The role of body fat in multiple sclerosis susceptibility and severity: A Mendelian randomisation study

Mona M Almramhi[1,2], Catherine S Storm[1], Demis A Kia[1], Rachel Coneys[3], Burleen K Chhatwal[1] and Nicholas W Wood[1]

Mona M. Almramhi, MSc[1,2], Catherine S. Storm, BSc[1], Demis A. Kia, MBBS[1], Rachel Coneys, BSc[3], Burleen K. Chhatwal, BSc[1], Nicholas W. Wood PhD, FRCP, FMedSci [1]

[1]Department of Clinical and Movement Neurosciences, University College London Queen Square Institute of Neurology, London, United Kingdom.

[2] Department of Medical Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

[3] Department of Neurodegenerative diseases, University College London Queen Square Institute of Neurology, London, United Kingdom.

* Correspondence:

NW Wood

n.wood@ucl.ac.uk

MM Almramhi

mmalmaramhi@kau.edu.sa

ABSTRACT

Objective

The objective of this study was to explore the potential causal associations of body mass index, height, weight, fat mass, fat percentage and non-fat mass in the whole body, arms, legs and trunk (henceforth, “anthropometric measures”) with multiple sclerosis risk (MS) and severity. We also investigated the potential for reverse causation between anthropometric measures and MS risk.

Methods

We conducted a two-sample univariable, multivariable and bidirectional Mendelian randomisation (MR) analysis.

Results

A range of features linked to obesity (body mass index, weight, fat mass and fat percentage) were risk factors for MS development and worsened the disease’s severity in MS patients. Interestingly, we were able to demonstrate that height and non-fat mass have no association with MS risk or MS severity. We demonstrated that the association between anthropometric measures and MS is not subject to bias from reverse causation.

Conclusions

Our findings provide evidence from human genetics that a range of features linked to obesity is an important contributor to MS development and MS severity, but height and non-fat mass are not. Importantly, these findings also identify a potentially modifiable factor that may reduce the accumulation of further disability and ameliorate MS severity.

INTRODUCTION

Obesity is reported as a risk factor for many metabolic, inflammatory and autoimmune diseases in terms of incidence, disease severity and outcomes [1] . Evidence shows that excess body fat is accompanied by inflammation and alterations in immune cell function, reflected in an increase in circulating pro-inflammatory proteins, elevated leukocyte, neutrophil and monocyte counts and impaired immune cell function, leading to an increased risk of severe infectious disease [2, 3].

In multiple sclerosis (MS), a link has been demonstrated between obesity, the risk of developing MS and a worsening disability level in MS patients. Recent observational and Mendelian randomisation (MR) studies have shown that obesity in childhood or early adulthood, as measured by elevated body mass index (BMI), is associated with the risk of developing MS[4-7]. Although BMI is commonly used to identify obese persons due to its ease of calculation and cost-effectiveness [8], its use has been criticised [9]. BMI does not distinguish between the contributions of fat and non-fat tissue (e.g., lean tissue mass) to body weight [10] which might contribute to the misclassification of certain groups of individuals. For instance, athletes might be classified as obese due to their higher BMI, but the BMI increase comes from higher lean muscle mass, not from accumulated fat [11]. In addition, BMI does not capture the location of body fat and non-fat, which have been shown to play an essential role in predicting the risk of several diseases. For example, increased abdominal fat is associated with cancer, stroke and cardiometabolic disease, whereas decreased lean mass of the arms and legs is associated with increased falls and frailty [12].

Although MR studies have been employed to investigate the relation between obesity (BMI) and MS risk, the MR approach has not yet been used to investigate the relation between

obesity and the progression of disability in MS. Observational studies of obesity and the progression of disability in MS patients have reported inconsistent findings. Whereas some studies report evidence supporting the association between higher BMI, fat mass (FM), fat percentage (FP) and disability progression in MS [8, 13], other studies have identified no evidence of an association between disability progression in MS and BMI, FM or FP [14, 15].

The MR approach is an epidemiological technique that utilises genetic variants as proxies to investigate the causal role of a modifiable exposure on an outcome of interest [16]. Therefore, MR studies are less prone to bias from confounders, reverse causation and other biases that raise questions regarding the findings from observational studies. Most importantly, MR distinguishes correlation from a causation that simple regression analysis cannot answer [17].

