Interaction of Mitochondrial Polygenic Score and Lifestyle Factors in LRRK2 p.Gly2019Ser Parkinsonism

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ABSTRACT: Background: A mitochondrial polygenic score (MGS) is composed of genes related to mitochondrial function and found to be associated with Parkinson's disease (PD) risk.
Objective: To investigate the impact of the MGS and lifestyle/environment on age at onset (AAO) in LRRK2 p.-Gly2019Ser parkinsonism (*LRRK2*-PD) and idiopathic PD (iPD).
Methods: We included N = 486 patients with *LRRK2*-PD and N = 9259 with iPD from the Accelerating Medicines Partnership® Parkinson's Disease Knowledge Platform (AMP-PD), Fox Insight, and a Tunisian Arab-Berber founder population. Genotyping data were used to perform the MGS analysis. Additionally, lifestyle/environmental data were obtained from the PD Risk Factor Questionnaire (PD-RFQ). Linear regression models were used to assess the relationship between MGS, lifestyle/environment, and AAO.
Results: Our derived MGS was significantly higher in PD cases compared with controls ($P = 1.1 \times 10^{-8}$). We observed that higher MGS was significantly associated with earlier AAO in *LRRK2*-PD ($P = 0.047$, $\beta = -1.40$) and there was the same trend with a smaller effect size in iPD ($P = 0.231$, $\beta = 0.22$). There was a correlation between MGS and AAO in *LRRK2*-PD patients of European descent ($P = 0.049$, $r = -0.12$) that was visibly less pronounced in Tunisians ($P = 0.449$, $r = -0.05$). We found that the MGS interacted with caffeinated soda consumption ($P = 0.003$, $\beta = -5.65$) in *LRRK2*-PD and with tobacco use ($P = 0.010$, $\beta = 1.32$) in iPD. Thus, patients with a high MGS had an earlier AAO only if they consumed caffeinated soda or were non-smokers.
Conclusions: The MGS was more strongly associated with earlier AAO in *LRRK2*-PD compared with iPD. Caffeinated soda consumption or tobacco use interacted with MGS to predict AAO. Our study suggests gene–environment interactions as modifiers of AAO in *LRRK2*-PD. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: mitochondrial genes; polygenic score; gene–environment interaction

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The age at onset (AAO) of Parkinson's disease (PD), as well as the risk of developing the disease, are known to be affected by genetic and environmental factors.[1-5] In terms of genetics, monogenic forms and strong risk factors account for ~10% of PD cases.[6] Among these cases, the most common monogenic cause is the leucine-rich repeat kinase 2 (LRRK2) p.Gly2019Ser mutation. Besides monogenic forms and other genetic variants, PD can be explained by the interplay of complex genetics and lifestyle or environmental factors. One way to assess the cumulative effect of genetic variants on disease risk or AAO is by deriving and using a polygenic score (PGS).[7,8] Previously, a mitochondrial polygenic score (MGS) was derived and composed of genes involved in mitophagy, mitochondrial bioenergetics, and proteostasis pathways.[9] This meant a higher genetic score (a higher cumulative burden) was found to be associated with a higher risk for PD. Biologically, mitochondria are essential key players in PD pathogenesis. In particular, respiratory chain, mitophagy, and mitochondrial biogenesis impairment are associated with PD.[10]

LRRK2 localizes to the cytosol as well as to the mitochondria in the cells. Additionally, fibroblasts derived from patients with LRRK2-PD showed reduced NADH dehydrogenase activity and increased mitochondrial mass, mtDNA copy number, and nuclear factor erythroid 2-related factor 2 (Nrf2) expression.[11] In macrophages, the LRRK2 p.Gly2019Ser mutation interferes with mitochondrial homeostasis and alters cell death pathways.[12] A recent study reported that the seeding of p.Ala53Thr alpha-synuclein oligomerization happens especially at mitochondrial membranes in neurons, which can lead to respiratory chain impairments and a subsequent increase in reactive oxygen species (ROS).[13] As alpha-synuclein pathology is an important hallmark in LRRK2-PD and idiopathic PD (iPD), this is another molecular link between PD pathogenesis and mitochondrial impairment.

PD susceptibility has consistently been associated with lifestyle and environmental factors. Several meta-analyses have highlighted the protective association between smoking and PD risk.[14] It has been demonstrated that smoking status correlates with later AAO in iPD.[1,15,16] Additionally, caffeine and nonsteroidal anti-inflammatory drug (NSAID) consumption was associated with reduced iPD risk and later AAO.[1,14] Analogous to iPD, smoking and caffeine consumption are associated with later onset in LRRK2-PD.[5,17] In a study including affected and unaffected LRRK2 mutation carriers, NSAIDs (ie, aspirin and ibuprofen) users had reduced odds of developing PD.[18] In addition to lifestyle and environment, genetic modifiers of the AAO have been identified as well. There is evidence that variants in the DNM3[19] and CORO1C[20] genes are associated with AAO in LRRK2-PD.

The interaction of mitochondrial-related genes, lifestyle, and environment has not been thoroughly investigated. Importantly, mitochondria are at the interface of environmental impacts in the cell. Mitochondrial function can be affected by tobacco use, caffeine consumption, or pesticide exposure.[10,21-23] Smoking and vaping have been shown to be associated with mitochondrial gene dysregulation[24] and there is evidence that caffeine affects mitochondrial bioenergetics[22] and increases mitochondrial function.[23] Pesticides like rotenone or paraquat are known to increase mitochondrial dysfunction by inducing redox cycling or binding to complex I, which both result in the production of ROS.[25] Given the separate relevance of mitochondrial dysfunction and certain lifestyle factors and environmental exposure in PD pathogenesis, we hypothesize that there could be gene–environment interactions modulating their effect on AAO in LRRK2-PD and iPD. We included in our study patients with iPD and LRRK2-PD from the Accelerating Medicines Partnership® Parkinson's Disease Knowledge Platform (AMP-PD), Fox Insight, and a Tunisian Arab-Berber cohort. The AMP-PD and Fox Insight cohorts are publicly available datasets, providing genetic and metadata, and are an important resource for PD research. Additionally, as the frequency of LRRK2 p.Gly2019Ser is higher in Ashkenazi Jewish and Tunisian Arab-Berber populations,[26] the Tunisian cohort is specifically relevant to our research question.

