen, a consortium measuring adiponectin levels in 26

European or European-descent cohorts, were detected. Finally, as with any null finding, the width of the 95% confidence intervals gives a sense of what effect sizes can be excluded, given the large (2-SD) genetic increase in adiponectin levels.

In conclusion, using data from the largest genetic consortia for adiponectin and MS, we find that lifelong exposure to a substantially (2-SD) genetically-elevated adiponectin level has no clinically-relevant effects on MS susceptibility in individuals of European descent. Adiponectin is therefore not likely to be an ideal candidate target for MS prevention; however, its therapeutic potential for MS following disease onset remains to be determined. Additional studies will be necessary to ascertain which biological factors drive the causal association between body weight and MS.

Acknowledgements

We wish to thank the ADIPOGen Consortium, IMSGC, and the IMSGC/WTCCC2 study for access to their data.

ADIPOGen data is publicly available and can be accessed at <http://www.mcgill.ca/genepi/adipogen-consortium>. IMSGC data is publicly available and can be accessed at <https://www.immunobase.org/>. WTCCC2 data is available by application only.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding Statement

This work was supported by the MS Society of Canada [2627].

References

1. Friese M a, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat Rev Neurol* [Internet]. Nature Publishing Group; 2014;10(4):225–38. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24638138>
2. Versini M, Jeandel P-Y, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: not a passive bystander. *Autoimmun Rev* [Internet]. 2014 Sep [cited 2015 Dec 7];13(9):981–1000. Available from: <http://www.sciencedirect.com/science/article/pii/S1568997214001414>
3. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med* [Internet]. 2000 Sep 28;343(13):938–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11006371>
4. Compston A, Coles A. Multiple sclerosis. *Lancet* [Internet]. 2008 Oct 25 [cited 2014 Jul 11];372(9648):1502–17. Available from: <http://www.sciencedirect.com/science/article/pii/S0140673608616207>
5. Munger KL, Bentzen J, Laursen B, Stenager E, Koch-Henriksen N, Sørensen TIA, et al. Childhood body mass index and multiple sclerosis risk: a long-term cohort study. *Mult Scler* [Internet]. 2013 Sep 1 [cited 2015 Nov 24];19(10):1323–9. Available from: <http://msj.sagepub.com.proxy3.library.mcgill.ca/content/19/10/1323.long>
6. Munger KL, Chitnis T, Ascherio A. Body size and risk of MS in two cohorts of US women. *Neurology* [Internet]. 2009 Nov 10;73(19):1543–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19901245>
7. Hedström AK, Olsson T, Alfredsson L. High body mass index before age 20 is associated with increased risk for multiple sclerosis in both men and women. *Mult Scler* [Internet]. 2012 Sep;18(9):1334–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22328681>
8. Langer-Gould A, Brara SM, Beaber BE, Koebnick C. Childhood obesity and risk of pediatric multiple sclerosis and clinically isolated syndrome. *Neurology* [Internet]. 2013 Feb 5;80(6):548–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24101749>
9. Mokry LE, Ross S, Timpson NJ, Sawcer S, Davey Smith G, Richards JB. Obesity and Multiple Sclerosis: A Mendelian Randomization Study. *PLoS Med* [Internet]. 2016 Jun;13(6):e1002053. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27351487>

10. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* [Internet]. Nature Publishing Group; 2006 Oct 22 [cited 2015 Mar 2];6(10):772–83. Available from: <http://www.nature.com.proxy3.library.mcgill.ca/nri/journal/v6/n10/full/nri1937.html>
11. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *J Am Med Assoc* [Internet]. 2003;289(14):1799–804. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12684358>
12. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical Decrease of an Adipose-Specific Protein, Adiponectin, in Obesity. *Biochem Biophys Res Commun* [Internet]. 1999 Apr [cited 2015 Oct 31];257(1):79–83. Available from: <http://www.sciencedirect.com/science/article/pii/S0006291X99902553>
13. Piccio L, Cantoni C, Henderson JG, Hawiger D, Ramsbottom M, Mikesell R, et al. Lack of adiponectin leads to increased lymphocyte activation and increased disease severity in a mouse model of multiple sclerosis. *Eur J Immunol* [Internet]. NIH Public Access; 2013 Aug 1 [cited 2015 Dec 7];43(8):2089–100. Available from: [/pmc/articles/PMC3901539/?report=abstract](http://pmc/articles/PMC3901539/?report=abstract)
14. Piccio L, Stark JL, Cross AH. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J Leukoc Biol*. 2008;84(4):940–8.
15. Kraszula Ł, Jasińska A, Eusebio M-O, Kuna P, Głąbiński A, Pietruczuk M. Evaluation of the relationship between leptin, resistin, adiponectin and natural regulatory T cells in relapsing-remitting multiple sclerosis. *Neurol Neurochir Pol* [Internet]. 2012 [cited 2015 Dec 7];46(1):22–8. Available from: <http://www.sciencedirect.com/science/article/pii/S0028384314600918>
16. Hietaharju A, Kuusisto H, Nieminen R, Vuolteenaho K, Elovaara I, Moilanen E. Elevated cerebrospinal fluid adiponectin and adipsin levels in patients with multiple sclerosis: a Finnish co-twin study. *Eur J Neurol* [Internet]. 2010 Feb [cited 2015 Dec 7];17(2):332–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19538214>
17. Palavra F, Marado D, Mascarenhas-Melo F, Sereno J, Teixeira-Lemos E, Nunes CC, et al. New markers of early cardiovascular risk in multiple sclerosis patients: oxidized-LDL correlates with clinical staging. *Dis Markers* [Internet]. Hindawi Publishing Corporation; 2013 Jan [cited 2015 Dec 8];34(5):341–8. Available from: [/pmc/articles/PMC3809749/?report=abstract](http://pmc/articles/PMC3809749/?report=abstract)
18. Penesova A, Vlcek M, Imrich R, Vernerova L, Marko A, Meskova M, et al. Hyperinsulinemia in newly diagnosed patients with multiple sclerosis. *Metab Brain Dis* [Internet]. 2015 Aug [cited 2015 Dec 8];30(4):895–901. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25809135>

19. Mokry LE, Ahmad O, Forgetta V, Thanassoulis G, Richards JB. Mendelian randomisation applied to drug development in cardiovascular disease: a review. *J Med Genet* [Internet]. BMJ Publishing Group Ltd; 2015 [cited 2016 Sep 22];52(2):71–9. Available from: <http://jmg.bmj.com/content/52/2/71.full.pdf>
20. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* [Internet]. 2008 Apr 15 [cited 2015 May 12];27(8):1133–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17886233>
21. Dastani Z, Hivert M-F, Timpson N, Perry JRB, Yuan X, Scott RA, et al. Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. *PLoS Genet* [Internet]. Public Library of Science; 2012 Jan 1 [cited 2015 Dec 15];8(3):e1002607. Available from: [/pmc/articles/PMC3315470/?report=abstract](http://pmc/articles/PMC3315470/?report=abstract)
22. Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kempainen A, Cotsapas C, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet* [Internet]. NIH Public Access; 2013 Nov 1 [cited 2015 Mar 18];45(11):1353–60. Available from: [/pmc/articles/PMC3832895/?report=abstract](http://pmc/articles/PMC3832895/?report=abstract)
23. Sawcer S, Hellenthal G, Pirinen M, Spencer CCA, Patsopoulos NA, Moutsianas L, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* [Internet]. Europe PMC Funders; 2011 Aug 11 [cited 2014 Jul 9];476(7359):214–9. Available from: [/pmc/articles/PMC3182531/?report=abstract](http://pmc/articles/PMC3182531/?report=abstract)
24. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Leong A, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. Muraro PA, editor. *PLOS Med* [Internet]. Public Library of Science; 2015 Aug 25 [cited 2015 Aug 26];12(8):e1001866. Available from: [/pmc/articles/PMC4549308/?report=abstract](http://pmc/articles/PMC4549308/?report=abstract)
25. Walter K, Min JL, Huang J, Crooks L, Memari Y, McCarthy S, et al. The UK10K project identifies rare variants in health and disease. *Nature* [Internet]. Europe PMC Funders; 2015 Sep 14 [cited 2016 Jun 27];526(7571):82–90. Available from: <http://www.nature.com/doifinder/10.1038/nature14962>
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am J Hum Genet* [Internet]. Elsevier; 2007 Sep [cited 2016 Jun 22];81(3):559–75. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0002929707613524>

27. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* [Internet]. 2015 Apr 1 [cited 2015 Nov 17];44(2):512–25. Available from: <http://ije.oxfordjournals.org.proxy3.library.mcgill.ca/content/44/2/512.long>
28. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* [Internet]. BMJ Group; 1997 Sep 13 [cited 2015 Oct 27];315(7109):629–34. Available from: </pmc/articles/PMC2127453/?report=abstract>
29. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert M-F, Warren LL, et al. Mendelian Randomization Studies Do Not Support a Causal Role for Reduced Circulating Adiponectin Levels in Insulin Resistance and Type 2 Diabetes. *Diabetes* [Internet]. American Diabetes Association; 2013 Oct 1 [cited 2016 Jun 22];62(10):3589–98. Available from: <http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db13-0128>
30. Huckins LM, Boraska V, Franklin CS, Floyd JAB, Southam L, Sullivan PF, et al. Using ancestry-informative markers to identify fine structure across 15 populations of European origin. *Eur J Hum Genet* [Internet]. Nature Publishing Group; 2014 Oct 1 [cited 2015 Dec 16];22(10):1190–200. Available from: </pmc/articles/PMC4169539/?report=abstract>
31. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* [Internet]. Wiley-Blackwell; 2013 Nov 1 [cited 2015 Dec 14];37(7):658–65. Available from: </pmc/articles/PMC4377079/?report=abstract>
32. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2015. Available from: <https://www.r-project.org/>
33. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao T-S, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. *Proc Natl Acad Sci U S A* [Internet]. 2004 Jul 13 [cited 2016 Feb 17];101(28):10308–13. Available from: <http://www.pnas.org.proxy3.library.mcgill.ca/content/101/28/10308.long>
34. Zhornitsky S, McKay KA, Metz LM, Teunissen CE, Rangachari M. Cholesterol and markers of cholesterol turnover in multiple sclerosis: relationship with disease outcomes. *Mult Scler Relat Disord* [Internet]. 2016 Jan [cited 2016 Feb 20];5:53–65. Available from: <http://www.sciencedirect.com/science/article/pii/S2211034815300122>
35. Natarajan R, Hagman S, Hämäläinen M, Leppänen T, Dastidar P, Moilanen E, et al. Adipsin Is Associated with Multiple Sclerosis: A Follow-Up Study of Adipokines. *Mult Scler Int* [Internet]. Hindawi Publishing Corporation; 2015 Jan [cited 2015 Dec 8];2015:371734. Available from: </pmc/articles/PMC4655075/?report=abstract>

36. Fantuzzi G. Adiponectin in inflammatory and immune-mediated diseases. Cytokine [Internet]. NIH Public Access; 2013 Oct 1 [cited 2016 Feb 8];64(1):1–10. Available from: /pmc/articles/PMC3770746/?report=abstract
37. Chen H, Assmann JC, Krenz A, Rahman M, Grimm M, Karsten CM, et al. Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. J Clin Invest [Internet]. 2014 May [cited 2016 Feb 23];124(5):2188–92. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4001545&tool=pmcentrez&rendertype=abstract>
38. Bielekova B, Lincoln A, McFarland H, Martin R. Therapeutic Potential of Phosphodiesterase-4 and -3 Inhibitors in Th1-Mediated Autoimmune Diseases. J Immunol [Internet]. American Association of Immunologists; 2000 Jan 15 [cited 2016 Feb 4];164(2):1117–24. Available from: <http://www.jimmunol.org/content/164/2/1117.full>

Figure 1. Mendelian randomization estimate of the association of adiponectin level with risk of MS. Estimates obtained using a fixed-effects model.