In this study, we aimed to conduct an MR analysis to assess the causal effects of the lifelong genetically elevated BMI, height, weight, FM, FP and non-fat mass (NFM) on MS risk and severity to better understand the effect of obesity on MS in adults. Further, we performed a bidirectional MR analysis to assess whether the lifelong genetically increased risk of MS influences anthropometric measures.

METHODS

Data for anthropometric-related measures

We tested whether each anthropometric measure is a causal risk factor for MS risk and MS severity by considering 21 anthropometric measures (Table 1) divided into two categories. The adiposity-related measures included BMI, weight, FP and FM for the whole body, the upper limbs (right arm, left arm), lower limbs (right leg, and left leg) and trunk. The second category included height and NFM for the whole body, upper limbs, lower limbs and trunk. The summary statistics data (β-coefficients and standard errors) for each measure were obtained from Neale Lab (http://www.nealelab.is/uk-biobank/), which has conducted the most up-to-date genome-wide association analysis (round 2) on 4,236 phenotypes with a sample size of 361,194 persons of white-British ancestry from the UK Biobank (see supplementary).

Data for MS

We obtained summary statistics data for MS risk from the latest genome-wide association study meta-analysis by the International Multiple Sclerosis Genetics Consortium (IMSGC), which included a total of 47,429 cases with MS and 68,374 healthy controls (discovery plus replications cohorts) [18]. In this dataset 233 variants were identified to be associated with MS risk, in which 200 variants were outside of the Major Histocompatibility Complex (MHC) region, 32 variants were within MHC and one variant on chromosome X.

To assess the effect of anthropometric measures on MS risk, we used the discovery cohort of MS GWAS, which included 14,802 cases with MS and 26,703 healthy individuals. Due to complex linkage disequilibrium structures and a high potential for pleiotropy in the MHC region, 12 Mbps around this region (from 24 to 35 mega base pairs of chromosome 6; GRCh37) were excluded from the discovery MS risk dataset.

To explore whether MS influences anthropometric measures (bidirectional MR analysis), we selected 200 susceptibility variants that are located outside of the MHC region as GWAS for MS risk[18]. With MS-associated variants as the exposure, we then obtained corresponding effect estimates from anthropometric measures as the outcome.

For MS severity, we also obtained summary statistics data from IMSGC, who performed association analysis on 7,069 cases with MS to identify genetic variants that might influence MS severity; the rate of disability progression in these cases was measured by MS severity score [19]. No variants with strong evidence (p value $< 5 \times 10^{-8}$) of an association were found in this severity-based analysis. Therefore, we were unable to conduct a bidirectional MR between MS severity and anthropometric measures. The participants in both MS cohorts were of European ancestry. The severity of MS refers to the rate of disability progression [20]. If the patient accumulated disability at a faster-than-average rate compared to the patients with similar disease duration, then he/she experienced rapid disease progression (severe MS) [20]. By contrast, the inverse is true if the patient has a lower-than-average disability relative to their peers with similar disease duration; in that case, the patient is classified as having mild MS [20].

Selection and Validity Assessment of Genetic Variants

We selected all genetic variants (single-nucleotide polymorphism) that were robustly associated at genome-wide significance (p value $< 5 \times 10^{-8}$) with the anthropometric measures. Selected variants were taken forward to harmonised with MS data, excluding palindromic variants and clumping for linkage disequilibrium ($r^2 < 0.001$). Steiger filtering was then computed to remove genetic variants that explain more of the outcome variances than the exposure variances in an effort to guard against using pleiotropic variants as genetic instruments [21-23]. Pleiotropic variants manifest a horizontal pleiotropy when they influence the

outcome through a pathway other than the exposure of interest [21].We then used the mean *F*-statistic and the proportion of variance explained (R^2) to evaluate the strength of the selected variants (see supplementary). The value of the mean *F*-statistic >10 has been proposed for determining the suitability of genetic variants for MR analysis to avoid a weak instrument [24-26].

