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Inducible nitric oxide synthase gene methylation and parkinsonism in manganese-exposed welders

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Abstract

Introduction—Neurologist-assessed parkinsonism signs are prevalent among workers exposed to manganese (Mn)-containing welding fume. Neuroinflammation may possibly play a role. Inducible nitric oxide synthase, coded by *NOS2*, is involved in inflammation, and particulate exposure increases the gene's expression through methylation of CpG sites in the 5' region.

Methods—We assessed DNA methylation at three CpG sites in the *NOS2* exon 1 from blood from 201 welders. All were non-Hispanic Caucasian men 25–65 years old who were examined by a neurologist specializing in movement disorders. We categorized the workers according to their Unified Parkinson Disease Rating Scale motor subsection 3 (UPDRS3) scores as parkinsonism

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Conflict of interest

The authors declare that they have no conflict of interest.

cases (UPDRS3 = 15; n = 49), controls (UPDRS3 < 6; n = 103), or intermediate (UPDRS3 = 6 to <15; n = 49).

Results—While accounting for age, examiner and experimental plate, parkinsonism cases had lower mean *NOS2* methylation than controls (*p*-value for trend = 0.04), specifically at CpG site 8329 located in an exonic splicing enhancer of *NOS2* (*p*-value for trend = 0.07). These associations were not observed for the intermediate UPDRS3 group (both *p*-value for trend 0.59).

Conclusions—Inflammation mediated by inducible nitric oxide synthase may possibly contribute to the association between welding fume and parkinsonism, but requires verification in a longitudinal study.

Keywords

DNA methylation; inhalation exposure; manganese; nitric oxide synthase type II; occupational exposure; parkinsonian disorders; welding

1. Introduction

Historically, occupational exposure to manganese (Mn) has been associated with parkinsonism among highly exposed workers, such as Mn miners and ore crushers. Modern occupational Mn exposures are substantially lower, but welders are frequently exposed to Mn above current occupational standards [1]. While records-based studies provide little evidence that contemporary welders have an increased risk of Parkinson disease [2], studies that investigate parkinsonism more generally and employ neurologists in the assessment of this broader outcome demonstrate that signs of parkinsonism are prevalent among workers exposed to welding fume [3, 4] and other occupational sources of Mn [5]. Among welders, characteristic signs of this movement disorder are bradykinesia and rigidity [3].

Welders demonstrate increased signal on T1 weighted MRI in the basal ganglia [6], a finding generally believed to indicate Mn accumulation. Basal ganglia dysfunction likely contributes to the association between parkinsonism and Mn-containing welding fume [6–8]. Although the exact pathophysiologic mechanism of Mn neurotoxicity has not been fully elucidated, inflammation may play a role. In contemporary Mn mine workers, Mn exposure is associated with microglia cell density in the globus pallidus [8], the portion of the basal ganglia traditionally considered a target of Mn neurotoxicity. Glial cell activation enhances the uptake of Mn into neurons [9], which in turn may contribute to neuronal death [10]. In addition, activation of glial cells, particularly microglia, might damage adjacent neurons through increased expression of pro-inflammatory mediators [11, 12]. These include nitric oxide, which is produced by nitric oxide synthase [13]. There are three such enzymes in humans, including inducible nitric oxide synthase (iNOS). Inhaled welding fumes, at concentrations consistent with potential workplace exposures, increase levels of messenger RNA for microglial markers and iNOS in the striatum and midbrain of rats [14]. Moreover, mice without the gene coding for iNOS are less susceptible to the neurotoxic effects of Mn [15].

The gene coding for iNOS in humans, *NOS2*, is primarily regulated at the transcriptional level, at least in part via methylation of CpG dinucleotides [16]. Specifically, hypermethylation of CpG sites in the 5' promoter region of the gene decreases its expression [16, 17] and is associated with lower breath nitric oxide [18] suggesting lower iNOS activity. Conversely, hypomethylation of *NOS2* may be associated with greater iNOS activity. Most studies indicate that exposure to fine and coarse particulate, including metal-rich particulate, is associated with lower *NOS2* methylation in or near the gene's promoter region [19–23]. Therefore, in a sample of workers from a well-characterized cohort of welders with a relatively high prevalence of parkinsonism [3], we assessed the association between *NOS2* methylation and parkinsonism. Our hypothesis was that compared to welders with normal neurological exams, parkinsonian welders would have lower *NOS2* methylation (Figure 1).

