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Original Article

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Environment-wide association study of elevated liver enzymes: results from the Korean National Environmental Health Survey 2018–2022

Youngchan Chi [1], **Jong-Tae Park** [1], **Sewhan Na** [1,2], **and Kyeongmin Kwak** [1*]

[1]Department of Occupational and Environmental Medicine, Korea University Ansan Hospital, Ansan, Korea
[2]Department of Environmental Health Sciences, Seoul National University Graduate School of Public Health, Seoul, Korea

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***Correspondence:**
Kyeongmin Kwak
Department of Occupational and Environmental Medicine, Korea University Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Korea.
Email: pathfinder81@korea.ac.kr

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ORCID iDs
Youngchan Chi
https://orcid.org/0000-0002-1715-8184
Jong-Tae Park
https://orcid.org/0000-0002-0687-3609
Sewhan Na
https://orcid.org/0000-0003-1184-7955
Kyeongmin Kwak
https://orcid.org/0000-0001-6296-8058

Abbreviations
AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; BPA: bisphenol A; BPS: bisphenol S; CI: confidence interval; cont.: container;

ABSTRACT

Background: Environmental exposure is characterized by low concentration, chronic, and complex exposure. Traditional epidemiological studies show limitations in reflecting these characteristics since they usually focus on a single or very limited number of exposure factors at a time. In this study, we adopted the methodology of environment-wide association study (EWAS) to figure out the association of human liver function with various environmentally hazardous substances.
Methods: We analyzed 2,961 participants from the Korean National Environmental Health Survey Cycle 4 (2018–2020). Using generalized linear model (GLM) analysis, we analyzed the association of 72 variables with 3 liver function indices (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and gamma glutamyl transferase [GGT]). Finally, we visualized our results with Manhattan plot.
Results: In GLM analysis, perfluorooctanesulfonate were positively associated with ALT (odds ratio [OR]: 2.2; 95% confidence interval [CI]: 1.39–3.46; $p_{adjusted}$ = 0.0147) and perfluorodecanoic acid showed positive association with GGT (OR: 2.73; 95% CI: 1.36–5.5; $p_{adjusted}$ = 0.0256). Plasma mercury showed positive association with GGT (OR: 1.45; 95% CI: 1.14–1.84; $p_{adjusted}$ = 0.0315). Using a plastic container while keeping food in the refrigerator was associated with elevated GGT compared to using a glass container (OR: 1.51; 95% CI: 1.16–1.95; $p_{adjusted}$ = 0.0153). 2-ethyl-5-oxohexyl phthalate, showed a negative trend with all 3 indices, with AST (OR: 0.54; 95% CI: 0.39–0.73; $p_{adjusted}$ = 0.00357), ALT (OR: 0.5; 95% CI: 0.34–0.75; $p_{adjusted}$ = 0.036), GGT (OR: 0.55; 95% CI: 0.4–0.76; $p_{adjusted}$ = 0.00697). Bisphenol S and frequent use of sunblock cream showed negative association with ALT (OR: 0.77; 95% CI: 0.66–0.89), and GGT (OR: 0.25; 95% CI: 0.11–0.55), respectively.
Conclusions: We conducted an exploratory study on environmental exposure and human liver function. By using EWAS methodology, we identified 7 factors that could have potential association with liver function.

Keywords: Liver enzyme; Environmentally hazardous chemicals; Complex exposure; EWAS

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DEHP: di-2-ethylhexyl phthalate; EWAS: environment-wide association study; FDR: false discovery rate; GGT: gamma glutamyl transferase; GLM: generalized linear model; GWAS: genome-wide association study; IRB: Institutional Review Board; KoNEHS: Korean National Environmental Health Survey; MEHP: mono (2-ethylhexyl) phthalate; MEOHP: 2-ethyl-5-oxohexyl phthalate; med.: medicine; OR: odds ratio; PAH: polycyclic aromatic hydrocarbon; PBA: 3-phenoxybenzoic acid; PFAS: polyfluorinated substances; PFDeA: perfluorodecanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctanesulfonate; rec.: recent; 3m: three months.