MR Statistical Analysis

Univariable MR Analysis

We used inverse variance weighting (IVW) using a multiplicative random-effects model to account for heterogeneity among the causal estimates obtained from Wald estimates as the primary analysis method [27, 28]. For sensitivity analyses, we used MR-Egger and weighted median approaches. MR-Egger yields an unbiased estimate even if all the genetic variants have pleiotropic effects, while the weighted median provides consistent estimates even when up to 50% of genetic variants exhibit horizontal pleiotropy [27, 28]. Heterogeneity was assessed using Cochran's Q and quantified with the I^2 statistic; a *p* value of Cochran's Q < 0.05 provides evidence of heterogeneity [28, 29]. We used the MR-Egger intercept to detect unbalanced horizontal pleiotropy across the genetic variant; a *p* value of intercept < 0.05 provides evidence of pleiotropy [27, 28].

Multivariable MR Analysis

We noted in Figure 1 that many of the genetic variants have pleiotropic effects and influence more than one of the anthropometric measures. This violates the key assumption for MR, which is that the genetic variants should not be associated with outcomes through a pathway rather than the exposure of interest [16]. To account for pleiotropy and determine whether several of the anthropometric-related measures affect MS through the same pathway or whether these measures have direct (i.e. independent) effects, we conducted multivariable

MR (MVMR) analyses using the IVW method[30]. For these analyses, we used the genetic instruments for each anthropometric measure that retained a significant effect on MS in the previous univariable analyses. If there was evidence of residual heterogeneity or unbalanced pleiotropy in the estimates from univariable analyses, the MR-Radial method was used to identify pleiotropic variants [31]. Variants with a large Cochran's Q statistic (a Cochran's Q *p* value < 0.05) were removed, and the MVMR analysis was then conducted. Due to the different selection criteria in the univariable and MVMR analyses, the number of genetic instruments can be different. Evidence of attenuation in estimates of any of the anthropometric measures in MVMR compared to the corresponding univariable MR estimates indicates a mediated effect executed through the other anthropometric measures included in MVMR analyses [32]. Evidence of significant estimates of any of the anthropometric measures in MVMR indicates a direct effect that does not execute through the other anthropometric measures [32].

Interpretation of Findings

In the absence of evidence of horizontal pleiotropy, we used the IVW estimates as the most reliable indicator of the underlying causal relationship. If there was evidence of horizontal pleiotropy, we used the estimate from MR-Egger and the weighted median (as both proposed to correct for horizontal pleiotropic effects). Finally, we applied the false discovery rate (FDR)-adjusted *p* value on the IVW results to account for multiple comparisons [33]. Exposures with significant adjusted *p* values of ≤ 0.05 were defined as exposures with potential evidence of a causal effect.

RESULTS

Table 1 shows the exact numbers of genetic variants, sample size, R^2, the mean *F*-statistic and the means standard deviations (SD) for the anthropometric measures. Weak instrument bias is likely negligible in this data since the mean *F*-statistic is greater than 10. Figure 1 shows the genetic correlations among the 21 anthropometric measures.

Influence of Genetically Raised Anthropometric Measures on the Risk of MS

Table 2 displays the odds ratio for MS risk per one SD increase in each of the anthropometric measures. The IVW results showed that genetically raised BMI, weight, FM and FP in the whole body, trunk, arms (left and right) and legs (left and right) were causally associated with an increased MS risk. For the sensitivity analyses, the results from the weighted median method further replicated the direction and significance of the IVW results, thereby providing additional confidence in the IVW results. The results from MR-Egger were also similar to the IVW results in the direction of the causal associations with a moderate increase in the effect estimates; however, confidence intervals (CI) were wider, resulting in a number of estimations crossing the null. The I^2 statistic indicated a slight degree of heterogeneity; however, Cochran's Q *p* values were not significant, except for the weight. The MR-Egger intercept indicated no evidence for horizontal pleiotropy except for the FM in the right and left legs where the intercept *p* values were significant ($p < 0.05$), suggests horizontal pleiotropy effect. The pleiotropy-corrected causal estimates from the MR-Egger and weighted median for these measures were moderately increased relative to the IVW estimates, but still significant and in the same direction, further supporting the causal role of FM in the legs on MS risk. For height and NFM in the whole body, trunk, arms and legs, the MR results found no evidence for a relationship between these measures and MS risk.

