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ORIGINAL ARTICLE

Differential metabolic profiles by Hispanic ethnicity among male Tucson firefighters

Tuo Liu[1] · Melissa A. Furlong[1] · Justin M. Snider[2] · Malak M. Tfaily[3] · Christian Itson[3] · Shawn C. Beitel[1] · John J. Gulotta[4] · Krishna Parsawar[5,6] · Kristen Keck[5,6] · James Galligan[7] · Douglas I. Walker[8] · Jaclyn M. Goodrich[9] · Jefferey L. Burgess[1]

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Abstract

Introduction Firefighters face regular exposure to known and probable human carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), benzene, and formaldehyde, leading to an increased risk of various cancers compared to the general population. Hispanic and black firefighters are at increased risk of additional cancers not elevated in non-Hispanic white firefighters, yet biological pathways underlying these differences are unknown.

Objectives The study objectives were to evaluate differences in the urinary metabolome between Hispanic and non-Hispanic firefighters, pre-and post-fireground exposure.

Methods To investigate the metabolic patterns, we employed a comprehensive metabolomics pipeline that leveraged liquid chromatography coupled with high-resolution mass spectrometry. We applied linear mixed effects regression to identify the differential metabolites at an FDR < 0.05 among 19 Hispanic and 81 non-Hispanic firefighters. We also performed over-representation analysis using Mummichog to identify enriched pathways at FDR < 0.05.

Results Out of 175 features in HILIC(−) mode and 1847 features in RP(+) mode, we found 26 and 276 differential urinary features, respectively, when comparing Hispanic and non-Hispanic firefighters. We noted pathway enrichment in tryptophan and galactose metabolism. However, post-exposure, we did not observe differences in the metabolomic response by ethnicity despite differing fireground exposures.

Conclusion Dysregulation in the tryptophan and galactose pathway is an important contributor to cancer risks and may explain the increased cancer risk among Hispanic firefighters.

Keywords Firefighters · Urinary metabolomics · Differential metabolites · Occupation · Cancer risk

1 Introduction

According to the National Fire Protection Association, there were approximately 1.0 million firefighters in the United States (US) in 2020, of whom 90,000 are women (NFPA, 2022). Firefighters are exposed to known and probable human carcinogens (Daniels et al., 2015), including but not limited to polycyclic aromatic hydrocarbons (PAHs) (Kenneth et al., 2014), benzene (Kenneth et al., 2014), formaldehyde (Keith, 2011), and phthalates (Kolena et al., 2020),

✉ Melissa A. Furlong
mfurlong@arizona.edu

[1] Department of Community, Environment, and Policy, Mel and Enid Zuckerman College of Public Health, University of Arizona, 1295 N Martin Ave, Tucson, AZ 85724, USA

[2] School of Nutritional Sciences and Wellness, University of Arizona, Tucson, USA

[3] Department of Environmental Science, University of Arizona, Tucson, USA

[4] Tucson Fire Department, Tucson, USA

[5] Cancer Center, University of Arizona, Tucson, USA

[6] Analytical and Biological Mass Spectrometry Core, University of Arizona, Tucson, USA

[7] College of Pharmacy, University of Arizona, Tucson, USA

[8] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

[9] Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, USA

and experience a higher risk for select cancers, including skin melanoma (Lee et al., 2020), lung (Daniels et al., 2015), leukemia (Daniels et al., 2015), kidney (Rebecca et al., 2015), and prostate (Grace et al., 2006; Lee et al., 2020; Rebecca et al., 2015). In general, firefighters in the US experience excess overall cancer mortality (SMR = 1.14; 95% CI 1.10–1.18) as compared to the general US population (Demers et al., 2022; Robert et al., 2014). Specifically, according to studies of firefighters in Florida (Fangchao et al., 2006; Lee et al., 2020), Washington (Paul et al., 1994), California (Michael, 2007; Rebecca et al., 2015), and other US cities (Daniels et al., 2015; Dongmug et al., 2008; Robert et al., 2014), male firefighters were at increased risk of skin melanoma, urine tract cancers, mesothelioma and other types of cancers, of which kidney, leukemia, colon, prostate, and testicular cancer have less consistent results in the literature (Soteriades et al., 2019). Consequently, the International Agency for Research on Cancer (IARC) recently classified firefighters' occupational exposure as carcinogenic to humans with sufficient evidence for mesothelioma and bladder cancer (Demers et al., 2022).

In addition, Hispanic firefighters may be at increased risk for various cancers. In the US, Hispanics experience overall lower all-cancer incidence rate but higher incidence rates of cervical, stomach, liver, and gall bladder cancer than non-Hispanic Whites (Haile et al., 2012; Miller et al., 2018), and Hispanic firefighters may be at similarly increased risk. In a large-scale, case–control study assessing cancer risk among male firefighters, Tsai et al. (2015) reported that firefighters of other ethnicity/race (two thirds of whom were Hispanic), as compared to non-Hispanic white firefighters, had statistically significantly increased risks for a total of six cancers, including melanoma, prostate, testicular, bladder, kidney, and brain cancer (Tsai et al., 2015). We had previously shown that xenobiotic metabolizing genes and cancer-related genes were differentially methylated in Hispanic compared to non-Hispanic firefighters (Goodrich et al., 2021a). We also previously showed that firefighters experience differential metabolite expressions at baseline and after fires (Furlong et al., 2023), but did not explore whether these differed by Hispanic ethnicity. Societal conditions within fire departments and potentially differential task assignments to Hispanic firefighters have been hypothesized as possible reasons which might lead to the difference in the cancer risks (Tsai et al., 2015). Other factors, such as diet and environmental exposure, could also play an important role.