2. Materials and Methods

2.1 Participants and assessment of parkinsonism

Prior to study conduct, we obtained Human Subjects approval from Washington University (St. Louis, MO) and the University of Washington (Seattle, WA), and written informed consent from each participant. All participants were part of an on-going study in the U.S. Midwest examining the association between welding and parkinsonism [3]. Recruitment has been detailed previously [24]. Briefly, we used a union membership list to contact employees and retirees from three welding worksites: two shipyards and one heavy equipment fabrication shop. One of two neurologists specializing in movement disorders (B.A.R. and S.R.C.) examined each participant using a standardized neurological exam that included the Unified Parkinson Disease Rating Scale motor subsection 3 (UPDRS3) [3, 25]. At the time of the exam, we asked each participant to complete a structured questionnaire [24] on demographics and work history, and to provide a blood sample. We stored all blood samples at -80°C .

At the time of laboratory analysis, 437 whole blood specimens were potentially available for the present work. We selected a subsample based on demographic characteristics to minimize the potential for confounding. Specifically, most participants in the cohort were non-Hispanic Caucasian men [3], and therefore we focused on this demographic group to avoid confounding by race, ethnicity and sex. In addition, as expected parkinsonism was very strongly associated with age, so we restricted our study to workers age 25–65 to minimize the potential for confounding by age. This also had the benefit of largely restricting to active workers exposed for a sufficient period of time to have developed parkinsonism. Because this was a pilot study, we then applied to the cohort a design similar to a nested case-control study: We excluded specimens obtained at a repeat exam and selected workers based on UPDRS3 category. In total we included 201 workers from one of three UPDRS3 groups: UPDRS3 score < 6 ($n = 103$, hereafter controls), UPDRS3 score > 8 to 12 ($n = 49$, hereafter intermediate UPDRS3 group), and UPDRS3 15 ($n = 49$, hereafter parkinsonism cases). These categories parallel our previous classification [3] except that we were unable to include all workers in the intermediate category (UPDRS3 6

to UPDRS3 <15) so we only retained workers with UPDRS3 scores most clearly distinct from those of both cases and controls.

2.2 Assessment of NOS2 methylation

We (P.L.S., F.M.F.) collected DNA from whole blood using the QIAamp DNA blood kit (Qiagen, Germantown, MD). We then bisulfite-treated 500ng of purified DNA using Qiagen's EpiTect Fast DNA Bisulfite Kit and diluted to a concentration of 10ng/μl. We assessed *NOS2* methylation at the three CpG sites as in a recent study [26] of apprentice welders: sites 8309 (CpG site 1), 8314 (CpG site 2) and 8329 (CpG site 3). These CpG sites are in exon 1 (Genbank: AF017634), bordering the 5' promoter region and immediately adjacent to a transcription factor binding site and overlapping an exonic splicing enhancer.

We designed the assay using Qiagen's Assay Design Software, and obtained primers from Eurofins MWG Operon (Huntsville, AL). The sequences were gggtgagtataaatatttttggtgtag (forward primer), biotin-taaaactaccaatccctcat (reverse primer), and tgggtgtagtggtttata (sequencing primer). Each 25μl PCR reaction consisted of 12.5μl 2× Pyromark PCR Master Mix (Qiagen), 5 pmol forward primer, 5 pmol reverse primer, 15 – 20ng of bisulfite-treated DNA and water. Thermocycling conditions were 15 min at 95°C followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C, with a final extension of 10 minutes at 72°C. After visual determination of a single band on an agarose gel, 8μl of the PCR product was used in a Qiagen Q24 Pyrosequencing Assay according to the manufacturer's protocol. DNA methylation status was determined using a Pyromark Q24 instrument and final results were analyzed using Pyromark Q24 software. We included duplicate sample(s) and positive and negative laboratory controls on each experimental plate. The inter-assay coefficient of variation for mean *NOS2* methylation was 2.4% and the intra-assay coefficient of variation was 0.98%.

We ensured that all three UPDRS3 groups were represented on each experimental plate, while maintaining blinding of the lab. Complete methylation data were available for all participants; most had complete data in the initial attempt, but a repeat attempt was required for 4 cases, 5 controls, and 2 in the intermediate UPDRS3 group.