For the MVMR analyses, we fitted a model with adiposity-related measures that retained an effect on MS risk in the univariable MR models. The MVMR-IVW revealed that compared with univariable estimates, the direct estimates were slightly lower for the BMI, weight, FM and FP at different body parts and slightly larger for the FP in the right arm and the FM in the trunk but still significant. Meanwhile, the direct estimates for the FM in the left leg were attenuated, resulting in a wider 95% CI that overlapped null. Thus, the observed effects for left leg FM in the univariable MR analyses are more likely operating through the pathways of other adiposity-related measures.

Influence of Genetically Raised MS Risk on Anthropometric Measures

We further conducted a bidirectional MR to assess the causal relationship between MS risk and anthropometric measures, as shown in Table 3, which displays the β-coefficients for each anthropometric measure per log odds increase in MS risk. After removing six genetic instruments not found in the anthropometric data, 37 genetic instruments with incompatible alleles and one genetic instrument for being palindromic, a total of 97 instruments were used for this analysis. The IVW and MR-Egger results revealed that a genetic predisposition to MS has no significant effect on any of the anthropometric measures investigated here, except for the weighted median, where the *p* values for some of the anthropometric measures were significant. Since there was no evidence of pleiotropy from the MR-Egger intercept, IVW is more robust for detecting the true causal effect than the weighted median. There was significant evidence of heterogeneity as reflected by the I^2 statistic and Cochran's Q *p* values. Since the pleiotropy is balanced, the heterogeneity is more likely due to the non-collapsibility of the odds ratio [23]. Further, the heterogeneity in these analyses was accounted for by using the IVW multiplicative random-effects model [28].

Influence of Genetically Raised Anthropometric Measures on MS Severity

Table 4 displays the β-coefficients for MS severity per one SD increase in each anthropometric measure. The IVW results showed that genetically raised BMI, weight and FM in the whole body, trunk, arms and legs were causally associated with an increase in MS severity. For sensitivity analyses, the estimates were slightly increased in the MR-Egger estimator, while they were nearly identical to the IVW in the weighted median estimator. The MR-Egger and weighted median replicated the IVW direction of the estimates but did not reach statistical significance due to wide CIs. The Cochran's Q, I^2 statistic and MR-Egger intercept indicated no evidence for heterogeneity or horizontal pleiotropy. Thus, causal estimates were more convincing in the IVW results.

For the FP, the MR findings revealed evidence that genetically raised trunk FP is causally associated with an increase in MS severity; however, it did not pass the FDR. By contrast, we did not detect any significant association between FP in the whole body or the other limbs (arms and legs) and MS severity. The power to detect a significant association here would seem to be low due to the small proportion of variance explained by the genetic variants associated with FP in the whole body and limbs (R^2 = 0.8–1.4%) compared with the corresponding values for other anthropometric measures that retained an effect on MS severity (Table 1). For height and NFM in the whole body, trunk, arms and legs, we found no evidence of the causal role of these measures on MS severity.

We took the measures that retained an effect on MS severity in the univariable MR model forward and further fitted an MVMR model. The MVMR-IVW revealed that BMI, weight, and FM in the whole body, trunk, legs and left arm have a significant direct effect on MS severity, but the estimates are slightly lower than the total estimates in the univariable MR analyses. The direct effect for FM in the right arm attenuated to the null after adjusting for the other adiposity-

related measures suggests that the effects of FM in the right arm on MS severity were more likely operating through the pathways of other adiposity-related measures.

DISCUSSION

The purpose of the present study was to explore the causal role of anthropometric measures on MS to obtain a better understanding of the impact of excessive fat (obesity) and non-fat mass on MS risk and severity. Our study provides evidence from human genetics that obesity-related measures are an important contributor to MS development and greater disability progression, but height and NFM are not.

Our MR findings first confirmed that a higher BMI leads to a greater risk of developing MS but found no evidence that MS risk influences BMI or the other anthropometric measures. This finding supports previous observational and MR studies that found an association between elevated BMI and an increase in the risk of developing MS [4-7].