It is unclear, however, whether the biological response to fires varies by ethnicity. In this study, we sought to strengthen our understanding of underlying biological changes explaining cancer risk differences by Hispanic ethnicity using an untargeted metabolomics approach. Coupled with a baseline and post-fire exposure sampling scheme, we profiled and compared untargeted urinary metabolite profiles in firefighters of Hispanic and non-Hispanic ethnicity. We hypothesized that metabolic profiling would reveal differences in the urine metabolome of Hispanic and non-Hispanic firefighters and that the differences might reveal biological implications for cancer risk disparity by ethnicity. Results from this study may serve as a resource for advancing understanding of cancer risk difference among firefighters by ethnicity and provide a direction for future mechanistic or longitudinal studies.

2 Methods

2.1 Study population and sample preparation

Our analysis included 100 male firefighters from the Tucson Fire Department. Detailed study recruitment and urine sample collection were reported previously (Christiane et al., 2021). Briefly, urine samples were gathered from firefighters who had not participated in a fire response for at least four days (baseline). Subsequently, the same firefighters responded to a structural fire (post-fire) and urine samples were collected 2–4 h afterwards. The urine samples were transported on ice from the Tucson Fire Department to the University of Arizona, the specific gravities (SGs) were measured by refractometry, and samples aliquoted and stored at – 80 °C. Urine samples were prepared by spiking a ^{13}C labeled internal standard mix to a 1:1 solution of urine and ice-cold acidified methanol. The product mixtures were vortexed, centrifuged, and extracted in duplicate and stored at – 80 °C until further analysis. Several quality control samples (QC) and blanks were employed for machine calibration.

2.2 High-resolution metabolomics

High resolution metabolomics (HRM) analysis was performed in two sample sets within a three month period, using liquid-chromatography with high-resolution mass spectrometry (LC-HRMS; Thermo Scientific Exploris Orbitrap 480 Thermo Scientific, Waltham, MA). Duplicate sample extracts were randomized into batches across the two sample sets, except for those from Hispanic firefighters (N = 21) which were all analyzed during the first set. To boost metabolic feature coverage, sample extracts were analyzed in two modes using a dual-column, dual-polarity approach that included reverse-phase (RP) C18 chromatography with positive electrospray ionization (ESI) (RP(+)), along with hydrophilic interaction (HILIC) chromatography with negative ESI (HILIC(−)).

We performed HRM following adapted methods from Najdekr et al. (2019). Briefly, after injecting 1.0 µL of sample, we conducted RP separation using a 1.8 µm, 2.1 × 150 mm HSS T3 Column (ACQUITY Premier HSS T3 Column) and a methanol gradient (A = 99.9% water, 0.1% formic acid; B = 99.9% water, 0.1% formic acid). This involved an initial 3-min period with 99% A and 0.1% B, followed by a linear increase to 50% B at 11 min and a subsequent increase to 95% B held for 2 min. For HILIC separation, a 1.7 µm, 2.1 mm × 150 mm Amide column (Waters ACQUITY Premier BEH Amide Column) was employed. The mobile phase consisted of a gradient of 10 mM ammonium formate and acetonitrile (A = 10% water, 90% ACN, 10 mM ammonium formate, 0.1% formic acid; B = 50% water, 50% ACN, 10 mM ammonium formate, 0.1% formic acid). Like RP, the HILIC separation began with a 3-min period of 99% A and 0.1% B, followed by a linear increase to 50% B at 11 min and an additional increase to 95% B held for 2 min. The flow rate of the mobile phase was maintained at a constant 0.3 mL/min for both modes. The mass spectrometer operated at a resolving power of 60,000 with a mass-to-charge ratio (m/z) range of 65–1000 Da.

Lab blanks and standard QCs were run before AcquireX samples at the beginning of each batch. A pooled "total" QC sample was run after every 30 sample injections to access instrument variability and aid in normalization. "Batch" QC's were run at the end of the corresponding batch. Finally, a standard library QC was run every 30 samples to access retention time variation during the annotation process. Additionally, the mass spectra of the internal standards were manually inspected using the software Skyline (Brendan et al., 2010) to access batch effects.

2.3 Metabolite annotation

Metabolic features were uniquely defined by their mass-to-charge ratio (m/z), retention time and relative abundance. Annotation was accomplished using Compound Discoverer 3.3 (Thermo Scientific). To ensure annotation quality, a mass tolerance of 5 ppm was applied Mass spectra were then annotated against both in-house and online libraries. Annotations were prioritized as follows: (1) in-house standards library (MetaSci, Inc), (2) MzCloud, (3) Masslist, (4) ChemSpider, (5) Metabolika, At the time of this analysis, 300 metabolites from the MetaSci library were confirmed using our in-house library.

Annotation algorithms tend to forcefully search for matches at the risk of generating false positives. We thus limited the mass difference to be less than 5 ppm. To comply with the reporting standards for metabolite annotation (Schymanski et al., 2014) and indicate annotation strength, we adopted a modified confidence score for annotation using a self-defined match strength with the in-house database (MzVault), and the online databases (MzCloud, Chemspider, Metabolika, Masslist). The scoring framework was as follows: we assigned a score of 5 for MzVault and MzCloud, 4 for Chemspider, 3 for Metabolika, and 3 for Masslist to the feature if a full match with respective libraries was achieved; we assigned a score of 2 to the features if a partial match was achieved; if no match, we then assigned a score of 0 to the feature. As a result, each feature had 5 scores for relevant libraries, and the sum was interpreted as the overall annotation confidence.

2.4 Data preprocessing and statistical analysis

Ion intensity missing values were imputed using random forest algorithm as implemented in the Compound Discoverer Software (Thermo Scientific, Waltham, MA). Biological variation from individual levels of hydration was removed by multiplying the normalized ion intensities by corresponding specific gravity factors (SGF) defined as $\mathrm{SGF} = \frac{1.02-1.00}{SG-1.00}$ (Jean-François et al., 2015). Since our samples were analyzed in two sets, we visually checked and adjusted for potential batch-wise variation, if any. All metabolic features' ion intensities were then log2 transformed. Median scaling was applied to remove unwanted variation as implemented in the R package NormalizeMets (Alysha et al., 2018).

