Metabolic syndrome does not influence the phenotype of LRRK2 and GBA related Parkinson’s disease

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In order to evaluate the influence of the metabolic syndrome (MS) (obesity, hypertension, elevated triglycerides, reduced levels of HDL cholesterol and glucose impairment) on the phenotype of LRRK2 and GBA Parkinson’s disease (PD), and on the prevalence of prodromal features among individuals at risk, we collected laboratory test results, blood pressure, demographic, cognitive, motor, olfactory and affective information enabling the assessment of each component of MS and the construction of the MDS prodromal probability score. The number of metabolic components and their levels were compared between participants who were separated based on disease state and genetic status. One hundred and four idiopathic PD, 40 LRRK2-PD, 70 GBA-PD, 196 healthy non-carriers, 55 LRRK2-NMC and 97 GBA-NMC participated in this study. PD groups and non manifesting carriers (NMC) did not differ in the number of metabolic components (p = 0.101, p = 0.685, respectively). LRRK2-PD had higher levels of triglycerides (p = 0.015) and higher rates of prediabetes (p = 0.004), while LRRK2-NMC had higher triglyceride levels (p = 0.014). NMC with probability rates for prodromal PD above 50% had higher frequencies of hypertriglyceridemia and prediabetes (p < 0.005, p = 0.023 respectively). While elevated triglycerides and prediabetes were more frequent among LRRK2 carriers, MS does not seem to influence GBA and LRRK2-PD phenotype.

Parkinson’s disease (PD) is clinically diagnosed based on cardinal motor features with variability related to age of onset, disease progression, response to levodopa and non-motor profile. In recent years a better understanding of the prodromal phase of PD has emerged enabling the construct of the Movement Disorders task force research criteria for prodromal PD1. Among Jews from Ashkenazi (AJ) decent, mutations in the glucocerebrosidase (GBA) and Leucine Rich Repeat Kinase 2 (LRRK2) genes are present among more than 1/3 of the PD population2 with specific phenotype for each mutation group3,4. Penetrance estimations for these mutations are far from complete5-8 due to genetic polymorphisms, environmental causes as well as inflammatory mechanisms9-11.

Metabolic Syndrome (MS) is defined as the presence of three out of five components: abdominal obesity or elevated body mass index (BMI), elevated serum triglycerides, low serum HDL, high blood pressure and prediabetes (drug treatment for any of the last four conditions also fulfills the criteria)9-11. MS is significantly related to atherosclerosis and vascular cerebral disease12 and to neurodegeneration13, however its influence on PD clinical phenotype, progression and risk for future development of the disease is still unclear. While Leehey et al. detected a faster progression in motor symptoms among PD patients who had MS14, the association between MS and risk for PD is conflicting: with one cohort study indicating lower risk15, and another pointing towards increased risk16.

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The association between the components of MS and PD is not clear either. Diabetes mellitus has been suggested to represent a risk for PD but this remains equivocal\(^\text{23,24}\). Possible mechanisms connecting the two states include neuroinflammation, mitochondrial dysfunction and increased oxidative stress\(^\text{25}\). Higher levels of triglycerides and LDL were found to be associated with lower risk for PD\(^\text{26}\). The role of hypertension in PD is conflicting as well\(^\text{21,22}\). These discrepancies result from the choice of population, sample size, comorbid diseases, follow-up periods, analytical techniques and statistical power.

Mutations in the GBA gene influence the accumulation of ceramide, a sphingolipid which participates in cellular signaling. The accumulation of ceramide impairs insulin action and promotes apoptosis potentially linking insulin resistance and inflammation\(^\text{3}\) However, to date the relationship between these factors and PD have not been studied.

In order to better characterize the factors that influence the specific phenotypes associated with GBA and LRRK2 PD as well as those that might contribute to disease risk, we assessed the prevalence of MS and its’ different components among genetically determined patients with PD and non-manifesting carriers of mutations in the LRRK2 or GBA genes (NMC) and correlated PD phenotype and future probability for developing PD with the presence of MS. We hypothesized that PD patients with MS would have worse motor and cognitive phenotypes due to comorbidity burden with potential vascular and inflammatory implications, specifically among GBA-PD, and that an increase of metabolic components burden would be associated with higher MDS probability scores for developing PD through similar mechanisms.