BMI does not differentiate between fat and non-fat tissues and between fat stored in different parts of the body. Thus, BMI can partially be used to study obesity. Therefore, we used a range of anthropometric measures that enable capture of the fat stored in different compartments of the body and to discriminate between fat and non-fat tissues. Our MR findings suggest that people with greater fat stored in the whole body, arms, legs and trunk are at a high risk of developing MS. On the other hand, our findings indicated that height or having an increase in NFM are unlikely to put an individual at high risk of getting MS. This lack of evidence of associations between height, NFM and MS risk is unlikely due to low power since the number of GWAS-associated genetic instruments for height and NFM and the variance they explained are greater than the corresponding values—for example, for FP or FM in the legs, which retained a significant causal association with MS risk.

Of course, recommending a healthy weight (or BMI) to all people is important for lowering their risk of a host of diseases, and we can now add MS to this list. However, in terms of advising and managing patients who already have a diagnosis of MS, understanding whether fat and/or non-fat mass may play a role is perhaps even more important. Therefore, we were particularly interested in identifying any causal effect of anthropometric measures on MS severity. Our MR findings suggest that obesity is a significant contributor to disability progression, and therefore severity, in MS, as evident by MR results for BMI, weight and FM. These findings support previous observational studies that found that higher BMI and fat accumulation in the whole body, arm, leg and trunk are significantly associated with greater disability in MS patients [8, 13]. Our findings are also in line with observational studies that identified no link between NFM and disability progression in MS [8]. By contrast, our findings are in disagreement with the results of other observational studies that identified no evidence of an association between disability progression in MS and adiposity-related measures, with respect to BMI, FM or FP [14, 15]. The lack of association in these studies is more likely due to the small sample size, which ranged from 27 to 150 participants, which reduces the power to detect the true effect.

This study also had some key limitations. Firstly, Body composition differs between men and women, with men generally having greater muscle mass and women having proportionally more fat mass [34]. Body composition is also affected by the ageing process, which is characterized by an increase in total body FM and a concomitant reduction in lean mass and bone density, which are independent of general and physiological changes in body weight and BMI [35]. These changes in body composition could induce differences in the causal relation between anthropometric-related measures and MS risk/severity in men and women of different ages. Due to the lack of GWAS results based on sex/age for MS, we were unable to predict such differences or determine which anthropometric-related measure could strongly predict the

risk of MS developing or MS disability worsening among obese men and women. Secondly, changes in fat and non-fat tissues distribution occur during childhood and adolescence. Due to the lack of genetic instruments associated with fat and non-fat measures for children and adolescents, we cannot extrapolate the role of fat burden across age groups in the development of MS or predict whether BMI can be informative to represent body fatness in childhood and adolescence. Thirdly, we could not conduct a bidirectional MR analysis between anthropometric measures and MS severity due to the absence of variants with strong evidence of association with MS severity. Therefore, we have not ruled out a possible bidirectional causal relationship between anthropometric measures and MS severity. Fourthly, the other important issue that might affect the anthropometric-MS severity association is collider bias. This phenomenon occurs when the studied sample is only restricted to cases (as with MS severity), leading to either induced associations or distorted associations between a risk factor and the progression data, depending on the direction of the relationship between the risk factor and disease onset [36]. Our results showed that the direction of the relationship between BMI, weight, FM and MS severity is the same as with MS risk; thus, caution is needed in interpreting the association between these measures and MS severity, as these relationships might be susceptible to collider bias. Fifthly, MR estimates reflect the lifelong effects of a risk factor in contrast to the short-term effects captured in observational studies.

In conclusion, our findings provide evidence that obesity is an important contributor to MS development and MS severity, but height and non-fat mass are not. These findings expand our understanding of the role of anthropometric measures in MS aetiology. Importantly, these findings also identify a potentially modifiable factor that may reduce the accumulation of further disability and ameliorate MS severity.

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Authors' contributions

Mona M. Almramhi, design of the study, conducting the analysis, interpretation of results, drafting the manuscript for intellectual content. Authors Nicholas W. Wood, Catherine S. Storm, Demis A. Kia, Rachel Coneys and Burleen K. Chhatwal, contributed equally to interpretation of the results and critical revision of the manuscript for intellectual content.