We calculated the percentage of missing value and a coefficient of variation (CV) by taking the average of duplicate ion intensities for each metabolic feature. We then applied a filtration of missing percentage $\leq 75\%$ and CV ≤ 0.20 to all metabolic features.

An analytical pipeline for our study is outlined in the Supplemental Fig. 5. To identify potential differential expression patterns by ethnicity, we drew side-by-side heatmaps with preprocessed expression signals and grouped then by ethnicity category. Metabolic features with annotation confidence > 0 that passed the filtration requirements described above were included in subsequent statistical analyses. Additionally, to identify potential batch effects, we mapped the total abundance by sample injection order/time and visually inspected whether there were clear trends across batches or over time (Supplemental Figs. 6, 7). We treated preprocessed metabolites' relative abundance as the response and Hispanic ethnicity (yes/no) as the main predictor while adjusting for BMI (kg/m2), age (years), years of firefighting (years), rank, and sample type (baseline and post-fire), and fit a linear mixed effects model with a random effect for participant to account for the repeat sampling from the participants. In the actual differential analysis, we conducted a complete-case analysis and

excluded observations with missing demographic values from the model. We defined differential metabolites by a p value of ≤ 0.05 for the Hispanic term in the mixed model. Multiple testing was adjusted by applying false discovery rate (FDR) adjustment at 0.05 to control for false positives. Metabolites with FDR < 0.05 and annotation confidence score ≥ 10 were presented in table and used to plot the heatmap to discern potential metabolic profile patterns by Hispanic ethnicity.

To evaluate whether biological response to ethnicity varied by fireground exposure status, we evaluated interactions between Hispanic ethnicity and fireground exposure status (baseline, post-fire). We added this interaction term to our main model and used an FDR cutoff of 0.05 to identify significant interactions. Outliers and influential observations were also inspected.

2.5 Pathway analysis

We performed pathway analyses for both HILIC(−) and RP(+) samples to investigate the biological implications gleaned from the difference in metabolic profiles by ethnicity, using Mummichog (version 2) (Li et al., 2013) as implemented on MetaboAnalyst (version 6.0) (Xia et al., 2009). Mummichog uses a set of permutation-based computational algorithms that take advantage of the collective power of metabolic networks to identify overrepresented pathways without metabolite identification from user input, and therefore accelerates the metabolomics workflow(Li et al., 2013). All annotated metabolic signals were included in the pathway analysis as the reference set. P values from the main model were provided to signals that passed our filtration and a p value of 1 was provided to those that did not pass. To reduce false positive matches, we restricted all annotated metabolites to be matched in primary ions, and only pathways with minimum size of 3 were investigated. We defined the significance level as a p value of 0.05. Only significant pathways with p value < 0.05 were investigated. Overrepresentation analysis results were interpreted in conjunction with pathway maps from Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa, 2000).

Data preprocessing, statistical analyses, and visualization were performed in the R programming environment, version 4.3.0 (R Core Team, 2023).

2.6 Targeted metabolite analysis

Nine hydroxylated urinary metabolites of PAHs (PAH-OHs) were measured in all participants, including 1-naphthol, 2-naphthol, 2-fluorenol, 3-fluorenol, 9-fluorenol, 2-phenanthrol, 4-phenanthrol, 1-phenanthrol and 3-phenanthrol, and 1-hydroxypyrene, as previously described (Christiane et al., 2021). The sum of these PAH-OHs were compared post-exposure between the Hispanic and non-Hispanic study participants using a T-test.

3 Results

Participant demographics including age, BMI, rank, and years of firefighting experience are listed in Table 1. A total of 100 firefighters from Tucson Fire Department were recruited and 200 urine samples (100 baseline, 100 post-fire) were extracted and measured in duplicate, which gave 399 usable urine extracts (200 baseline; 199 post-fire, 1 sample loss during sample preprocessing). The mean age of participants was 40.05 (9.91) years for Hispanic firefighters (N = 19) and 37.07 (8.38) years for non-Hispanic firefighters (N = 81). Hispanic firefighters had a non-significantly higher mean BMI score compared to non-Hispanic firefighters in our sample population, 28.53 and 27.68, respectively. Although there were no significant differences by Hispanic ethnicity across rank (captain, engineer, firefighter, trainee, and paramedic) categories, Hispanic firefighters had a larger proportion of engineers and a lower percentage of captains and firefighters than non-Hispanic firefighters. Hispanic firefighters also had more years of experience than non-Hispanic firefighters (10.58 vs 8.76 years), though the difference was not statistically significant.

3.1 Metabolomics analysis

Combining baseline and post-exposure urine samples, metabolomics analysis detected 175 and 1847 potential metabolites by HILIC(−) and RP(+) mode, respectively. Metabolites were first identified by comparison to an in-house library containing 300 confirmed metabolites (MzVault). To augment metabolite annotation, we incorporated online libraries, including MzCloud, Chemspider, Metabolika, Masslist. The annotation score scheme was reported previously (Furlong et al., 2023). After filtration

Table 1 Summary statistics of male Tucson Fire Department firefighters

	Hispanic (N = 19)	Not Hispanic (N = 81)	P value
Age	40.05 (9.91)	37.07 (8.38)	0.181[a]
BMI			0.279[a]
N-Miss	0	1	
Mean (SD)	28.53 (2.29)	27.68 (3.21)	
Rank			0.615[b]
Chief/Engineer	9 (47.4%)	33 (40.7%)	
Firefighter	10 (52.6%)	48 (59.3%)	
Years as firefighter			0.305[a]
N-Miss	0	1	
Mean (SD)	10.58 (6.91)	8.76 (6.90)	

[a]Linear model ANOVA, [b]Fisher's exact test for count data

using annotation confidence, CV, and missingness, a total of 137 and 1007 features from HILIC(−) and RP(+) mode, respectively, were fed into the linear mixed effects model.