2.3 Statistical analysis

All statistical analyses were conducted in Stata Version 11 (College Station, TX). We constructed logistic regression models to separately compare two groups – parkinsonism cases and the intermediate UPDRS3 group – to controls with regard to percent *NOS2* methylation. In our primary analysis, we defined percent *NOS2* methylation as mean percent methylation across the three CpG sites [26]. In a secondary analysis, we considered percent methylation at individual CpG sites [20], as these sites have been observed to be differentially methylated in the brain despite their close proximity [27]. We modeled *NOS2* methylation linearly, but to verify the appropriateness of this approach, we also constructed tertiles of *NOS2* methylation. We calculated p-values for trend based on the former.

We adjusted *a priori* for age because of its association with both *NOS2* methylation [28] and UPDRS3 score [3]. We also adjusted *a priori* for experimental plate for pyrosequencing

reactions [20, 22], specifically conditional logistic regression while grouping on experimental plate. We examined whether inclusion of any of the following factors in the model further altered odds ratios (ORs) or 95% confidence intervals (CIs) by > 10%: age squared; examiner; current and former tobacco smoking; consumption of caffeine, coffee and alcohol; body mass index and timing of blood collection. This included year, day of the week, season and heating degree days in the study region on the day blood was collected. We accordingly adjusted all models for examiner in addition to age and experimental plate. Other considered factors, including smoking, did not alter results and therefore were not included in models. We also did not adjust for welding exposure because our hypothesis was that DNA methylation is in the causal pathway between welding fume exposure and parkinsonism (Figure 1), and therefore is inappropriate to include in the model as a potential confounder. We repeated all analyses while excluding workers who had been retired for > 1 year because *NOS2* methylation appears to change relatively rapidly [26, 29, 30], and the presence of parkinsonism could affect a worker's ability to work in a welding related job. Results from the larger cohort study [3] were consistent with such a potential healthy worker effect. In addition, we verified results were similar when we excluded all participants with co-morbidities associated with vascular parkinsonism (stroke, hypertension, heart disease and/or diabetes). Specifically, participants with a history of stroke were excluded from the original cohort [3], and then we conducted sensitivity analyses here in which we excluded participants with any of the other three conditions.

We also explored whether, as hypothesized (Figure 1), welding fume exposure was associated with *NOS2* methylation. Using the workers' self-reported work histories, we calculated cumulative duration (years) of occupational exposure to welding fume. This exposure metric is the one most strongly associated with parkinsonism among this cohort [3] and thus was our primary exposure of interest. We also considered the current job classification (welder, welder helper, around welding, not around welding) and retirement status to explore the influence of intensity and recentness of exposure on *NOS2* methylation.

3. Results

3.1 Participants

On average, participants with DNA methylation data had worked nearly 20 years in a job with exposure to welding fume (mean 19.8 years, standard deviation 13.8 years). Parkinsonism cases were older than controls and were less likely than participants in the intermediate UPDRS3 group to be a welder at the current or most recent job (Table 1). Proportionally more controls had ever smoked tobacco regularly compared to the other UPDRS3 groups.

3.2 *NOS2* methylation and parkinsonism

The *NOS2* CpG sites we assessed were highly methylated; all controls had > 90% methylation at each of the three CpG sites, and the overall mean *NOS2* methylation was 95.5% (standard deviation 1.3%). Methylation appeared similar in cases (mean 95.4%, standard deviation 1.5%) as compared to the other groups (each with mean 95.5%, standard deviation 1.2–1.3%). However, after accounting for age, examiner and especially

experimental plate, there was a clear inverse association between *NOS2* methylation and parkinsonism (p -value for trend = 0.04, Table 2). Compared to the lowest tertile of *NOS2* methylation, the prevalence of parkinsonism was 59% lower in the middle tertile of *NOS2* methylation and 76% lower in the upper tertile of *NOS2* methylation. A weaker inverse association was suggested when comparing the intermediate UPDRS3 group to controls, but there was no evidence of a dose-response association (p -value for trend = 0.59).