Table 1. Characteristics of SNPs used in Mendelian randomization analyses

Locus*	SNP	Chromosome	Location	hg 19 position	Adiponectin					MS					
					Adiponectin-increasing allele	Other allele	Allele frequency	Effect on adiponectin† (β)	p-value for association with adiponectin**	Proxy SNP	Proxy - ADIPOGen SNP correlation (r ²)	Adiponectin-increasing allele	SNP source	Effect on MS (β)	p-value for association with MS
<i>GNL3</i>	rs1108842	3	5' UTR	52720080	C	A	0.5	0.03	1.4E-13	-	-	C	IMSGC/WTCCC2	-0.003	0.88
<i>CDH13</i>	rs12922394	16	intronic	82672327	C	T	0.9	0.08	2.0E-15	-	-	C	IMSGC/WTCCC2	-0.005	0.88
<i>TSC22D2</i>	rs1597466	3	intergenic	150055561	G	T	0.9	0.03	1.9E-08	-	-	G	IMSGC/WTCCC2	0.021	0.50
<i>CMIP</i>	rs2925979	16	intronic	81534790	C	T	0.7	0.04	1.2E-20	-	-	C	IMSGC/WTCCC2	0.006	0.76
<i>TRIB1</i>	rs2980879	8	intergenic	126481475	T	A	0.7	0.03	7.1E-09	rs4871603	0.82	T	IMSGC/WTCCC2	0.016	0.40
<i>GPR109A</i>	rs601339	12	intergenic	123174743	G	A	0.2	0.03	7.8E-10	rs2454722	0.99	G	IMSGC/WTCCC2	0.025	0.26
<i>ADIPOQ</i>	rs6810075	3	intergenic	186548565	T	C	0.6	0.06	1.2E-43	rs1648707	0.85	A	IMSGC Immunochip	-0.031	0.08
<i>DNAH10</i>	rs7133378	12	intronic	124409502	A	G	0.3	0.02	1.3E-09	rs7973683	0.90	A	IMSGC/WTCCC2	0.002	0.91
<i>PDE3A</i>	rs7955516	12	intergenic	20498036	C	A	0.4	0.02	4.5E-08	rs7303397	0.93	G	IMSGC/WTCCC2	-0.017	0.38
<i>LYPLAL1</i>	rs3001032	1	intergenic	219727779	C	T	0.3	0.02	3.6E-08	rs1572505	0.89	A	IMSGC/WTCCC2	0.020	0.31

*when possible, plausible biological candidates have been listed; otherwise, closest genes have been designated

**taken from ADIPOGen sex-combined analyses in European populations

†estimated from models using natural-log-transformed adiponectin

IMSGC: International Multiple Sclerosis Genetics Consortium

WTCCC2: Wellcome Trust Case Control Consortium 2

SUPPLEMENTARY METHODS

PubMed Search

To investigate possible pleiotropic mechanisms of the selected adiponectin-altering SNPs on MS, the following term categories were searched on the PubMed database: gene name(s), gene mutations, encoded protein name(s), encoded protein name(s) + multiple sclerosis, encoded protein name(s) + autoimmune disease.

For rs1108842: “GNL3,” “GNL3 mutations,” “guanine nucleotide binding protein-like 3,” “nucleostemin,” “guanine nucleotide binding protein-like 3 multiple sclerosis,” “nucleostemin multiple sclerosis,” “guanine nucleotide binding protein-like 3 autoimmune disease,” “nucleostemin autoimmune disease”

For rs12922394: “CDH13,” “CDH13 mutations,” “T-cadherin,” “H-cadherin,” “cadherin-13,” “T-cadherin multiple sclerosis,” “H-cadherin multiple sclerosis,” “cadherin-13 multiple sclerosis,” “T-cadherin autoimmune disease,” “H-cadherin autoimmune disease,” “cadherin-13 autoimmune disease”

For rs1597466: “TSC22D2,” “TSC22D2 mutations,” “TSC22 domain family member 2,” “TSC22 domain family member 2 multiple sclerosis,” “TSC22 domain family member 2 autoimmune disease”

For rs2925979: “CMIP,” “CMIP mutations,” “c-maf inducing protein,” “c-maf inducing protein multiple sclerosis,” “c-maf inducing protein autoimmune disease”

For rs2980879: “TRIB1,” “TRIB1 mutations,” “tribbles pseudokinase 1,” “tribbles pseudokinase 1 multiple sclerosis,” “tribbles pseudokinase 1 autoimmune disease”

For rs601339: “GPR109A,” “HCAR2,” “hydroxycarboxylic acid receptor 2,” “hydroxycarboxylic acid receptor 2 multiple sclerosis,” “hydroxycarboxylic acid receptor 2 autoimmune disease”

For rs7133378: “DNAH10,” “DNAH10 mutations,” “dynein heavy chain 10 axonemal,” “dynein heavy chain 10 axonemal multiple sclerosis,” “dynein heavy chain 10 axonemal autoimmune disease”

For rs7955516: “PDE3A,” “PDE3A mutations,” “phosphodiesterase 3A,” “phosphodiesterase 3A multiple sclerosis,” “phosphodiesterase 3A autoimmune disease”

For rs3001032: “LYPLAL1,” “LYPLAL1 mutations,” “lysophospholipase-like protein 1,” “lysophospholipase-like protein 1 multiple sclerosis,” “lysophospholipase-like protein 1 autoimmune disease”