3.2 Differential analysis

After adjustment for age, BMI, rank, and years in firefighting service, we identified 26 and 276 metabolites from HILIC(−) and RP(+) mode respectively, that were differentially expressed by ethnicity by a p value ≤ 0.05. Of these, 10 and 109, respectively, remained significant after adjustment for multiple testing. Metabolites that met the FDR < 0.05 and high annotation confidence (≥ 10) cutoffs are presented in Table 2. Out of 26 differential metabolites from HILIC(−) mode, 14 were upregulated and 12 downregulated; out of 276 differential metabolites from RP(+) mode, 191 were upregulated and 85 downregulated, both at raw p value 0.05 level (Fig. 1). After adjusting for multiple testing by controlling family-wise error rate at FDR 5% level, 10 metabolites remained statistically differential from HILIC(−) mode, including d-Sorbitol, sebacic acid, 5-morpholino-2,4(1H,3H)-pyrimidinedione, L-homocitrulline, deoxyguanidinoproclavaminic acid, 3-alpha-20-alpha-dihydroxy-5-beta-pregnane3-glucuronide, diethyl tartrate, val-glu, perchloric acid, and 6-hydroxy-3,6,9-trimethyl-2-oxo-2,3,3a,5,6,9b-hexahydro-4H-furo[3′,4′:6,7]cyclohepta[1,2-b]furan-4-yl acetate; 109 metabolites remained statistically differential from RP(+) mode. A complete list of differential metabolites, along with their p values and FDR corrected q values, can be found in Supplemental Tables 3 and 4.

In the heatmaps for the differential metabolites' expression at FDR < 0.05 level by ethnicity group (Fig. 2), we observed clear differences in the metabolite expression profiles comparing Hispanics to non-Hispanics. This difference was consistent regardless of fire exposure status (pre-, and post-fire). A combination of lollipop plot and side-by-side boxplot show the magnitude, effect size, and direction of the difference in metabolite expression by ethnicity group, (Supplemental Figs. 6, 7) was included in Supplemental material.

3.3 Pathway analysis

The pathway over-representation analysis identified 28 potentially relevant biological functions comparing Hispanic to non-Hispanic firefighters (Fig. 3). Among these, 2 were associated with HILIC(−), and 26 were linked to RP(+) modes. At 0.05 level, only 2 metabolic pathways remained significantly enriched. Specifically, tryptophan metabolism

Table 2 Differential metabolites for Hispanic compared to non-Hispanic Firefighters (N = 100 participants, 199 samples, 197 complete cases used in the main model))

Mode	Metabolite[a]	Formula	MW	RT (min)	Annotation confidence	Slope	P value	FDR
HILIC(−)	5-morpholino-2,4(1H,3H)-pyrimidinedione	C8H11N3O3	197.080	10.764	10	0.386	0.000	0.006
HILIC(−)	D-Sorbitol	C6H14O6	182.079	8.672	14	0.559	0.001	0.027
HILIC(−)	Sebacic acid	C10H18O4	202.120	1.520	12	– 0.328	0.002	0.047
RP(+)	Cytosine	C4H5N3O	111.043	5.645	13	0.339	0.000	0.007
RP(+)	α-Aspartylphenylalanine	C13H16N2O5	280.106	9.581	12	0.635	0.000	0.007
RP(+)	6-Methoxyquinoline	C10H9NO	159.068	5.991	10	0.403	0.000	0.009
RP(+)	OPEO	C16H26O2	250.193	19.555	10	– 0.682	0.000	0.012
RP(+)	α-Aspartylphenylalanine	C13H16N2O5	280.106	9.255	12	0.518	0.001	0.018
RP(+)	*N*-Acetyl-L-arginine dihydrate	C8H16N4O3	216.122	2.136	12	0.438	0.002	0.026
RP(+)	Oxybenzone	C14H12O3	228.079	14.255	10	– 1.324	0.002	0.027
RP(+)	HU-331	C21H28O3	328.204	16.103	10	0.486	0.002	0.027
RP(+)	Methylimidazoleacetic acid	C6H8N2O2	140.059	1.394	12	0.318	0.003	0.035
RP(+)	L-Norleucine	C6H13NO2	131.095	3.815	10	0.487	0.003	0.037
RP(+)	5-Aminolevulinic acid	C5H9NO3	131.058	3.297	15	0.363	0.004	0.038
RP(+)	Cantharidin	C10H12O4	196.074	13.487	12	0.272	0.004	0.039[a]

MW molecular weight, *RT* retention time; annotation confidence had a maximum score of 20, with a higher number signifying higher confidence; *FDR* false discovery rate

HILIC(−) hydrophilic interaction chromatography with negative electrospray ionization, RP(+): reverse-phase with positive electrospray ionization

[a]We included in this table metabolites with annotation confidence score ≥ 10 and FDR < 0.05; slope is the coefficient estimate for the Ethnicity term in the linear mixed effects model with random intercept for participant. The slope coefficient estimates are equal to log2 ratio of the relative abundance of Hispanic to non-Hispanic samples; direction of the slope implies whether the metabolite was up- or down-regulated when comparing Hispanic to non-Hispanic firefighters. Models were controlled for age, BMI, rank, and years of firefighting