Methods

Study Demographics, Genetics, and Environmental Data

Three datasets with genetic, environmental, and lifestyle data were included in this study: AMP-PD, Fox Insight, and a cohort from the Tunisian Arab-Berber population. In total, 9745 patients were included in our study: 486 patients with LRRK2-PD (AMP-PD: 127, Fox Insight: 154, Tunisian cohort: 205) and 9259 patients with iPD (AMP-PD: 2077, Fox Insight: 6949, Tunisian cohort: 233). Within the group of patients with iPD, individuals carrying risk variants for PD were excluded (ie, SNCA p.Ala53Thr, GBA p.Asn370Ser, and PRKN p.Arg275Trp) as far as the data were available from the cohorts. For patients with LRRK2-PD, the mean AAO was 58.2 years (SD = 11.1) and the mean age at examination (AAE) was 66.7 years (SD = 12.4). The mean AAO of patients with iPD was 61.2 years (SD = 10.2) and the mean AAE was 65.2 years (SD = 9.6) (Table S1).

In order to explore the relatedness of patients from the AMP-PD, Fox Insight, and Tunisian cohorts, we performed an identity-by-descent (IBD) analysis with PLINK and a PI_HAT > 0.1875, indicating at least second-degree relatives.

AMP-PD contains whole-genome sequencing (WGS) data from four harmonized cohorts[27] (Supplementary Text S1). The majority of the patients of the AMP-PD cohort were of European descent (~95%) and the remaining ~5% were of Arab, African American, Hispanic, Asian, Native Hawaiian, or Alaskan descent, with self-reported ethnicity/race. The group of patients with genetic ancestry different from European/White in the AMP-PD cohort is too small and therefore we excluded them from our analysis. The Fox Insight dataset is a cohort within The Michael J. Fox Foundation (MJFF) and the genetic data (array-based genotyping) were provided by 23andMe, as previously described.[28] All patients with PD included from the Fox Insight cohort in this study were of self-reported European ancestry. Lastly, we included a cohort recruited from the Tunisian Arab-Berber population with array-based genotyping data, as previously described.[19]

In the Fox Insight and Tunisian cohorts, lifestyle and environmental information were assessed with the PD Risk Factor Questionnaire (PD-RFQ) for tobacco use, caffeine consumption, and pesticide exposure[29] (Supplementary Text S2 and Table S2). However, the AMP-PD dataset did not assess environment and lifestyle data with the PD-RFQ. Therefore, available environmental/lifestyle data in the AMP-PD cohort were not used to maintain consistency and we used the more detailed data of the Fox Insight and Tunisian cohorts.

Mitochondrial Polygenic Score Analysis

The genetic datasets from AMP-PD, Fox Insight, and the Tunisian cohort were stored in a binary PLINK format.[30] The same quality control filtering steps were applied to all three datasets (minor allele frequency >0.01, missingness per sample <0.02, missingness per SNP [single-nucleotide polymorphism] <0.05 and Hardy-Weinberg equilibrium $>1 \times 10^{-50}$) using PLINK v1.9. The Fox Insight dataset was imputed using the Michigan Imputation Server[31] in combination with the Haplotype Reference Consortium v1.1 reference panel.[32] As the Tunisian dataset is of North African background, we performed the imputation on our in-house computer cluster, using SHAPEIT[33] and IMPUTE2[34] in combination with the 1000 Genomes Project Phase 3 reference panel.[35] Genotyping data for AMP-PD was obtained from WGS.

The MGS was calculated using the PLINK score function. Based on the larger secondary mitochondrial function gene list published by Billingsley et al.[9] we calculated the MGS (for a detailed description see Supplementary Text S3). In order to harmonize the MGS between cohorts, we only used SNPs that were consistently present across all three datasets. Subsequently, we included ~15,000 SNPs for the MGS used in this

study (MGS SNPs and corresponding weights can be obtained from https://github.com/LuethTheresa/MitochondrialPolygenicScoreAndAgeAtOnset). The obtained MGS was standardized to a mean of 0 and a standard deviation (SD) of 1.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism v9.4.0 and R v4.0.3.[36,37] The analysis of the association between MGS and AAO was interpreted for significance in the complete study group, based on the presence of the 'a priori' hypothesis on the association between MGS and AAO in *LRRK2*-PD. The association with AAO was tested for significance in *LRRK2*-PD patients and the significance level was set at $P = 0.05$. All other analyses in this study were exploratory and P-values were not corrected for multiple testing.

First, we aimed to investigate the association between AAO and MGS in *LRRK2*-PD and iPD (Fig. S2). Therefore we used correlation analyses and multiple regression analyses. In our linear regression models, we used AAO as a dependent variable and the standardized MGS as an independent variable. We included sex and the first five principal components (PC1-5) from the principal component analysis (PCA) in the regression models to adjust for potential confounders (Supplementary Text S4). Analogous to the MGS, PC1-5 were standardized to a mean of 0 and a standard deviation (SD) of 1.

Next, we aimed to investigate interactions between lifestyle, environment (ie, tobacco use, caffeine consumption and pesticide exposure), and MGS on AAO. In order to do this we utilized multiple linear regression models as well. Lifestyle and environmental exposure were set as dichotomous independent variables (yes/no) in our linear regression models. For the investigation of lifestyle and exposure, the Fox Insight and Tunisian cohorts were included, as for AMP-PD the PD-RFQ was not available. All patients from the Fox Insight dataset were of European/White ancestry and all patients from the Tunisian dataset were of Tunisian/Arab ancestry. To visualize potential gene–lifestyle or gene–environment interactions, we performed Kaplan–Meier analyses. To assess the difference in AAO of patients with high or low MGS, a pairwise comparison was performed using the log-rank test. For the stratification, we defined 'high MGS' as higher or equal to the median MGS and 'low MGS' was defined as lower than the median MGS. Kaplan–Meier analyses were performed for all participants (unstratified for any lifestyle or environmental factor) and stratified by a specific factor (eg, consumed caffeinated soda yes or no).