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Competing interests

The authors declare that they have no competing interests.

Author Contributions

Conceptualization: Chi Y, Park JT, Kwak K; Data curation: Chi Y, Na S; Formal analysis: Chi Y, Kwak K; Project administration: Kwak K; Supervision: Kwak K; Validation: Park JT, Na S; Visualization: Chi Y; Writing - original draft: Chi Y, Kwak K; Writing - review & editing: Park JT, Kwak K, Na S.

BACKGROUND

According to a report by the European Environment Agent in 2020, there are nearly 100,000 chemicals on the market. Among them, fewer than 500 chemicals are appropriately regulated and are well known for their characteristics and potential hazards. However, the remaining chemicals have not been adequately studied.[1] The World Health Organization reported that environmental exposure is a major risk factor for death caused by chronic diseases.[2] Modern society cannot be isolated from the use of chemicals; therefore, it is essential to understand and identify the potential health risks of chemicals around us. Environmental exposure is characterized by low concentration, chronic, and complex exposure.[3,4] However, traditional epidemiological studies usually focus on a single or very limited number of exposure factors at a time.[5] This showed limitations in the study of simultaneous, multifactorial exposure to chemicals in an individual's daily life. Therefore, novel approaches have recently been introduced to appropriately reflect the characteristics of environmental exposure.

As a novel methodology, we applied and adopted the environment-wide association study (EWAS) method. EWAS are derived from a genome-wide association study (GWAS), an exploratory method for identifying genetic mutations related to certain diseases. In GWAS, genetic factors are considered independent variables; similarly, in EWAS, environmentally hazardous substances are regarded as independent variables.[5,6] In this manner, we can conduct exploratory research to find the associations of various chemicals with certain diseases on a broad scale.

Since the liver usually functions as the main defense organ in the body, a substantial number of environmentally hazardous chemicals are metabolized and detoxified in the liver.[7,8] These metabolites generated during detoxification cause liver damage. However, these processes usually do not present specific clinical manifestations, making clinical diagnosis difficult.[9] Therefore, liver damage caused by environmentally hazardous substances can easily remain silent for a long time. For example, per- and polyfluorinated substances (PFAS), a well-known endocrine-disrupting chemical, are detected in the serum of nearly all adults in the US.[10] As they accumulate in the liver with a fairly long half-life, this bioaccumulation is associated with long-term health effects on the human body.[11] In addition to PFAS, it is already well known that numerous chemicals, such as heavy metals and various organic solvents, are associated with human liver dysfunction.[12,14]

As mentioned above, humans are exposed to many chemicals daily, and a substantial amount of these chemicals are potential risk factors for liver function. Therefore, in this study, we attempted to comprehensively determine the association between various hazardous chemicals and human liver function.

METHODS

Participants

In this study, we collected baseline data from the 4th Korean National Environmental Health Survey (KoNEHS). The KoNEHS is a study conducted by the National Institute of Environment Research to evaluate the levels of exposure to various environmentally hazardous substances in the Korean population. This survey used stratified cluster sampling methods to secure a representation of the entire adult Korean population. The baseline

survey was conducted from 2018 to 2020 and 4,239 adults (age ≥ 19 years) were enrolled in this study. Participants who did not undergo blood or urine sample analyses (n = 1,266) were excluded. Due to coronavirus disease 2019, blood and urine sampling was not properly performed in 2020. Participants taking medication for hepatitis (n = 9) and who did not respond to the questionnaire on monthly income (n = 3) were also excluded from this study. Therefore, a total of 2,961 adults were analyzed (**Fig. 1**).