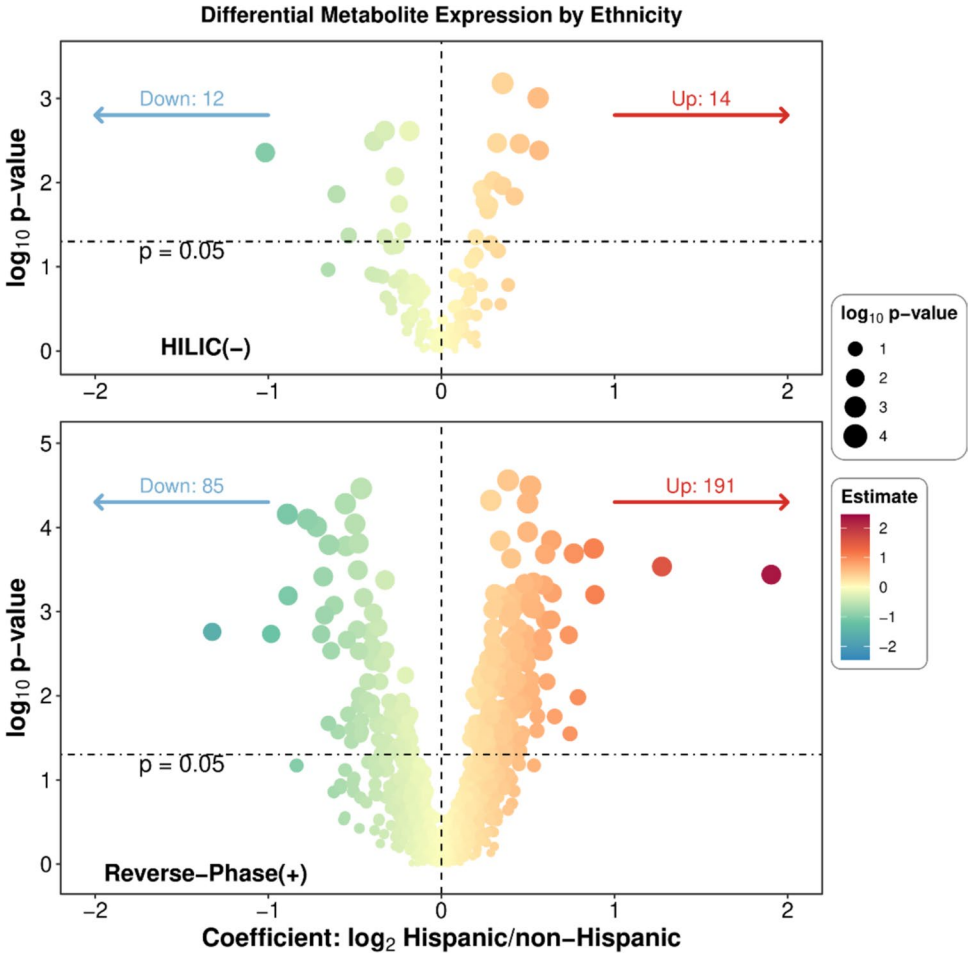

Fig. 1 Volcano plot for differential metabolites comparing Hispanic to non-Hispanic (reference group) firefighters. Estimate refers to the coefficient estimation for ethnicity term in the linear mixed effects model with random effect for participant, where the coefficient estimates are equal to log2 ratio of the relative abundance of Hispanic to non-Hispanic samples; direction of the coefficients implies whether the metabolite was up- or down-regulated. Models were controlled for age, BMI, rank, and years of firefighting. P values were log10 transformed for visualization purposes, and metabolites with smaller p values have larger dot sizes

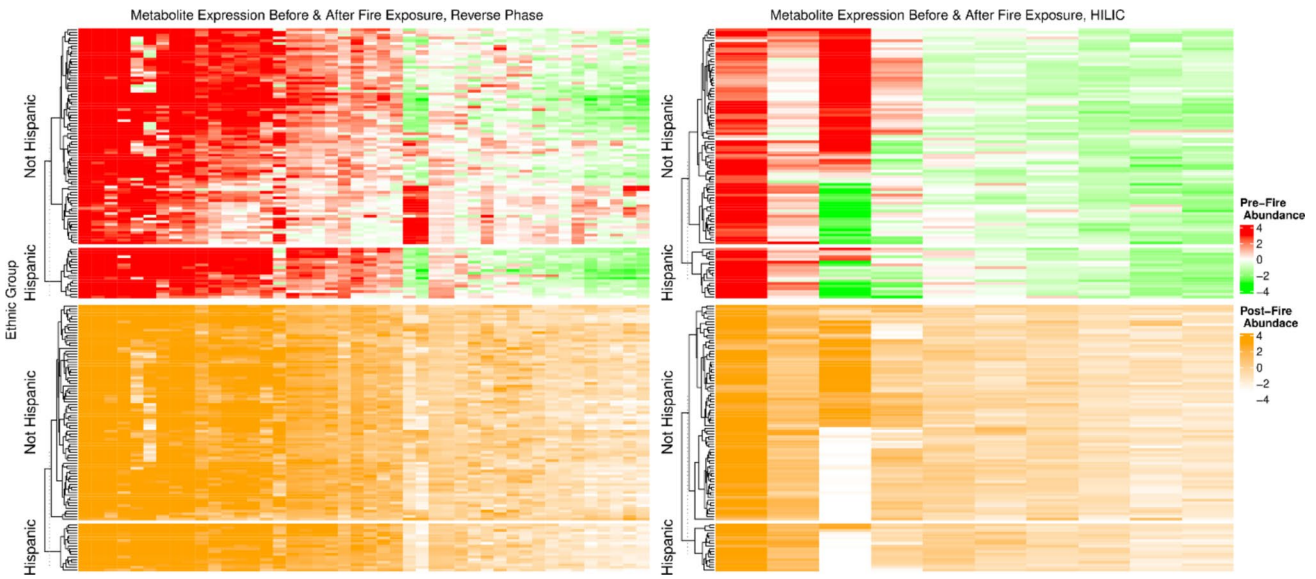

Fig. 2 Heatmap for differential metabolites with FDR < 0.05 by ethnicity and fire exposure status. The subplot on the left refers to the reverse phase mode and the one on the right refers to the HILIC mode. For both subplots, the expression pattern for differential metabolites were first plotted for pre-fire exposure, on top of post-fire exposure. Pre-fire comparison was drawn in red-green scheme and post-fire comparison was drawn in white-yellow scheme. The relative abundance for each feature is in log2 scale and centered