Results

Association between MGS and AAO in PD

We calculated MGS, based on the larger (>1300 gene names) secondary mitochondrial function gene list published by Billingsley et al.,[9] using LDpred2 and our in-house cohorts. We successfully replicated the finding that a higher MGS is associated with a higher risk for PD (odds ratio [OR] = 1.25 per 1 SD of the MGS; Supplementary Text S3). Additionally, we observed that patients with iPD had a higher MGS compared with healthy controls in the AMP-PD cohort ($P = 1.1 \times 10^{-8}$; Fig. 1A).

We analyzed the association between the MGS and the AAO in patients with *LRRK2*-PD. To visualize the relationship between the MGS and AAO we performed a correlation analysis (Fig. 1B). The MGS was inversely correlated with the AAO ($r = -0.19$, $P = 4.2 \times 10^{-5}$, N = 477). The higher the MGS, and thereby the higher the cumulative burden of variants associated with mitochondrial dysfunction, the earlier the AAO in *LRRK2*-PD. We then investigated this relationship using multivariable linear regression models and confirmed the significant negative association ($\beta = -1.40$, 95% CI = -2.77 to -0.02, $P = 0.047$; Table 1). Thus, if the MGS is increased by 1 SD the AAO is approximately one and a half years earlier in *LRRK2*-PD. As the AAO is earlier in females compared with males[38] and the AAO and MGS vary between the three cohorts and ethnicities, we included sex and the first five principal components as covariates in the regression models.

Interestingly, when stratifying the data for the two ethnicities/races (ie, European/White or Tunisian/Arab) to analyze the MGS and AAO relationship, a negative correlation of the same magnitude as before was observed for *LRRK2*-PD patients of European descent ($r = -0.12$, $P = 0.049$; Fig. S1A). However, when looking at the patients of Tunisian Arab-Berber descent, the negative correlation is visibly not as pronounced ($r = -0.05$, $P = 0.449$). Subsequently, we utilized the linear regression model to investigate the association between MGS and AAO in *LRRK2*-PD patients stratified by ethnicities/races as well. The association was in the same direction as in the whole study group in European ($\beta = -0.42$, 95% CI = -2.64 to 1.81, $P = 0.715$) or Tunisian ($\beta = -1.06$, 95% CI = -2.67 to 0.55, $P = 0.197$) *LRRK2*-PD patients, but with a smaller effect size and a $P > 0.05$. The diminished association could be due to reduced sample sizes in the subgroups.

We observed a visibly weaker negative correlation between MGS and AAO in iPD ($r = -0.05$, $P = 1.2 \times 10^{-5}$, N = 9114; Fig. 1C) and we could not validate the association using the regression model in the whole study group.

When stratifying the data of the iPD patients for the two ethnicities/races, again the negative correlation between MGS and AAO of patients with European/White ancestry was of the same magnitude as before ($r = -0.04$, $P = 5.2 \times 10^{-4}$, Fig. S1B). However, there was a trend for a positive correlation in patients with Tunisian/Arab ancestry ($r = 0.10$, $P = 0.120$). We also used the linear regression model to analyze the association between MGS and AAO in iPD patients stratified by ethnicities/races. The negative association was present in patients with European/White ancestry ($\beta = -0.38$, 95% CI = -0.73 to -0.02, $P = 0.038$) but not in patients with Tunisian/Arab ancestry ($\beta = 1.18$, 95% CI = -0.74 to 3.10, $P = 0.231$).

Effect of Lifestyle Factors and Environmental Exposure on AAO

We focused our analysis on known protective (smoking and caffeine consumption) and risk factors
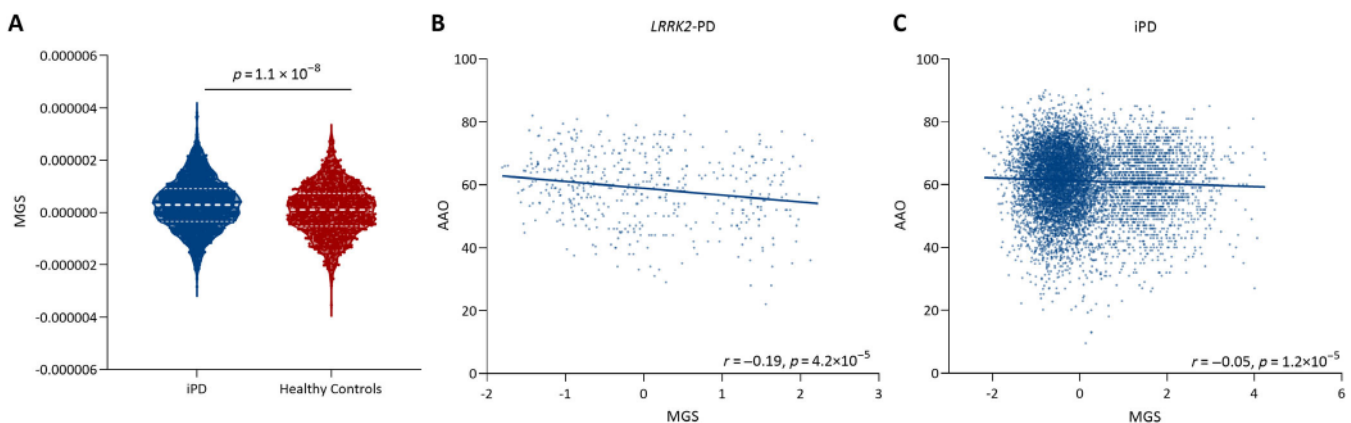
FIG. 1. Relationship between Parkinson's affection status, age at onset (AAO), and mitochondrial polygenic score (MGS). (A) The violin plot shows the difference in the MGS between patients with idiopathic PD (iPD) and healthy controls. The dashed lines indicate the median and interquartile range. P = Mann–Whitney U test exploratory P-value. The correlation plots show the association between MGS and AAO in patients with Parkinson's disease carrying the LRRK2 p.Gly2019Ser mutation (*LRRK2*-PD) (B) or patients with iPD (C). r = Spearman's rank correlation coefficient, P = Spearman's exploratory P-value. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 *Association between the mitochondrial polygenic score and the age at onset in patients with LRRK2-PD and idiopathic Parkinson's disease*