The association between parkinsonism and *NOS2* methylation was most evident for the third CpG site (8329). This association was not attenuated when we excluded retirees (Table 2, p -value for trend = 0.01), or conducted a variety of sensitivity analyses (exclusion of participants with co-morbidities associated with vascular parkinsonism, exclusion of samples for which the assay failed initially, application of the smooth function to the continuous methylation data, or adjustment for methylation at the other CpG sites in *NOS2*, data not shown in tables). Among the non-retired workers, each absolute increase of 1% of the CpG site 8329 was associated with a 33% (95% CI 8% to 51%) lower prevalence of parkinsonism.

3.3 Welding exposure and *NOS2* methylation

Because results for the third CpG site (8329) were particularly robust, and methylation of this CpG site is perhaps the most plausibly related to *NOS2* expression given its location in the gene, we explored whether welding exposure was associated with methylation at this CpG site. In linear regression models accounting for age and experimental plate, workers with recent exposure to welding fume had somewhat lower *NOS2* 8329 methylation than workers who had been retired for > 1 year (0.72% lower absolute methylation) or who were still at the worksite but not around welding fume in their current job (0.51% lower absolute methylation), although confidence intervals were wide because most workers remained exposed (Table 3). Locally-weighted scatterplot smoothing revealed an inverse association between total duration of welding fume exposure and *NOS2* 8329 methylation among workers with < 10 years of cumulative exposure, but no association otherwise. Accordingly, likelihood ratio test (p -value = 0.02) indicated that we must include a spline at 10 years duration (in addition to a linear term for duration) to adequately capture the association between duration of welding fume exposure and *NOS2* 8329 methylation. Therefore we stratified by duration of exposure, and observed 0.16% (95% CI 0.02% to 0.29%) lower methylation of *NOS2* 8329 per year of exposure among workers with < 10 years of exposure (p -value for trend = 0.03) and no association among workers who had already experienced 10 years of exposure (0.004%, 95% CI -0.03% to 0.04%, p -value for trend = 0.81).

4. Discussion

In workers from welding worksites subject to contemporary guidelines for workplace exposures, we observed greater UPDRS3 scores in relation to lower *NOS2* methylation. Interestingly, when we focused on participants who were employed at the welding worksite at the time of blood collection, this association was particularly evident for the third CpG site (8329), which is located in an exonic splicing enhancer of *NOS2*. This short motif is involved in the splicing of hetero-nuclear RNA or pre-mRNA into messenger RNA. It is also adjacent to the 5' promoter region. Because lower *NOS2* methylation in the promoter is

associated with greater *NOS2* expression [16, 17] and perhaps greater iNOS activity [18], these results are consistent with the hypothesis that parkinsonism associated with exposure to welding fume could be mediated, in part, through the nitric oxide pathway (Figure 1). Moreover, our results suggest that at least in the initial years of exposure, methylation at the *NOS2* 8329 CpG site continually drops with each additional year of on-the-job welding fume exposure. Although this contrasts with results in 38 welding school apprentices [26], our study adds to the mounting evidence [19–23] that particulate exposure reduces promoter region *NOS2* methylation in humans. This would be expected if *NOS2* methylation contributes to the occurrence of parkinsonism in workers exposed to welding fume (Figure 1). Thus, insofar as our results are not due to chance, inflammation, and iNOS in particular, may play a role in the high prevalence of parkinsonism among welders. As detailed above, experimental and animal studies support the hypothesis that iNOS plays a role in Mn neurotoxicity [11, 12] [14, 15].

In the present study we were not able to assess *NOS2* methylation in brain tissue, nor did we assess *NOS2* expression, iNOS activity or nitric oxide levels. However, we hypothesize that these are correlated with *NOS2* methylation in blood (Figure 1). Blood DNA methylation may be a good surrogate for DNA methylation in the brain [27], and methylation of *NOS2* is associated with *NOS2* expression [16, 17]. Therefore, it is intriguing that using DNA obtained from blood we observed an association in the hypothesized direction even though this association may have been attenuated by the use of DNA from blood rather than brain, and the use of DNA methylation as a surrogate for gene expression.