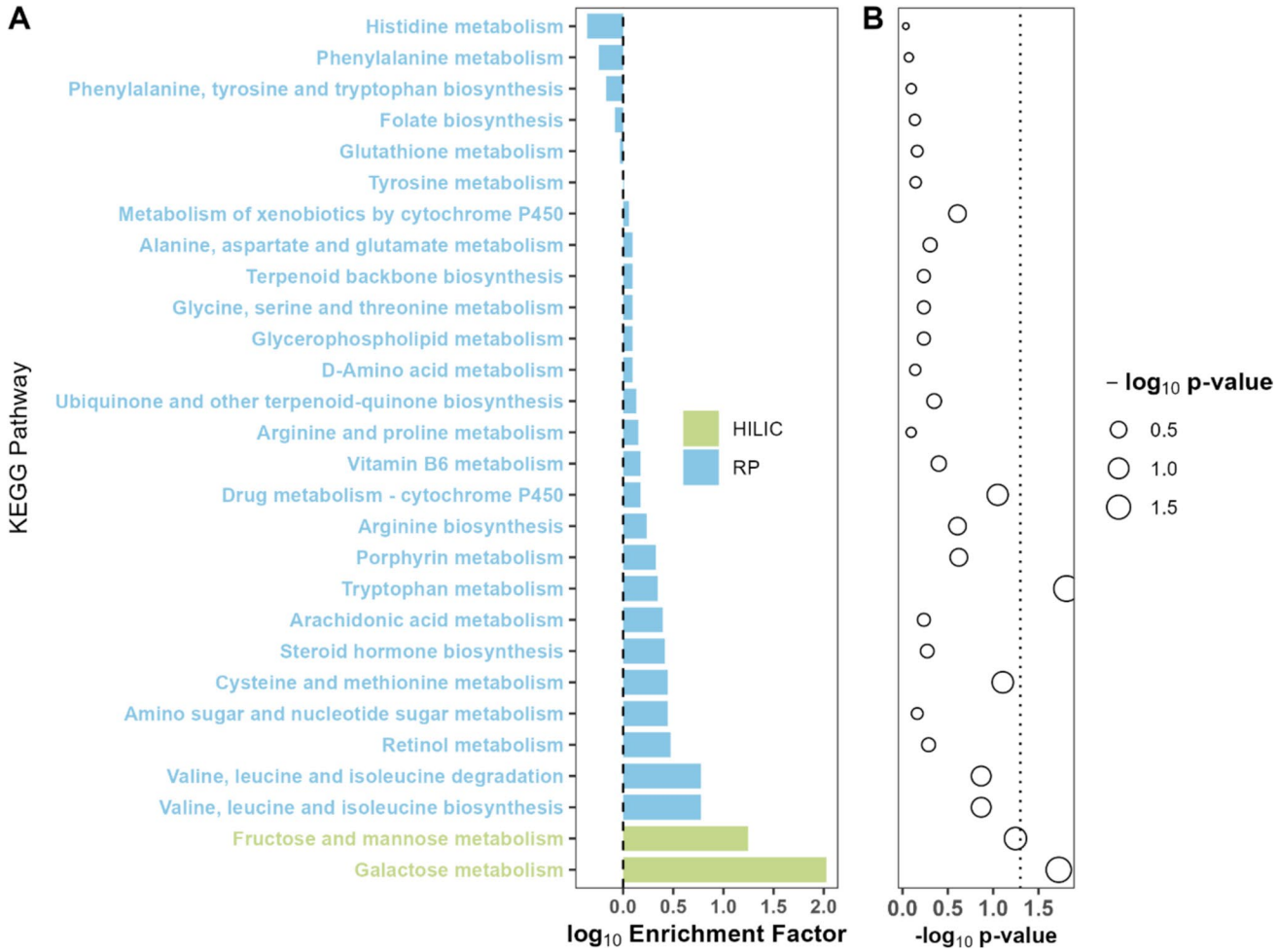

Fig. 3 Pathway overrepresentation analysis of Hispanic versus non-Hispanic firefighters based on mixed effects model coefficients and statistical significances; **A** *HILIC*(−) and RP(+) mode are colored in green and blue, respectively; dashed line indicates enrichment factor = 1, where enrichment factor is calculated as the ratio of the number of significant hits from user input to the expected hits within a pathway, and enrichment factor below 1 indicates the pathway is underrepresented and over 1 overrepresented; **B** the p values are calculated by Fisher test against null hypothesis; the dotted line indicates p value = 0.05 and bigger circle size indicates a low p value and thus higher significance

from RP(+) mode exhibited an enrichment factor of 2.21, while galactose metabolism from HILIC(−) mode showed a substantial enrichment factor of 53.00. The galactose pathway stayed significantly enriched after adjustment for multiple testing.

3.4 Sensitivity analysis

Sensitivity analyses results (Supplemental Table 5) indicated no statistically significant interaction between the ethnicity effect and the exposure effect at FDR 0.05 level. Refitting the model after identifying and removing outliers did not substantially change the associations between ethnicity and metabolites' relative abundance. For batch effects, we plotted the relative abundance by samples and observed only random fluctuations in the expression pattern, for both intra and inter batch, by sample injection, and there was no clear pattern in the expression pattern across the two sets (Supplemental Figs. 8–11).

3.5 Targeted metabolite analysis

The distribution by ethnicity of the total post-exposure concentrations of the nine urinary PAH-OHs are shown in Supplementary Fig. 12. The geometric mean values were 4.27 ± 0.26 (ng/L) and 4.33 ± 0.45 (ng/L), respectively. These values were not significantly different ($p = 0.4$).