### Results

A total of 562 subjects participated in this study. Data of 104 iPD patients, 40 LRRK2-PD and 70 GBA-PD patients is presented in Tables 1 and 2.

Both GBA-PD and LRRK2-PD had longer disease duration and higher LEDD compared with iPD (\(p < 0.001\) and \(p < 0.002\), respectively). LRRK2-PD had higher UPSIT scores (\(p = 0.008\)), higher rates of prediabetes (\(p = 0.004\)) and higher triglyceride levels (\(p = 0.015\)) which were correlated with disease duration (\(r = 0.332, p = 0.036\)) and LEDD (\(r = 0.432, p < 0.001\)).

Groups did not differ in the mean number of metabolic components (\(p = 0.101\)), nor in the frequency of MS (presence of any three components as cutoff) (\(p = 0.211\)). In the linear regression model, which was constructed to estimate the relationship between MS and its’ components (as dependent variables) and genotype, sex, age, RBDQ, UPDRS-III, MoCA, UPSIT, disease duration, LEDD and NMSQ (independent variables), the number of MS components was associated with age and iPD status (Table 3). There was no association between patients’ characteristics, obesity or low HDL levels. Prediabetes was associated with age and iPD status which accounted for 45.8% of the variance. Hypertension was associated with age and LRRK2-PD status, which accounted for 34.9% of the variance. Hypertriglyceridemia was associated with age and the score on the RBDQ questionnaire accounting for 44.8% of the variance.

Seventy PD patients (32.7%) in our cohort had MS. This group was older (69.32 ± 8.14 vs. 63.66 ± 10.54; \(p < 0.001\)) and had a higher age of diagnosis (66.41 ± 8.42 vs. 60.59 ± 10.19; \(p < 0.001\)) but did not differ in any other disease or genetic characteristics.

One hundred ninety-six healthy non GBA-LRRK2-carriers were compared with 55 LRRK2-NMC and 97 GBA-NMC (Tables 4 and 5).

| Table 1. PD cohort characteristics. iPD – idiopathic Parkinson’s Disease, m-male, f-female, LEDD- LevoDopa Equivalent Daily Dose, MDS-UPDRS- Movement Disorder Society – Unified Parkinson’s Disease Rating Scale, MoCA – Montreal Cognitive Assessment, UPSIT- University of Pennsylvania Smell Identification Test, RBDQ- REM sleep Behavior Disorder Questionnaire, NMSQ- Non-Motor Symptoms questionnaire, SCOPA-AUT- Scale of Outcome in Parkinson’s Disease – Autonomic Dysfunction, BDI- Beck Depression Inventory. *Differences between iPD and LRRK2-PD and GBA-PD, †Differences between LRRK2-PD and GBA-PD |
| --- |
| **IPD** | **LRRK2-PD** | **GBA-PD** | **significance** |
| **#** | 104 | 40 | 70 |
| **Age(y)** | 66.61 (10.43) | 64.82 (10.03) | 64.30 (9.78) | 0.302 |
| **Disease duration (y)** | 2.55 (1.98)* | 3.64 (2.52) | 3.98 (2.65) | 0.001 |
| **Sex m/f** | 71/33 | 24/16 | 46/24 | 0.644 |
| **LEDD** | 277.11 (279.11)* | 432.67 (398.41) | 468.16 (428.85) | 0.002 |
| **Orthostatic Hypotension (%)** | 17.30% | 10.00% | 22.85% | 0.233 |
| **MDS-UPDRS** | 38.14 (18.41) | 35.35 (16.70) | 43.77 (21.89) | 0.077 |
| **MoCA** | 23.86 (3.75) | 24.53 (4.44) | 23.54 (3.85) | 0.455 |
| **UPSIT** | 17.12 (9.87) | 20.83 (9.47)* | 14.51 (9.31) | 0.008 |
| **RBDQ** | 3.06 (2.56) | 3.08 (2.38) | 3.95 (3.53) | 0.291 |
| **NMSQ** | 7.46 (4.62) | 6.74 (3.78) | 8.55 (5.45) | 0.083 |
| **SCOPA-AUT** | 16.93 (10.09) | 18.03 (11.74) | 17.11 (10.85) | 0.478 |
| **BDI** | 8.00 (6.13) | 6.93 (5.50) | 9.31 (7.35) | 0.169 |
| **Hypotension (%)** | 17.30% | 10.00% | 22.85% | 0.233 |
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participants with MS did not have a higher risk to develop PD based on the probability score (p = 0.073).