Parameter	LRRK2-PD			iPD		
	Estimate	95% CI	*P*-value	Estimate	95% CI	*P*-value
Complete cohort (*LRRK2*-PD: N = 473, iPD: N = 8986)[1]						
MGS	**−1.40**	**−2.77, −0.02**	**0.047★**	**−0.22**	**−0.57, 0.14**	**0.231**
Sex: male	1.80	−0.15, 3.75	0.071	0.94	0.51, 1.36	1.5×10^{-5}★
PC1	−0.72	−2.30, 0.85	0.369	−0.44	−0.79, −0.08	0.015★
PC2	−0.35	−1.53, 0.82	0.556	0.21	0.001, 0.42	0.049★
PC3	0.75	−0.46, 1.97	0.225	0.52	0.31, 0.73	1.2×10^{-6}★
PC4	−0.48	−1.47, 0.50	0.400	0.00	−0.21, 0.21	0.975
PC5	0.38	−0.61, 1.37	0.455	0.19	−0.02, 0.40	0.080
Patients with European/White ancestry (*LRRK2*-PD: N = 269, iPD: N = 8753)[1]						
MGS	**−0.42**	**−2.64, 1.81**	**0.715**	**−0.38**	**−0.73, −0.02**	**0.038★**
Sex: male	0.76	−1.64, 3.15	0.536	0.94	0.51, 1.36	1.4×10^{-5}★
PC1	−0.62	−2.97, 1.73	0.605	−0.02	−0.38, 0.34	0.914
PC2	0.11	−1.42, 1.64	0.890	0.32	0.11, 0.53	0.003★
PC3	−0.12	−1.39, 1.16	0.855	0.00	−0.22, 0.21	0.969
PC4	−1.14	−2.36, 0.08	0.069	−0.04	−0.25, 0.17	0.703
PC5	−0.33	−1.58, 0.92	0.606	0.21	0.002, 0.41	0.052
Patients with Tunisian/Arab ancestry (*LRRK2*-PD: N = 204, iPD: N = 233)[1]						
MGS	**−1.06**	**−2.67, 0.55**	**0.197**	**1.18**	**−0.74, 3.10**	**0.231**
Sex: male	3.36	0.07, 6.64	0.046★	−0.89	−4.73, 2.96	0.652
PC1	0.07	−1.57, 1.71	0.934	−0.25	−2.16, 1.65	0.795
PC2	−0.68	−2.33, 0.98	0.424	−0.53	−2.45, 1.39	0.587
PC3	−0.69	−2.33, 0.95	0.411	1.32	−0.62, 3.26	0.183
PC4	0.51	−1.12, 2.14	0.541	1.20	−0.72, 3.11	0.222
PC5	0.81	−0.84, 2.45	0.336	−1.24	−3.17, 0.68	0.206

Note: Baseline categories: Sex = female.

★*P* < 0.05.

[1]glm(formula = AAO ∼ MGS + Sex + PC1 + PC2 + PC3 + PC4 + PC5, family = Gaussian). Bold type denotes independent variable of interest and non-bold denotes included covariates.

Abbreviations: AAO, age at onset; CI, confidence interval; iPD, idiopathic Parkinson's disease; *LRRK2*-PD, patients with Parkinson's disease that carry the LRRK2 p.Gly2019Ser variant; MGS, mitochondrial polygenic score; PC, principal component.

(pesticide exposure) in PD using regression models including sex and study cohort as a covariate.

Tobacco Use

We observed no association of smoking with AAO in *LRRK2*-PD ($\beta = 3.02$, 95% CI = −1.03 to 7.07, $P = 0.146$). In iPD, smoking was associated with later AAO ($\beta = 1.50$, 95% CI = 0.60–2.40, $P = 0.001$). Thus, tobacco users had a one and a half years later AAO compared with non-users.

Caffeine Consumption

To thoroughly assess the relationship between caffeine and AAO in PD, we analyzed coffee, black tea, green tea, and caffeinated soda consumption. In *LRRK2*-PD, the only caffeinated beverage associated with later AAO was black tea ($\beta = 5.62$, 95% CI = 1.66–9.58, $P = 0.006$), meaning that patients that consumed black tea had a 5 years later AAO compared with *LRRK2*-PD patients that did not consume black tea.

In patients with iPD, coffee consumption was associated with later AAO ($\beta = 2.20$, 95% CI = 1.10–3.29, $P = 8.8 \times 10^{-5}$), meaning that patients that consumed

coffee had a 2 years later AAO compared with patients that did not. Green tea, however, was not associated with AAO in *LRRK2*-PD or iPD. Caffeinated soda was not associated with AAO in *LRRK2*-PD ($\beta = -3.83$, 95% CI = -7.86 to 0.19, $P = 0.064$) but with earlier AAO in iPD ($\beta = -2.25$, 95% CI = -3.33 to -1.17, $P = 4.6 \times 10^{-5}$). Thus, patients with iPD that consumed caffeinated soda had a 2 years earlier AAO compared with patients that did not.

Pesticide Exposure

We did not observe an association between pesticide exposure and AAO in *LRRK2*-PD or iPD.

Interactions Between Lifestyle, Environment, and MGS on AAO

Next, we explored the interaction of lifestyle/environment and MGS on AAO in a linear regression model (Table 2).

MGS and Tobacco Use

We detected no interaction between MGS and tobacco use in *LRRK2*-PD. However, there was an interaction between MGS and tobacco use in patients with iPD ($\beta = 1.32$, 95% CI = 0.32–2.32, $P = 0.010$, Table 2). In other words, our results suggest that the association between the MGS and AAO is dependent upon tobacco use. To further investigate this interaction we analyzed the association between MGS and AAO on tobacco users and non-users separately. There was no association in tobacco users. Conversely, we saw a negative association in tobacco non-users ($\beta = -0.79$, 95% CI = -1.41 to -0.17, $P = 0.013$; Table S3). Thus, an increase of 1 SD in the MGS is associated with approximately 1 year earlier AAO in patients with iPD that did not use tobacco.

To visualize this interaction, we performed Kaplan–Meier analyses, which showed an earlier AAO in iPD patients with a high MGS who had not used tobacco (Fig. 2B). The median AAO of patients that did not use tobacco and had a high MGS was 61.7 years compared with iPD patients with a low MGS that did not use tobacco at 62.3 years ($P = 0.115$).