We also note there are limitations to the cross-sectional study design. *NOS2* methylation appears to change rather rapidly [26, 29, 30]. While this lends support to our results that suggest that *NOS2* methylation may be noticeably altered with relatively recent changes in welding fume exposure, it also means that we cannot be certain that *NOS2* methylation contributed to parkinsonism, or rather the reverse occurred. Greater UPDRS3 scores could be associated with reduced mobility, and we cannot rule out the possibility that mobility affects *NOS2* methylation. In particular, it is known that exercise can affect methylation of a variety of genes in humans [31]. Another consideration when interpreting the results of the present work is that the results may have been influenced by a healthy worker effect, as evidenced by a U-shaped dose-response association between welding and parkinsonism [3], which would be expected since tremor and bradykinesia associated with parkinsonism would make highly demanding fine motor tasks difficult to perform. However, similar to the overall study findings [3], we would anticipate that any healthy worker effect may have attenuated the associations, here between *NOS2* methylation and parkinsonism, and between duration of exposure and *NOS2* methylation. Finally, we assessed DNA methylation from whole blood and it is possible that the differences we observed could be due to different proportions of blood cell types across UPDRS3 groups, but we have no reason to believe that blood cell types would differ according to UPDRS3 score.

Since parkinsonism in Mn neurotoxicity is presumably mediated through neuropathologic changes, studying DNA methylation of *NOS2* in human brain tissue of welders and other Mn-exposed workers would provide the most definitive data. This type of specimen is not widely available in working populations in the U.S. since neuropathologic tissue can only be

obtained at death. Future studies of parkinsonism and methylation of *NOS2* and other potentially relevant genes, such as inflammatory and metal transporter genes, might benefit from the inclusion of DNA from both blood and brain. Only the former can be collected longitudinally, and a longitudinal assessment of *NOS2* methylation will be required to strengthen our findings and those from any future studies utilizing brain tissue. While our study was focused on parkinsonism in welders, such studies might also be of interest in population-based studies of Parkinson's disease. Parkinson's disease shares some features of parkinsonism among Mn-exposed workers [3], and ambient Mn exposure may increase risk of Parkinson's disease [32]. *NOS2* has received considerable attention with regard to Parkinson's disease, but results of numerous human studies, which considered *NOS2* genotype but not methylation, have been inconclusive. One reason for that could have been a failure to well assess *NOS2* expression by genotype alone, as might be expected, given that methylation of the gene clearly contributes to its expression [16, 17].

5. Conclusions

Lower methylation of the gene coding for iNOS was associated with greater signs of parkinsonism among workers from welding worksites, suggesting that inflammation mediated by iNOS may possibly contribute to the high prevalence of parkinsonism observed previously in workers exposed to welding fume.

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Highlights

- We assess methylation of *NOS2* in 201 workers exposed to welding fume
- Exposure to manganese-containing welding fume is associated with *NOS2* methylation
- *NOS2* methylation is associated with parkinsonism among welders
- Inducible nitric oxide synthase may play a role in parkinsonism in welders

Figure 1. Hypothesized mechanism for association between welding and parkinsonism
Abbreviations: iNOS, inducible nitric oxide synthase; *NOS2*, gene coding for iNOS

Table 1

Characteristics of non-Hispanic men with DNA methylation data, by Unified Parkinson Disease Rating Scale motor subsection 3 (UPDRS3), Midwestern U.S. Welders Cohort, 2006–2013

	Parkinsonism (UPDRS3 ≥ 15) n = 49 n (%)	Intermediate (UPDRS3 > 8 to 12) n = 49 n (%)	Control (UPDRS3 < 6) n = 103 n (%)
UPDRS3, mean (SD)	20.3 (3.6)	10.1 (1.1)	3.0 (1.8)
Age, years, mean (SD)	53.7 (10.0)	49.6 (10.4)	47.7 (11.4)
Welding Worksite			
Worksite 1	25 (51)	23 (47)	36 (35)
Worksite 2	16 (33)	20 (41)	53 (51)
Worksite 3	8 (16)	6 (12)	14 (14)
Total duration welding exposure, years, mean (SD)	21.7 (13.5)	21.6 (12.6)	18.0 (14.4)
Welder ^a	14 (29)	25 (51)	44 (43)
Retiree (> 1 year)	6 (12)	3 (6)	7 (7)
Ever smoked tobacco	30 (61)	28 (57)	78 (76)
Currently smoke tobacco	19 (39)	16 (33)	41 (40)
Typically drink coffee daily	33 (67)	36 (73)	75 (73)
> 12 years of education ^b	12 (24)	11 (23)	20 (20)
First degree relative with Parkinson disease ^b	1 (2)	2 (4)	0 (0)

SD, standard deviation.

^aWelder at current/most recent job vs. welder helper or other worker at welding worksite.