hypertriglyceridemia (43.7% vs 72.0%, p = 0.364). Among the NMC, the number of metabolic risk factors was associated with sex and age but not with genetic status in the linear regression model which was constructed in order to estimate the relationship between MS and its’ components (as dependent variables) and genotype, sex, age, probability score, RBDQ, UPDRS-III, MoCA, LEDD, HbA1c, Hypo-HDL, and Obesity (BMI > 30) (p = 0.055). HbA1c was also correlated with the probability score (r = 0.005), prediabetes (26.7% vs. 48.0%, p = 0.023), but not in hypertension (60.8% vs. 76.0%, p = 0.095), obesity (20.1% vs 32.0%, p = 0.125), low HDL levels (21.6% vs. 24.0%, p = 0.140), lower MoCA scores (25.0% vs 16.6%, p = 0.026), lower LEDD (70.0% vs. 80.0%, p = 0.010) and higher SCOPA-AUT scores (10.0% vs 5.5%, p = 0.018).

Ten participants (3%) had a likelihood ratio for converting to PD (probability score) higher than 80% (4 iPD, 3 LRRK2-NMC, 3 GBA-NMC) placing them under the category of possible prodromal PD. Most of the cohort (57.8%) had a probability score of less than 2%. Splitting the non-manifesting groups based on the probability score resulted in 25 NMC with a score above 50 and 323 below it. Between group differences were noted in hypertriglyceridemia (43.7% vs 72.0%, p = 0.005), prediabetes (26.7% vs. 48.0%, p = 0.023), but not in hypertension (60.8% vs. 76.0%, p = 0.095), obesity (20.1% vs 32.0%, p = 0.125), low HDL levels (21.6% vs. 24.0%, p = 0.140) or the number of metabolic components (1.72 vs. 2.52, p = 0.087).

Ninety-Four (27.2%) non-manifesting participants had MS. This group was significantly older (59.3 ± 11.2 vs. 52.4 ± 9.9; p < 0.001), had less years of education (16.6 ± 2.7 vs. 17.5 ± 2.7; p = 0.015), lower MoCA scores (25.5 ± 3.7 vs. 26.6 ± 2.5; p = 0.007) and higher SCOPA-AUT scores (10.0 ± 8.9 vs. 7.8 ± 6.4; p = 0.039), however, participants with MS did not have a higher risk to develop PD based on the probability score (p > 0.170).

**Table 2.** PD cohort Metabolic syndrome components. iPD – idiopathic Parkinson’s Disease, HDL- High Density Lipoprotein, HbA1c – Hemoglobin A1C, BMI- Body Mass Index. *Significant after correcting for multiple comparisons with Bonferroni adjustment. †Differences between LRRK2-PD and GBA-PD, iPD.

**Table 3.** Linear regression Models for metabolic factors in PD. B(95%CI) and p values are presented for measures found significant in the model. *- significance driven by iPD; †- significance driven by LRRK2-PD. RBDQ- REM sleep Behavior Disorder Questionnaire, LEDD- LevoDopa Equivalent Daily Dose, UPDRS- Unified Parkinson’s Disease Rating Scale part III, MoCA – Montreal Cognitive Assessment, UPSIT- University of Pennsylvania Smell Identification Test, NMSQ- Non-Motor Symptoms questionnaire.