4 Discussion

In this study, we performed untargeted metabolomics to increase the understanding of differential health risk by ethnicity among male firefighters. This approach allowed us to profile the metabolome to identify differential expressions across 100 firefighters. Out of 175 HILIC(−) and 1847 RP(+) matched urinary metabolites, 26 from HILIC(−) and 276 from RP(+) were differentially expressed by Hispanic ethnicity. These differences were enriched for galactose metabolism and tryptophan metabolism pathways. However, the metabolomic response by Hispanic ethnicity did not vary by fireground exposure status, suggesting that differences by ethnicity may not be strongly related to differential responses to fires. Identification of these metabolites and pathways may help explain the increased risk for some cancers by Hispanic ethnicity among firefighters.

4.1 Health implication

In pathway analyses, d-sorbitol and galactitol were significant hits for the galactose metabolism pathway. D-sorbitol is a sugar alcohol obtained by reduction of glucose that has been commercially produced in packaged food and beverages as a sugar substitute. In our analysis, d-sorbitol was found in Hispanic firefighters at a statistically higher level than in non-Hispanic firefighters (slope = 0.56, $\frac{Hispanic}{non-Hispanic} = 2^{0.56} = 1.47$), which could be indicative of dietary differences between Hispanic and non-Hispanic firefighters. Galactitol was identified by compound matching of Mummichog. Galactitol is a reduction product formed from excess galactose in a reaction catalyzed by aldose reductase. Galactose is the hydrolysis product of lactose that is found primarily in dairy products. Studies have shown that chronic galactose exposure induces neurodegeneration and oxidative damage as well as acceleration of aging in mice (Cui et al., 2006; Zhou et al., 2015). Different levels of d-sorbitol and galactitol together could indicate differential exposures to environmental factors among firefighters such as dietary quality that chronically might contribute to the cancer risks observed across race/ethnicity, as discussed by Tucker et al. (2019). Dietary factors have been one of the major contributing risk factors for select cancers including colon, breast, and prostate, in addition, approximately 33% of cancers in the West could be associated with dietary factors (Sharma et al., 2013; Tucker & Flanagan, 2020). Although evidence of artificial sweeteners and cancer risk is weak and inconsistent (Debras et al., 2022; Nancy et al., 2023; Riboli et al., 2023), consumption of sugar substitutes could be linked to obesity which in turn is associated with various types of cancers that may help explain the cancer risk disparity among firefighters.

Tryptophan (TRP) is an essential amino acid obtained from dietary protein that plays an important role in various physiological activities including immunity and neurological functions. TRP is primarily metabolized by the kynurenine pathway (KP) catabolized by indoleamine 2,3-dioxygenase (IDO1, IDO2) whereas the rest takes part in the production of serotonin and tryptamine (Mellor et al., 2017; Platten et al., 2019). IDO was reported to be capable of suppressing T-cell activity by inhibiting T cell proliferation and thus restricting antitumor immune responses in pregnant mice (Munn et al., 1998; Platten et al., 2019). Disturbance of TRP metabolism, specifically TRP degradation catalyzed by IDO, thus can induce conditions ranging from neurodegeneration to cancer (Pilotte et al., 2012; Platten et al., 2019; Xue et al., 2023). Our pathway analysis indicated a higher level of TRP and 5-hydroxy-L-TRP among Hispanic firefighters than non-Hispanic firefighters (Fig. 4), which could be indicative of difference in intake of TRP from diet. However, the level of TRP is the product of dietary intake and innate TRP metabolism. If the difference in TRP levels persists over time, this could serve as a cue or starting point for cancer risk difference by Hispanic ethnicity among firefighters and a potential target for intervention.

A recent review reported that tryptophan metabolism was among the most consistently perturbed pathways when evaluating association of air pollution and untargeted metabolite expressions, and tryptophan with level-1 identification was reported down-regulated in both serum and plasma untargeted metabolomics studies (Liang et al., 2023). In addition, galactose metabolism was reported to be enriched after air pollution in 9 independent metabolomics studies (Liang et al., 2023). Although tryptophan was reported in biospecimens other than urine which is different from our bioassay, this could serve as an alternative explanation for ethnic/racial difference in cancer risks among firefighters since firefighters are exposed to carcinogens via inhalation and Hispanic firefighters may experience task assignment with more toxic exposure scenarios than non-Hispanic firefighters.

This work builds upon our previous study that focused on DNA methylation, a relatively stable epigenetic modification that contributes to carcinogenesis when dysregulated. We compared DNA methylation of > 700,000 loci between 31 Hispanic and 163 non-Hispanic white firefighters from three US states (Goodrich et al., 2021b). We reported DNA methylation differences at 76 loci including in genes involved in xenobiotic metabolism and cancer-related pathways. Altered regulation of these genes in Hispanic firefighters could influence the ability to process and protect against toxic