Variables

The variables were divided into 2 groups. First, environmentally hazardous chemicals can be identified using blood or urine tests. These substances can be categorized as heavy metals, polyaromatic hydrocarbons, phthalate metabolites, bisphenols, triclosan, parabens, nicotine derivatives, benzophenone, benzene metabolites, perfluorinated compounds, and phenolic benzoic acid. A total of 34 variables were investigated using blood and urine samples. Details are presented in **Supplementary Table 1**. The second group consisted of questionnaires. The KoNEHS survey contains many questionnaires that can indirectly reflect exposure to various chemicals. The responses to each questionnaire were mostly based on the frequency level, so they could be stratified. A total of 38 questionnaires were identified. The details of the questionnaires are provided in **Supplementary Table 2**.

Liver dysfunction group and variable handling

We distinguished the liver dysfunction group from the normal population based on elevated liver function indices (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and gamma glutamyl transferase [GGT]). We followed the cut-off values suggested by the KoNEHS Laboratory Procedure Manual. Values greater than AST, 34 IU/L; ALT, 49 IU/L; and GGT, 73 IU/L (for males) or 38 IU/L (for females) were defined as increased liver function indices; therefore, the liver dysfunction group. Urine samples for the measurement of various environmentally hazardous substances were adjusted by urine creatinine concentration. In general, environmental exposure to various substances is low-concentration exposure and

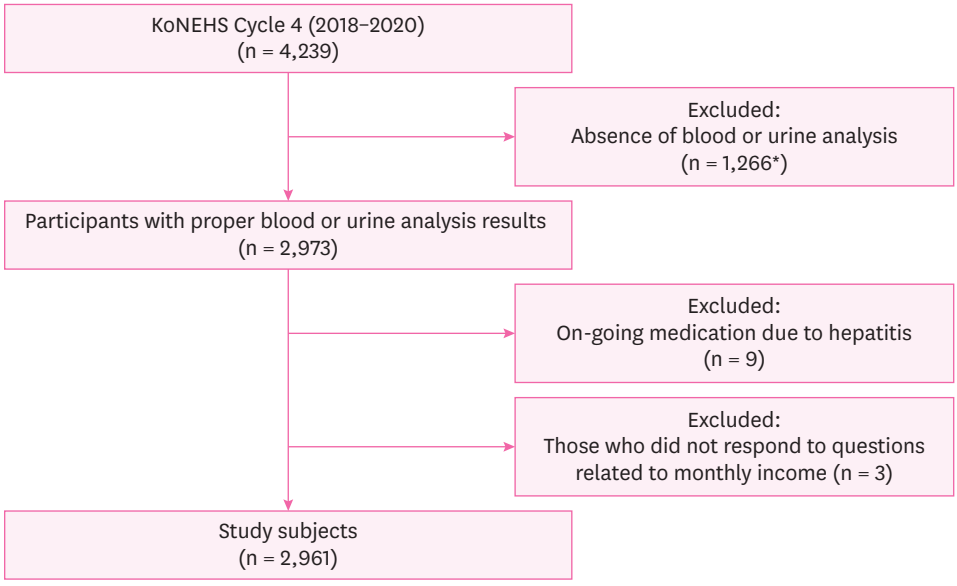

Fig. 1. Flow of study participants selection.
KoNEHS: Korean National Environmental Health Survey.
*Due to COVID-19, blood and urine sampling was not properly performed in 2020.

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shows skewness. Therefore, we log-transformed urine and blood samples for analysis.[15] As we mentioned, the questionnaire-based variables are composed of stratified answers based on frequency; we divide the high- and low-exposure groups by binary classification. Few nominal variables were analyzed in the factorization process.