| Number of metabolic factors | Prediabetes | Hypertension | hypertriglyceridemia | Low HDL | Obesity |
|-----------------------------|-------------|--------------|---------------------|---------|---------|
| Model                        | F = 4.928; p < 0.0001 | F = 4.068; p < 0.0001 | F = 2.121; p = 0.026 | F = 3.664; p < 0.0001 | F = 0.838; p = 0.593 |
| Genotype                    | 0.21(0.03–0.39); 0.02* | 0.095(0.009–0.180); 0.031* | 0.08(0.008–0.165); 0.032* |         |         |
| Sex                         | −0.14(−0.281–0.0) | 0.051 |                     |         |         |
| Age                         | 0.46(0.03–0.06); 0.0001 | 0.022(0.024–0.033); 0.001 | 0.011(0.003–0.018); 0.004 | 0.018(0.010–0.026); 0.0001 |         |
| Disease duration            | −0.82(−0.16–0.002); 0.055 |                     |         | 0.029(0.002–0.057); 0.037 |         |
| RBDQ                        |                     |                     |         |         |         |
| LEDD                        |                     |                     |         |         |         |
| UPDRS-III                   |                     |                     |         |         |         |
| MOCA                        |                     |                     |         |         |         |
| UPSIT                       |                     |                     |         |         |         |
| NMSQ                        |                     |                     |         |         |         |

LRRK2-NMC had higher triglyceride levels (p = 0.014) which were correlated with SCOPA-AUT (r = 0.410, p = 0.003) and with the probability score (r = 0.322, p = 0.019). HbA1c was also correlated with the probability score (r = 0.532, p = 0.010) among this group.

Among the NMC, the number of metabolic risk factors was associated with sex and age but not with genetic status in the linear regression model which was constructed in order to estimate the relationship between MS and its’ components (as dependent variables) and genotype, sex, age, probability score, RBDQ, UPDRS-III, MoCA, UPSIT and NMSQ (independent variables). As with PD, no association between participants’ characteristics, obesity and HDL levels were detected. Prediabetes was associated with age and UPDRS-III scores accounting for 38.8% of the variance. Hypertension was associated with age and male sex accounting for 35.7% of the variance. Hypertriglyceridemia was associated with age and male sex accounting for 41.9% of the variance (Table 6).
### Table 4. Non-manifesting carriers' characteristics. NMC- Non-Manifesting Carriers, m-male, f-female, MDS-UPDRS- Movement Disorder Society - Unified Parkinson's Disease Rating Scale, MoCA – Montreal Cognitive Assessment, UPSIT- University of Pennsylvania Smell Identification Test, RBDQ- REM sleep Behavior Disorder Questionnaire, NMSQ- Non-Motor Symptoms questionnaire, SCOPA-AUT- Scale of Outcome in Parkinson's Disease – Autonomic Dysfunction, BDI- Beck Depression Inventory. * Differences between LRRK2-NMC and GBA-NMC, control.

|                  | Control | LRRK2-NMC | GBA-NMC | Significance |
|------------------|---------|-----------|---------|--------------|
| Age (y)          | 54.57 (11.32) | 52.89 (10.58) | 54.27 (9.59) | 0.517         |
| Sex m/f          | 89/107  | 28/27     | 36/61   | 0.377         |
| Orthostatic Hypotension (%) | 7%      | 10.09%    | 10.30%  | 0.516         |
| MDS-UPDRS        | 5.46 (4.35) | 5.69 (4.31) | 6.06 (6.79) | 0.449         |
| MoCA             | 26.46 (2.77) | 26.43 (2.74) | 25.86 (3.27) | 0.308         |
| UPSIT            | 30.39 (6.82) | 31.42 (4.93) | 29.45 (6.89) | 0.163         |
| RBDQ             | 1.75 (1.67) | 1.42 (1.57) | 1.98 (1.96) | 0.281         |
| NMSQ             | 3.39 (3.48) | 2.75 (2.78) | 3.35 (3.58) | 0.699         |
| SCOPA-AUT        | 8.42 (7.22) | 7.98 (5.96) | 8.47 (7.90) | 0.252         |
| BDI              | 4.16 (5.26) | 3.61 (4.70) | 4.22 (5.65) | 0.304         |
| Probability of prodromal PD (%) | 10.95 (20.17) | 33.83 (31.54) | 16.09 (25.70) | 0.001         |