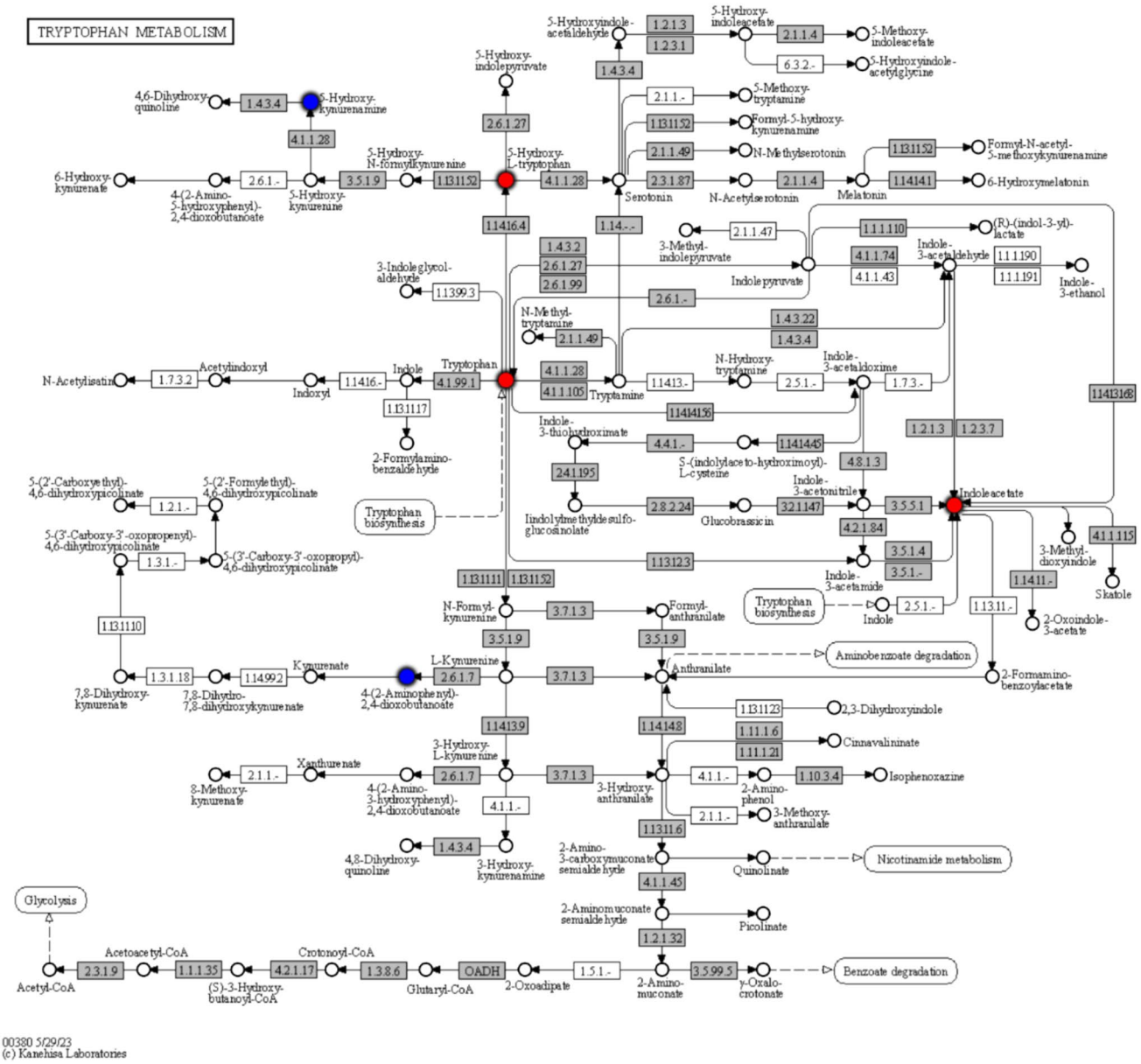

Fig. 4 Tryptophan metabolism with differential patterns by ethnicity. Red dots indicate up-regulated metabolites in Hispanic compared to non-Hispanic firefighters, while blue dots indicate down-regulated metabolites in Hispanics compared to non-Hispanics. Mummichog conducts overrepresentation analysis based on its own annotation results against empirical compounds for relevant pathway; thus, the matched compound may be different from our own annotations. Here we only present significant hits that matched with our own annotation via Compound Discoverer

exposures encountered at the workplace or home environment which could in turn influence downstream health risks.

This study also builds on our previous study of metabolic changes following fires in firefighters (Furlong et al., 2023). In that study, we also observed changes in tryptophan metabolism from baseline to post-fire, along with several other metabolomic changes indicating xenobiotic exposures and impacts on kidney function. In this study, we show that the response to fires does not differ by ethnicity at FDR < 0.05, although we may be underpowered to detect consistent interaction effects. However, tryptophan changes may be particularly important for Hispanic firefighters, given the double implication of changes by ethnicity and after fire.

Collectively, enrichment of galactose metabolism and tryptophan metabolism among Hispanic compared to non-Hispanic firefighters may be indicative of dietary differences between the groups or environmental air pollution difference in the workplace or the home. These findings may carry

practical implications in terms of cancer risk investigation among firefighters by race/ethnicity, such that difference in diet and exposure to air pollutants may play a critical role in the elevated risks among Hispanic firefighters. Importantly, intervention and prevention strategies could be developed to promote health and potentially reduce differences in external exposures that impact galactose and tryptophan metabolism.

4.2 Strengths and limitations

This study had several strengths. The firefighters were exposed to actual community fires with a wider range of burning materials than those used in training fires. Since we had repeat paired samples from firefighters, time invariant confounding was not a concern. We also expanded on prior evidence of differential DNA methylation by ethnicity in firefighters, to assess the metabolome. In addition, two separation modes (RP(+) and HILIC(−)) were used to boost metabolite identification, coupled with an ultra-high performance Orbitrap Exploris 480 Mass Spectrometer.

Limitations included annotation against an in-house library with a limited size and inability to adjust for time-variant covariates, such as medications and dietary factors. We also only had 19 Hispanic firefighters, which greatly reduced our statistical power to detect interactions between ethnicity and fire response on metabolite expression. Actual exposures likely varied by fire, although post-exposure total urinary PAH-OH levels were similar between Hispanic and non-Hispanic firefighters. Future studies in more controlled training settings could be used to reduce this variability.

5 Conclusion

To conclude, male firefighters showed a broad metabolic difference by ethnicity, including altered galactose and tryptophan metabolism that may be indicative of chronic dietary or environmental exposure differences among Hispanic and non-Hispanic firefighters. This interplay could collectively contribute to the differences in cancer risks among male firefighters by Hispanic ethnicity.

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Author contributions TL did the bulk of analysis and drafted the manuscript; MAF, JLB, supervised the analysis and results interpretation; KP, JMS, KK, and MMT supervised metabolomics analysis; CI helped with metabolomics analysis; SB and JG organized and supervised biological sampling; SB, JG, DW, and JMG supported results interpretation.

Data availability Data for this study is available upon request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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