Thus, tobacco non-users with a high MGS had a ~6 months earlier median AAO than iPD patients with a low MGS. Interestingly, the AAO was later in iPD patients with a high MGS that did use tobacco (high MGS: median AAO = 64.4; low MGS: median AAO = 63.7). In comparison, the median AAO was ~7 months earlier in iPD patients with a high MGS, unstratified for any lifestyle factors.

MGS and Caffeine Consumption

Next, we investigated the interaction between caffeine consumption, including coffee, black tea, green tea, and caffeinated soda.

There was a trend for an interaction between MGS and coffee consumption with the AAO of *LRRK2*-PD patients ($\beta = -4.06$, 95% CI = -8.26 to 0.13, $P = 0.060$). In addition, we detected a more pronounced interaction between MGS and caffeinated soda consumption ($\beta = -5.65$, 95% CI = -9.37 to -1.94, $P = 0.003$) in *LRRK2*-PD. To further investigate this interaction we analyzed the association between MGS and AAO in caffeinated soda drinkers and non-drinkers separately. There was a trend for a negative association in patients with *LRRK2*-PD that consumed caffeinated soda ($\beta = -2.30$, 95% CI = -7.99 to 3.39, $P = 0.432$; Table S4). Conversely, we saw a positive association between the MGS and AAO in patients that did not consume caffeinated soda ($\beta = 3.86$, 95% CI = 0.36–7.35, $P = 0.034$). To also visualize this potential interaction, we performed Kaplan–Meier analyses, which showed an earlier AAO in patients with a high MGS who consumed caffeinated soda (Fig. 2A) in *LRRK2*-PD. The median AAO of patients that consumed caffeinated soda and had a high MGS was 50.0 years compared with *LRRK2*-PD patients with a low MGS that consumed caffeinated soda at 61.5 years ($P = 0.005$). Thus, caffeinated soda consumers with a high MGS had a ~11 years earlier median AAO than *LRRK2*-PD patients with a low MGS. In comparison, the median AAO was only ~4 years earlier in *LRRK2*-PD patients, unstratified for any lifestyle factor. Analogously to the MGS and tobacco use interaction in iPD, the AAO was later in *LRRK2*-PD patients with a high MGS that did not consume caffeinated soda (high MGS: median AAO = 61.0; low MGS: median AAO = 54.85).

There was no interaction between the MGS, coffee, or caffeinated soda consumption in iPD. Furthermore, there was also no interaction between MGS, black tea, and green tea in either *LRRK2*-PD or iPD (Table S5).

MGS and Pesticide Exposure

Lastly, we investigated the interaction between MGS and pesticide exposure in a work and a non-work setting. However, we did not detect an interaction between MGS and pesticide exposure in *LRRK2*-PD or iPD (Table S5).

Discussion

Gene–environment interactions are relevant as onset modifiers of *LRRK2*-PD and iPD. The main strength of this study is the size of the study cohort consisting of three large cohorts. In addition, we utilized the

TABLE 2 *Interaction between the mitochondrial polygenic score, tobacco use and caffeine consumption, and the age at onset in patients with* LRRK2-*PD and idiopathic Parkinson's disease*

| Parameter	LRRK2-PD[1]			iPD[1]		
	Estimate	**95% CI**	**P-value**	**Estimate**	**95% CI**	**P-value**
Smoking (N = 144)				Smoking (N = 1759)		
Lifestyle factor	1.66	−2.85, 6.17	0.472	1.59	0.69, 2.49	0.001★
MGS	0.54	−2.70, 3.79	0.744	−0.78	−1.41, −0.14	0.017★
Sex: male	1.36	−2.87, 5.59	0.530	1.23	0.35, 2.12	0.006★
PC1	−4.59	−9.34, 0.16	0.061	−0.92	−1.50, −0.33	0.002★
PC2	1.76	−0.46, 3.97	0.123	0.01	−0.43, 0.45	0.965
PC3	0.53	−3.16, 4.23	0.778	0.88	0.38, 1.39	0.001★
PC4	0.77	−1.12, 2.66	0.427	0.11	−0.33, 0.55	0.629
PC5	−0.13	−1.87, 1.60	0.879	−0.09	−0.52, 0.35	0.688
MGS: lifestyle factor	**2.19**	**−1.49, 5.88**	**0.246**	**1.32**	**0.32, 2.32**	**0.010★**
Coffee consumption (N = 136)				Coffee consumption (N = 1676)		
Lifestyle factor	4.77	−0.57, 10.11	0.083	2.20	1.10, 3.29	9.1×10^{-5}★
MGS	4.09	0.36, 7.83	0.034★	−0.13	−1.17, 0.91	0.808
Sex: male	3.88	0.07, 7.70	0.048★	0.70	−0.22, 1.61	0.135
PC1	−3.54	−8.36, 1.29	0.153	−1.02	−1.62, −0.41	0.001★
PC2	1.22	−1.10, 3.54	0.304	−0.11	−0.57, 0.34	0.626
PC3	0.03	−3.81, 3.87	0.988	0.76	0.24, 1.28	0.004★
PC4	1.39	−0.59, 3.36	0.171	0.07	−0.39, 0.52	0.776
PC5	0.18	−1.63, 1.99	0.845	−0.08	−0.52, 0.37	0.736
MGS: lifestyle factor	**−4.06**	**−8.26, 0.13**	**0.060**	**−0.01**	**−1.19, 1.16**	**0.981**
Caffeinated soda consumption (N = 133)				Caffeinated soda consumption (N = 1406)		
Lifestyle factor	0.27	−4.48, 5.01	0.913	−2.24	−3.33, −1.16	5.0×10^{-5}★
MGS	3.69	0.54, 6.84	0.023★	−0.31	−1.24, 0.61	0.508
Sex: male	3.50	−0.20, 7.20	0.066	0.48	−0.51, 1.48	0.342
PC1	−3.63	−8.30, 1.04	0.131	−1.07	−1.72, −0.42	0.001★
PC2	0.80	−1.50, 3.10	0.498	−0.07	−0.57, 0.42	0.769
PC3	0.52	−3.23, 4.27	0.786	0.75	0.19, 1.32	0.009★
PC4	0.95	−0.94, 2.83	0.329	0.24	−0.25, 0.74	0.339
PC5	0.27	−1.49, 2.03	0.761	−0.27	−0.75, 0.22	0.283
MGS: lifestyle factor	**−5.65**	**−9.37, −1.94**	**0.003★**	**0.08**	**−1.04, 1.21**	**0.883**

★*P* < 0.05.