### Table 5. Non-manifesting carriers' Metabolic Syndrome components. NMC- Non-Manifesting Carriers, HDL- High Density Lipoprotein, HbA1c – Hemoglobin A1C, BMI- Body Mass Index. * Significant after correcting for multiple comparisons with Bonferroni adjustment. @ Differences between LRRK2-NMC and GBA-NMC, control.

|                  | Control | LRRK2-NMC | GBA-NMC | Significance |
|------------------|---------|-----------|---------|--------------|
| HDL (mg/Dl)      | 56.45 (15.76) | 56.44 (14.47) | 57.84 (17.16) | 0.694         |
| Triglycerides (mg/Dl) | 136.89 (75.66) | 146.36 (105.63) | 142.00 (75.01) | 0.014*        |
| HbA1c (%)        | 5.49 (0.48) | 5.45 (0.45) | 5.44 (0.44) | 0.683         |
| BMI (kg/m²)      | 26.36 (4.78) | 26.15 (4.26) | 26.66 (4.69) | 0.785         |
| Hypo-HDL (%)     | 18.97% | 23.63% | 24.21% | 0.525         |
| Hypertriglyceridemia (%) | 43.58% | 56.36% | 49.47% | 0.619         |
| Prediabetes (%)  | 30.25% | 21.81% | 25.26% | 0.393         |
| Hypertension (%) | 65.12% | 58.18% | 58.90% | 0.469         |
| Obesity (BMI > 30) (%) | 20.51% | 14.54% | 27.36% | 0.163         |

### Table 6. Linear regression Models for metabolic factors in unaffected carriers. B(95%CI) and p values are presented for measures found significant in the model. RBDQ- REM sleep Behavior Disorder Questionnaire, UPDRS- Unified Parkinson's Disease Rating Scale part III, MoCA- Montreal Cognitive Assessment, UPSIT- University of Pennsylvania Smell Identification Test, NMSQ- Non-Motor Symptoms questionnaire.

|                  | Number of metabolic factors | Prediabetes | Hypertension | Hypertriglyceridemia | Low HDL | Obesity |
|------------------|----------------------------|-------------|--------------|----------------------|---------|---------|
| Model            | F = 7.137; p < 0.001        | F = 5.857; p < 0.001 | F = 0.481; p < 0.001 | F = 7.030; p < 0.0001 | F = 0.619; p = 0.781 | F = 1.148; p = 0.329 |
| Genotype         | −0.459 (−0.745–0.173); 0.002 | −0.095 (−0.194–0.003); 0.058 | −0.171 (−0.279–0.064); 0.002 | −0.140 (−0.248–0.032); 0.002 | |
| Sex              | 0.048 (0.033–0.063); 0.0001 | 0.015 (0.010–0.020); 0.0001 | 0.014 (0.008–0.019); 0.0001 | 0.016 (0.011–0.022); 0.0001 | |
| Probability      | −0.042 (−0.077–0.007); 0.018 | −0.017 (−0.035–0.001); 0.001 | |
| RBDQ             | −0.042 (−0.077–0.007); 0.018 | |
| UPDRS-III        | −0.017 (−0.035–0.001); 0.001 | |
| MOCA             | −0.017 (−0.035–0.001); 0.001 | |
| UPSIT            | −0.017 (−0.035–0.001); 0.001 | |
| NMSQ             | −0.017 (−0.035–0.001); 0.001 | |

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*Significant after correcting for multiple comparisons with Bonferroni adjustment. Differences between LRRK2-NMC and GBA-NMC, control.
Discussion

We used an enriched cohort of GBA and LRRK2 PD patients and non-manifesting carriers in order to determine the association between the metabolic syndrome and its’ components with PD phenotype and study the influence of the metabolic syndrome on the prevalence of prodromal features of PD among individuals at risk for future disease. We did not detect a worse motor or non-motor profile among PD patients who had a concurrent diagnosis of MS. This finding was strengthened by the fact that both LRRK2-PD and GBA-PD patients were more advanced in their disease state with higher LEDD compared with iPD. The increase of metabolic components burden was not associated with a higher probability scores for PD among NMC and does not seem to influence the phenotype of G2019S LRRK2- PD or GBA-PD.