Statistical analysis

We analyzed the association between various variables of environmental exposure and elevated liver enzyme levels by modifying the EWAS approach proposed by Patel et al.[5,16] First, we used a generalized linear model (GLM) to associate the 72 environmental variables with the levels of AST, ALT, and GGT while adjusting for age, sex, smoking, alcohol intake, body mass index (BMI), and monthly income. We calculated odds ratio (OR) of each variable against each liver function indices through GLM, meaning odds of the outcome per unit increase in the value of the exposure. This study has the feature of multiple comparisons using various variables; consequently, there is a possibility of an increase in type I errors. To correct this problem, traditionally, few methods, such as Bonferroni correction, have been used. However, in the case of statistical analyses with a large number of variables, traditional methods are too conservative, resulting in diminished statistical power. Instead, the false discovery rate (FDR) is known to be a less conservative correction method compared to other methods and is widely used in GWAS-based studies.[17,18] Therefore, we used the FDR to adjust the *p*-values when judging the significance of the GLM results. We considered environmentally hazardous substances with FDR < 0.05 as potential risk factors for elevated liver enzyme levels. Previous studies using EWAS analysis used additional validation studies to ensure replicability in other data sets.[16,19] However, since the data set we used, KoNEHS, does not have a sufficiently large number of study subjects to properly validate with other sets of studies, we did not perform the additional validation process. Finally, we visualized the association results using a Manhattan plot. All statistical analyses were performed with R software (version 4.2.1 for Windows; R Foundation, Vienna, Austria).

Ethics statement

This study was conducted after obtaining approval from the Institutional Review Board (IRB) of Korea University Medical Center (IRB No. 2022AS0310).

RESULTS

General characteristics of the study population

A total of 2,961 adults were included in the analysis; 1,289 participants were male (43.5%), and the mean age of the participants was 52.01 (range, 19–82 years). The mean BMI was 25.03 kg/m^2. Among the 2,961 participants, 370 had elevated AST (12.5%), 195 had elevated ALT (6.6%), and 342 had elevated GGT (11.6%). In general, the elevation of liver function indices was more common in males than in females. Additionally, liver dysfunction was more prevalent in the overweight and obese groups. The heavy drinking (take alcohol more than 1–2 times a week) and current smoking groups also had higher levels of AST, ALT, and GGT. Elevation of AST levels was observed in the high-income group, but there were no significant differences in ALT and GGT levels (**Table 1**).

EWAS analysis

Table 2 shows the significant findings of the EWAS study, with an FDR < 0.05. Perfluorooctanesulfonate (PFOS) and perfluorodecanoic acid (PFDeA) were positively

Table 1. General characteristics of subjects

Characteristics	All (n = 2,961)	Dysfunction group					
		AST (n = 370)	*p*-value	ALT (n = 195)	*p*-value	GGT (n = 342)	*p*-value
Sex			< 0.001***		< 0.001***		< 0.01**
Male	1,289 (43.5)	215 (58.1)		134 (68.7)		175 (51.2)	
Female	1,672 (56.5)	155 (41.9)		61 (31.3)		167 (48.8)	
Age	52.01 ± 14.0	56.1 ± 13.6	< 0.001***	47.8 ± 14.9	< 0.001***	53.8 ± 12.8	0.092
BMI			< 0.001***		< 0.001***		< 0.001***
< 23	872 (29.5)	67 (18.1)		10 (5.1)		60 (17.5)	
23–25	679 (22.9)	67 (18.1)		32 (16.4)		68 (19.9)	
≥ 25	1,410 (47.6)	236 (63.8)		153 (78.5)		214 (62.6)	
Drinking			< 0.001***		< 0.05*		< 0.001***
None	912 (30.8)	109 (29.5)		46 (23.6)		72 (21.1)	
Group 1	473 (16.0)	46 (12.4)		23 (11.8)		38 (11.1)	
Group 2	524 (17.7)	52 (14.1)		38 (19.5)		35 (10.2)	
Group 3	588 (19.9)	76 (20.5)		48 (24.6)		70 (20.5)	
Group 4	301 (10,2)	53 (14.3)		26 (13.3)		72 (21.1)	
Group 5	163 (5.5)	34 (9.2)		14 (7.2)		55 (16.1)	
Smoking			< 0.001***		< 0.001***		< 0.001***
Never	1,905 (64.3)	193 (52.2)		94 (48.2)		189 (55.3)	
Quit	593 (20.0)	101 (27.3)		44 (22.6)		66 (19.3)	
Current	463 (15.7)	76 (20.5)		57 (29.2)		87 (25.4)	
Monthly income			< 0.001***		0.301		0.097
Group 1	1,343 (45.4)	208 (56.2)		81 (41.5)		170 (49.7)	
Group 2	1,618 (54.6)	162 (43.8)		114 (58.5)		172 (50.3)	