^bPercentages based on those with data (excludes 2 with missing education data, or 3 with missing data on family history of Parkinson disease).

Table 2

Parkinsonism and inducible nitric oxide synthase (NOS2) methylation,^a Midwestern U.S. Welders Cohort, 2006–2013

	<u>All workers</u>		<u>Excluding retirees</u>	
	Intermediate (UPDRS3 > 8 to 12)	Parkinsonism (UPDRS3 ≥ 15)	Intermediate (UPDRS3 > 8 to 12)	Parkinsonism (UPDRS3 ≥ 15)
	OR (95% CI) ^b <u>n = 49</u>	OR (95% CI) ^b <u>n = 49</u>	OR (95% CI) ^b <u>n = 46</u>	OR (95% CI) ^b <u>n = 43</u>
Mean NOS2 methylation^a				
Methylation tertile 1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Methylation tertile 2	0.59 (0.22–1.54)	0.41 (0.13–1.29)	0.56 (0.21–1.50)	0.32 (0.09–1.09)
Methylation tertile 3	0.61 (0.23–1.61)	0.24 (0.07–0.83)	0.53 (0.19–1.51)	0.13 (0.03–0.56)
<i>p</i> -value for trend ^c	<i>p</i> = 0.59	<i>p</i> = 0.04	<i>p</i> = 0.50	<i>p</i> = 0.02
NOS2 CpG Site 1^a				
Methylation tertile 1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Methylation tertile 2	1.91 (0.74–4.93)	1.10 (0.34–3.53)	1.90 (0.72–5.06)	0.70 (0.19–2.61)
Methylation tertile 3	0.84 (0.28–2.54)	0.72 (0.20–2.58)	0.86 (0.27–2.73)	0.67 (0.16–2.72)
<i>p</i> -value for trend ^c	<i>p</i> = 0.92	<i>p</i> = 0.11	<i>p</i> = 0.88	<i>p</i> = 0.10
NOS2 CpG Site 2^a				
Methylation tertile 1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Methylation tertile 2	0.75 (0.28–2.02)	0.46 (0.15–1.41)	0.70 (0.25–1.90)	0.43 (0.14–1.37)
Methylation tertile 3	0.77 (0.30–1.96)	0.83 (0.29–2.37)	0.68 (0.25–1.85)	0.77 (0.24–2.53)
<i>p</i> -value for trend ^c	<i>p</i> = 0.53	<i>p</i> = 0.32	<i>p</i> = 0.42	<i>p</i> = 0.29
NOS2 CpG Site 3^a				
Methylation tertile 1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Methylation tertile 2	0.72 (0.28–1.85)	0.53 (0.17–1.64)	0.67 (0.25–1.80)	0.34 (0.10–1.19)
Methylation tertile 3	0.78 (0.28–2.17)	0.40 (0.12–1.34)	0.72 (0.24–2.09)	0.22 (0.06–0.87)
<i>p</i> -value for trend ^c	<i>p</i> = 0.81	<i>p</i> = 0.07	<i>p</i> = 0.77	<i>p</i> = 0.01

^aMean and individual percent methylation at three CpG sites in NOS2 exon 1: CpG site 1 (8309), CpG site 2 (8314), CpG site 3 (8329).

^bOdds ratio and 95% confidence interval, relative to workers with UPDRS3 < 6 (N = 103 when including all workers, N = 96 when excluding retirees), adjusted for age (continuous), examiner and experimental plate.

^cBased on NOS2 methylation as a continuous measure.

Table 3Welding occupations and methylation of *NOS2* CpG Site 8329, Midwestern U.S. Welders Cohort, 2006–2013

	n = 201	Mean	β (95% CI)^a	β (95% CI)^a
	N	% methylation		
Classification of current/most recent job				
Not around welding	15	95.6	Reference	Reference
Around welding	95	95.7	−0.48 (−1.44, 0.47)	
Welder helper	8	95.5	−0.35 (−1.83, 1.14)	−0.51 (−1.43, 0.41)
Welder	83	95.5	−0.57 (−1.55, 0.41)	
Currently working at welding worksite				
No (retiree > 1 year)	16	96.4	Reference	
Yes	185	95.5	−0.72 (−1.66, 0.23)	–

^aLinear regression beta coefficient and 95% confidence interval, adjusted for age (continuous) and experimental plate