LRRK2-PD had elevated levels of triglycerides and higher rates of prediabetes, with no relationships to clinical phenotype. Among LRRK2-NMC higher levels of triglycerides were also detected. Both higher triglycerides and HbA1c were positively correlated with the probability score of prodromal PD among LRRK2-NMC. The MDS task force specified probable prodromal PD as a likelihood of 80%, but they also allowed, in specific research settings a more lenient cutoff.

Among NMC with high probability for future development of PD (>50%), disregarding genetic status, hypertriglyceridemia and the presence of prediabetes were detected, attesting to a possible contribution of these components of the MS to PD pathogenesis.

MS effect, as is that of its’ components, on risk for developing PD and severity of PD phenotype is still contested15,24, with conflicting reports regarding BMI22–24 and hypertension22,24. To this extent, our findings do not support a role for obesity on disease phenotype.

The association between diabetes mellitus (DM) and risk of PD is also still contested29,30 but a meta-analysis reported increased pooled relative risk of developing PD after DM27. DM and PD are associated with inflammation, oxidative stress and mitochondrial dysfunction, while glycation of alpha synuclein has been suggested to promote aggregation of this protein31. Prediabetic PD patients were found to have worse motor symptoms, faster motor progression and more severe cognitive decline compared to normo-glycemic PD patients29,32. 57% of our cohort of PD patients was prediabetic within range of previous studies29. LRRK2-PD had higher rates of prediabetes compared with both iPD and GBA-PD with no clinical impediment. NMC with high probability for future PD had higher rates of prediabetes as well, suggesting a possible role in the pathogenesis of PD.

High levels of triglycerides and LDL-C have been associated with decreased risk of PD among a cohort of Israeli adults33 as among other cohorts as well15,34. The phosphorylation of Rab8a by LRRK2 has been shown to alter the ability of lipid storage in PD, while its’ significance on total lipid levels and triglycerides has yet to be determined26. We observed higher levels of triglycerides among LRRK2-PD, LRRK2-NMC and non-genetic controls with increased probability rates for future development of PD; however, the clinical significance will require future corroborating studies.

Gaucher disease has been associated with insulin resistance36 and increased hepatic glucose output37, with lower levels of LDL and HDL cholesterol and higher levels of triglycerides38. We did not find increased prevalence of components of MS among GBA-PD nor could we detect any associations between these and the risk to develop PD among GBA-NMC. Based on our cross-sectional observational data we cannot suggest an effect of MS or its sub-components on the disease process in GBA-PD.

The strengths of this study include a relatively large and well-defined genetic cohort. Additionally, the prospective cohort design enables the exploration of MS and its components without relying on self-report of current medical conditions but rather on medication lists and laboratory results. Limitations include lack of information regarding smoking, alcohol consumption, and the timing of appearance of MS components relative to onset of PD. We did not assess the response of the different components of MS to the medical regimen but coded all participants who took MS component-related medications as positive for the component. Previous use of any MS related medication was not assessed. Blood samples were not collected after a night fast; however, this was uniform for all participants and is gaining acceptability in clinical studies39, nevertheless this might have caused bias in this research setting. The MDS-prodromal score incorporates genetic status into the model giving LRRK2 25 points and GBA between 2–10 points depending on the severity of the mutation1, thus LRRK2-NMC inherently have higher probability scores. This study collected data from both patients with PD and their first degree relatives with and without mutations in designated genes however; shared genetic background goes beyond mutations in the LRRK2 and GBA genes and could potentially influence our results.

Despite these limitations, the findings contribute to our knowledge of MS and its relation to PD. From a clinical perspective, MS is modifiable; hence understanding its impact on the risk of developing PD and the severity of PD is important to future personalized medicine. The importance of this topic, therefore, warrants further research.