[1]glm(formula = AAO ~ MGS × Lifestyle factor + Sex + PC1 + PC2 + PC3 + PC4 + PC5, family = Gaussian).

Note: Baseline categories: Sex = female. Bold type denotes independent variable of interest and non-bold denotes included covariates.

Abbreviations: AAO, age at onset; CI, confidence interval; iPD, idiopathic Parkinson's disease; *LRRK2*-PD, patients with Parkinson's disease that carry the LRRK2 p.-Gly2019Ser variant; MGS, mitochondrial polygenic score; PC, principal component.

thorough overlap of genetic, lifestyle, and environmental data of two cohorts to comprehensively investigate the relationship between MGS and AAO in PD. We see a robust relationship between the MGS and AAO in *LRRK2*-PD even after adjusting for potentially confounding covariates (ie, sex, cohort, or ethnicity represented by principal components 1–5). To our knowledge, we demonstrate a novel association between MGS and earlier AAO in *LRRK2*-PD. Furthermore, the diverse ethnic background of the patients in this study
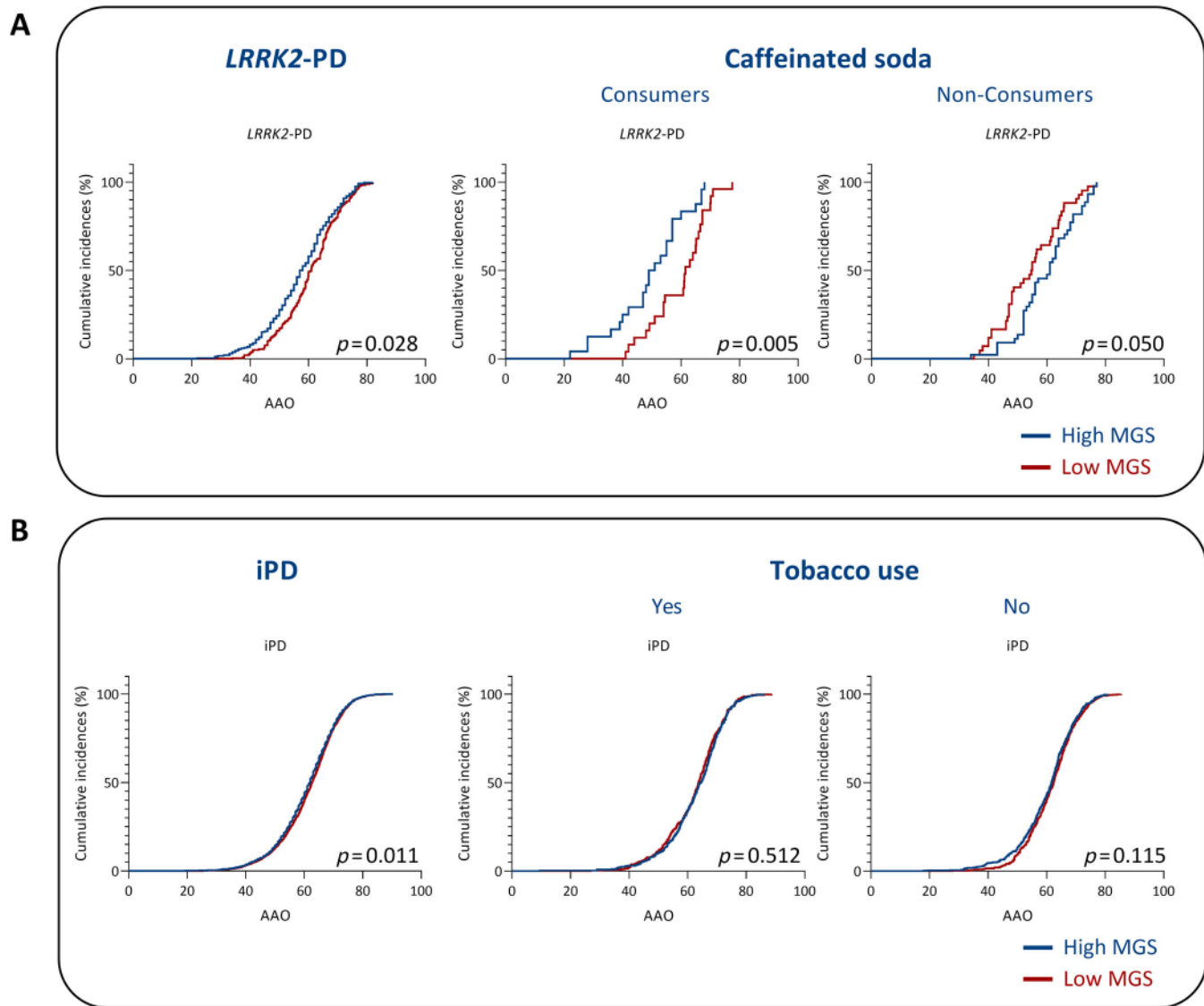
FIG. 2. Relationship between age at onset (AAO), mitochondrial polygenic score (MGS), and caffeine consumption or tobacco use. (A) Kaplan–Meier plots showing the difference in AAO in patients with Parkinson's disease carrying the LRRK2 p.Gly2019Ser mutation (*LRRK2*-PD) and high MGS or low MGS. The patients were plotted unstratified and stratified by caffeinated soda consumption. (B) Kaplan–Meier plots showing the difference in AAO of patients with idiopathic PD (iPD) and high MGS or low MGS. The patients were plotted unstratified and stratified by tobacco use. P = log-rank test exploratory *P*-value. [Color figure can be viewed at wileyonlinelibrary.com]

shows population-specific effects of the MGS. Though we see an overall association between the MGS and AAO, when separating the cohorts, the association was found to be more pronounced in the European cohorts and visibly weaker in the Tunisian/Arab cohort in the correlation analysis. It is well known that population- or ethnic-specific background is a key factor in polygenic scores and it is important for future studies to be inclusive of patients from diverse backgrounds.[39-41] To illustrate the importance of the study population in genetic scores, we performed a PCA using common SNPs. Additionally, we included the publicly available 1000 Genomes Project dataset as a validation for the clustering of the populations. In the PCA, the AMP-PD