Methods

This study evaluated cross-sectional demographic, laboratory and questionnaire data from subjects who participated in the BEAT-PD study (TLV-0204–16), a collaborative venture between Biogen and the Tel-Aviv Medical Center, which set out to characterize LRRK2 and GBA PD as well as NMC of these mutations. Patients were recruited consecutively if they were AJ, diagnosed with PD by a movement disorders specialist based on the UK brain bank criteria and were at Hoehn and Yahr stages 1–2. Patients were excluded if they had additional neurological or psychiatric disorders, a malignancy or were HIV, HBV or HCV positive. In addition, first degree relatives of patients with PD were recruited to this study if they were above the age of 40, were not diagnosed with PD and did not have a malignancy. Controls were invited to participants if they did not have PD and did not have a malignancy. The study was approved by the local ethical committee of the Tel-Aviv Medical Center, with all participants providing informed consent prior to participation and all methods performed in accordance with the relevant guidelines and regulations.
**Procedure.** All participants underwent genetic testing for the G2019S mutation in the LRRK2 gene and for the common AJ GBA mutations as described previously and were separated based on genetic status. Only heterozygote carriers were included in this study with dual mutation carriers and homozygote carriers excluded.

Disease severity was assessed using the MDS-Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) during ON medication. The Montreal Cognitive Assessment (MoCA) was used to assess global cognitive functions; mood was assessed using the Beck Depression Inventory (BDI). The Non-Motor Symptoms Questionnaire (NMSQ), Scale of Autonomic Function in PD (SCOPA-AUT) and the REM sleep Behavior Disorder Questionnaire (RBDQ) were collected. Olfaction was tested using the University of Pennsylvania Smell Identification Test (UPSIT). These measures were used to calculate the probability for prodromal PD (Likelihood Ratio Score) for all participants without a diagnosis of PD that were above the age of 50, based on the Movement Disorders task force guidelines. This measure has been validated by our group as by others and is updated based on relevant studies. Each non-PD subject was allocating a ratio between 0–100% for risk for future development of PD.

Blood pressure was measured in the supine and standing position with orthostatic hypotension categorized as a drop of 20 mm HG in systolic or 10 mm HG diastolic pressure after 5 minutes.

Demographic data on weight, height and full medication list was collected and Levodopa equivalent daily dose (LED) was calculated. Medications were separated into the following groups: anti-hypertensive, lipid lowering and anti-glycemic. Blood samples were collected and assessed for HbA1c, triglycerides and HDL cholesterol.

MS was diagnosed if at least three of the following five components were present: Hypertension- blood pressure above 130/85 mm HG in any position, or use of anti-hypertensive medication; Prediabetes- HbA1c above 5.7% or use of anti-glycemic medication; Obesity- if BMI > 30 kg/m²; Hypertriglyceridemia- triglycerides > 150 mg/dl or use of lipid lowering medications and Low HDL- 40 mg/dl for men and 50 mg/dl for women or use of lipid lowering medications.

**Statistical analysis.** Descriptive statistics (means and standard deviations (SD) for continuous variables, percent for categorical variables) were computed for all measures. The analysis was performed in a stepwise manner, first we evaluated differences between groups in all collected measures using mixed models (general linear) based on disease status: differences between PD patients based on genetic status and separately differences within the unaffected cohort based on genetic status. The analysis was adjusted for age and sex in both cohorts. For patients with PD, analysis was also adjusted for disease duration and LEDD. Measures that were significantly different between genetic groups within each cohort were then explored for their association with PD symptoms and signs using Pearson correlation coefficient. Differences in the prevalence of MS between each group within each cohort were calculated using chi square tests ($\chi^2$). In the next step, multiple linear regression models were constructed to estimate the relationship between MS and its’ components (as dependent variables) and genotype, sex, age, RBDQ, UPDRS-III, MoCA, UPSIT and NMSQ (independent variables). For the PD group the model also included disease duration and LEDD, while for subjects without PD, the association to the probability of prodromal PD score was also explored. Significance was determined for $p < 0.05$ for descriptive measures and corrected for multiple comparisons using Bonferroni adjustment for the metabolic components. Statistical analysis was performed using SPSS (SPSS version 22, Chicago, IL, USA).

**Data availability**

Anonymized data will be made available on request.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

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