cohort clustered together with the Fox Insight cohort and the European samples of the 1000 Genomes Project, as both consist of patients of mainly European/White ancestry (Fig. S3 and Supplementary Text S4). In the study that constructed the MGS that we used, the dataset consisted of participants of European ancestry.[9] However, the frequency of *LRRK2* p.Gly2019Ser is higher in Ashkenazi Jewish and Tunisian Arab-Berber populations.[26] This highlights the importance of deriving an MGS from these two founder populations, as it would be pertinent to further understanding the MGS effect. Combined international efforts will be required to generate, evaluate, and estimate an MGS in diverse populations. The lack of diverse cohorts in large-scale
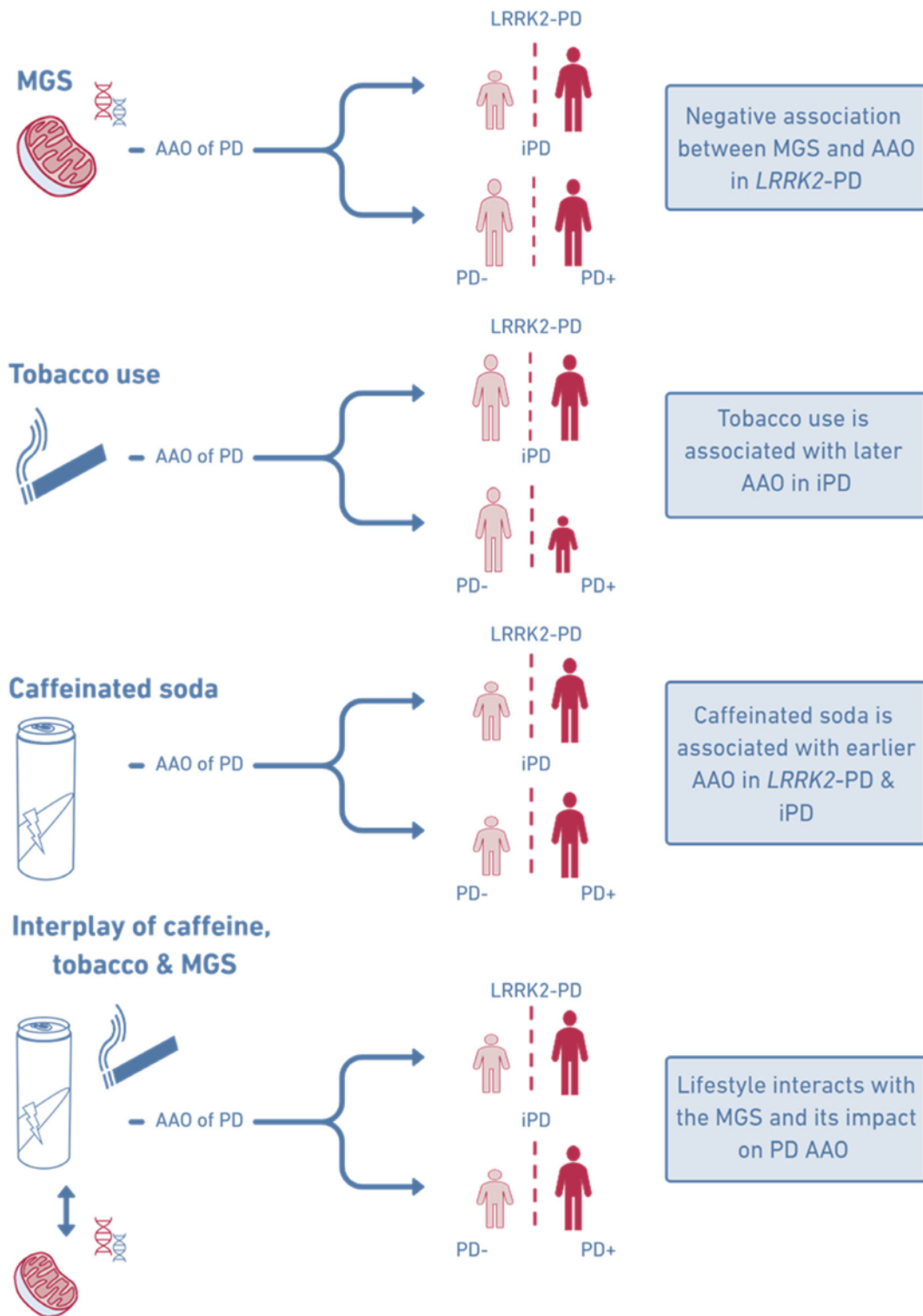
FIG. 3. Summary of study results. Caffeine consumption can be associated with later or earlier age at onset (AAO) in Parkinson's disease (PD), depending on the beverage. Furthermore, there is evidence for gene and lifestyle interactions, as in caffeine consumers and patients that did not use tobacco, in which the effect of the mitochondrial polygenic score (MGS) on AAO in PD is more pronounced. Abbreviation: *LRRK2*-PD, patients with Parkinson's disease carrying the LRRK2 p.Gly2019Ser mutation. [Color figure can be viewed at wileyonlinelibrary.com]

genetic studies is a well-known problem,[42,43] but more diversity is essential to overcome such limitations of polygenic scores.

Limitations of our study include potential bias that comes from different data reported in the three cohorts. In terms of genetics, genotyping data were either

obtained from arrays (Fox Insight and Tunisian cohort) or WGS (AMP-PD) that could contribute to batch effects. The AAO was earlier in the Tunisian cohort compared with the AMP-PD and Fox Insight cohorts. We observed that the percentage of early-onset PD patients (EOPD; AAO < 50 years) is higher than expected[44] within the Tunisian cohort, with ∼28% in the *LRRK2*-PD group and ∼ 32% in the iPD group. This difference could be a result of the different genetic and cultural backgrounds of the patients. However, we included the first five principal components as covariates in our analysis to adjust and counteract potential biases. Additionally, we compared the association between MGS and AAO in EOPD and late-onset PD patients (LOPD). The association between MGS and AAO observed for patients with *LRRK2*-PD was in the same direction as in patients with EOPD ($\beta = -1.13$, 95% CI = −2.61 to 0.35, $P = 0.136$; Table S6) and LOPD ($\beta = -0.86$, 95% CI = −1.99, 0.27, $P = 0.138$). Nevertheless, potential biases and confounding factors may impact the results of studies on age at onset, such as cultural differences, medical health availability, and family history of PD.

Relatedness remains problematic for the Tunisian cohort. After investigating IBD, the Fox Insight cohort had no related patients but there were three *LRRK2*-PD patients who were related within the AMP-PD cohort and eight *LRRK2*-PD patients in the Tunisian cohort. One in three marriages are consanguineous within the Tunisian population[19] and patients included in this study were partly recruited from families. Still, the minority of patients in our study (n = 11) are closely related and account for a minor fraction of the sample size.

The main environmental/lifestyle questionnaire used in our study is the validated PD-RFQ. However, the PD-RFQ was only available from the Fox Insight and Tunisian cohort. To harmonize the data as much as possible, AMP-PD was not included in our environment/lifestyle analyses. The PD-RFQ, though validated, also has its own caveats. For example, pesticide exposure in a non-work setting includes any exposure to chemicals utilized to kill insects, other pests, plants, weeds, mold, or mildew used in the house, garden, or on pets, which leads to an inflation of individual exposure. Diverse cultural preferences also exist that may not be captured by the lifestyle questionnaires: one example is the main source of caffeine intake (ie, coffee, tea, or soda), which varies significantly in different countries.[45] To overcome this caveat, we stratified our data for ethnicity/race and study cohort and performed interaction analyses only on iPD patients of the Fox Insight cohort that were all of European/White ancestry. Still, there was a trend for an interaction between MGS and tobacco use in predicting AAO ($\beta = 0.86$, 95% CI = −0.02 to 1.75, $P = 0.056$; Table S7).

A previous positive association between an estimated MGS and AAO was detected in addition to an association with PD risk.[9,46] However, herein we report a negative association with onset. It is important to note that we observe a negative association with AAO in patients with *LRRK2*-PD ($r = -0.19$, $P = 4.2 \times 10^{-5}$) and there was the same trend in iPD ($r = -0.05$, $P = 1.2 \times 10^{-5}$) with a small effect size. There may be three underlying reasons: (1) this study is based on a newly built MGS with the same mitochondrial-related gene list but alternative resulting variants and weights; (2) different cohorts for the idiopathic PD analyses were utilized (ie, Harvard Biomarker Study, McGill Parkinson's, Oslo Parkinson's Disease Study, Parkinson's Disease Biomarker's Program, Parkinson's Progression Markers Initiative, Spanish Parkinson's [IPDGC] part2, and German GWAS); and (3) lastly, the effect size was larger in *LRRK2*-PD, a more homogeneous cohort compared with iPD. The causes of the disease in iPD patients can be much more diverse and this heterogeneity may overshadow the subtle effect of the MGS, which may only be valid for certain subtypes of iPD. Another potential explanation is that mitochondrial biological implications are strongly related to disease onset in *LRRK2*-PD but not in iPD. Mitochondrial abnormalities are involved in the pathogenesis of *LRRK2*-PD, such as reduced NADH dehydrogenase activity, increased mitochondrial mass, mtDNA copy number, and nuclear factor erythroid 2-related factor 2 (Nrf2) expression.[11] Thus, an additional mitochondrial burden, reflected in a higher MGS, could lead to an earlier AAO in patients with *LRRK2*-PD.

Mitochondrial function can be affected by tobacco use, caffeine consumption, or pesticide exposure.[10,21-23] We, among others, have reported that caffeinated soda intake was associated with earlier AAO[5] or increased PD risk.[47] Hence, caffeinated soda appears to be different from other caffeinated beverages and potentially caffeine-independent mechanisms are driving these effects. For patients with *LRRK2*-PD, there was an interaction between MGS and caffeinated soda consumption. The median AAO was ∼11 years earlier in patients with a high MGS that consumed caffeinated soda. As the median AAO of *LRRK2*-PD patients unstratified for any lifestyle factor was only ∼4 years earlier, our data support a gene–lifestyle interaction between caffeine intake and MGS. Caffeine consumption is reported as a protective factor in PD, except for caffeinated soda, as described earlier. However, in rats, there is evidence that treatment with caffeine induces mitochondrial dysfunction in the neonatal brain.[48]

In addition to caffeine, tobacco use interacted with the MGS in patients with iPD exclusively. In contrast to caffeinated soda, tobacco is a protective factor in PD.[1,5,14] This could explain why we only observe an earlier AAO in iPD patients with a high MGS that did

not use tobacco, as the absence of this protective factor may enhance the vulnerability for a higher MGS.

Our results underline the importance of including lifestyle and environment when investigating genetic associations with AAO or disease risk. Gene–lifestyle or gene–environment interactions could significantly influence the association with these traits. A recent study demonstrated that genome-wide association study (GWAS) analyses could be affected by gene–environment correlations across geographic regions. The genetic correlations with socioeconomic status-related traits were significantly reduced when controlling for geographic regions.[49] Likewise, our study shows the differences between Tunisian Arab-Berbers and European/White ancestry though a more refined investigation is warranted.

In conclusion, there was an association between the MGS and earlier AAO in patients with *LRRK2*-PD and iPD, but with a visibly smaller effect size in the latter. Furthermore, we detected gene–lifestyle interactions in *LRRK2*-PD and iPD. Thus, lifestyle and environmental factors may interact with the MGS and affect its impact on the AAO in PD (Fig. 3). Our results highlight the importance of functional studies investigating the underlying molecular mechanisms leading to the interaction between MGS, caffeine consumption, and tobacco use. ●

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study. Data used in the preparation of this manuscript were obtained from the Fox Insight database (https://foxinsight-info.michaeljfox.org/insight/explore/insight.jsp) on 18/10/2020. For up-to-date information on the study, visit https://foxinsight-info.michaeljfox.org/insight/explore/insight.jsp. Data used in the preparation of this article were obtained from the Accelerating Medicine Partnership® (AMP®) Parkinson's Disease (AMP PD) Knowledge Platform. For up-to-date information on the study, visit https://www.amp-pd.org. The workflow and generated MGS are available: https://github.com/LuethTheresa/MitochondrialPolygenicScoreAndAgeAtOnset.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.