Values are presented as number (%) or mean ± standard deviation.
Monthly income (average monthly household income over the past year): Group 1, less than 3 million won; Group 2, equal or more than 3 million won.
Drinking: None, people who answered they do not drink; Group 1, people who drinks less than once in a month; Group 2, people who drinks 1–2 times in a month; Group 3, people who drinks 1–2 times in a week; Group 4, people who drinks more than 3 times in a week; Group 5, people who drinks almost everyday.
BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 2. Summary of statistically significant associations with abnormal liver function group

Hazardous substances	OR (95% CI)	*p*-value	FDR
Abnormal AST			
Phthalates			
MEOHP (μg/L)	0.54 (0.39–0.73)	8.40×10^{-5}	3.57×10^{-3}**
Abnormal ALT			
Phthalates			
MEOHP (μg/L)	0.5 (0.34–0.75)	8.46×10^{-4}	3.60×10^{-2}*
Bisphenols			
BPS (μg/L)	0.77 (0.66–0.89)	5.32×10^{-4}	1.51×10^{-2}*
Perfluorinated compounds			
PFOS (μg/L)	2.2 (1.39–3.46)	6.93×10^{-4}	1.47×10^{-2}*
Abnormal GGT			
Heavy metals			
Mercury (plasma) (μg/L)	1.45 (1.14–1.84)	2.59×10^{-3}	3.15×10^{-2}*
Phthalates			
MEOHP (μg/L)	0.55 (0.4–0.76)	2.46×10^{-4}	6.97×10^{-3}**
Perfluorinated compounds			
PFDeA (μg/L)	2.73 (1.36–5.5)	1.50×10^{-3}	2.56×10^{-2}*
Food container			
Plastic (compared to glass)	1.51 (1.16–1.95)	2.13×10^{-3}	1.53×10^{-2}*
Cosmetics			
Wearing sun block cream (≥ 1–2 times per week)	0.25 (0.11–0.55)	7.18×10^{-4}	3.02×10^{-2}*

Among 72 variables, one variable for AST, 3 variables for ALT, 5 variables for GGT were identified as statistically significant association. To correct possible type I error, *p*-values were adjusted with the methodology of FDR. Cut-off value of statistically significant result was FDR < 0.05.
OR: odds ratio; CI: confidence interval; FDR: false discovery rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; MEOHP: 2-ethyl-5-oxohexyl phthalate; BPS: bisphenol S; PFOS: perfluorooctanesulfonate; PFDeA: perfluorodecanoic acid.
*FDR < 0.05; **FDR < 0.01.

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associated with liver function indices. PFOS was positively associated with ALT levels (OR: 2.2; 95% confidence interval [CI]: 1.39–3.46; $p_{adjusted}$ = 0.0147). Furthermore, PFDeA and GGT were positively associated with GGT (OR: 2.73; 95% CI: 1.36–5.5; $p_{adjusted}$ = 0.0256). In the heavy metals category, high plasma mercury levels were significantly associated with higher GGT levels (OR: 1.45; 95% CI: 1.14–1.84; $p_{adjusted}$ = 0.0315). Using a plastic container while keeping food in the refrigerator was associated with elevated GGT compared to using a glass container (OR: 1.51; 95% CI: 1.16–1.95; $p_{adjusted}$ = 0.0153). 2-ethyl-5-oxohexyl phthalate (MEOHP), one of the phthalate metabolites, showed a negative trend with all 3 indices, with AST (OR: 0.54; 95% CI: 0.39–0.73; $p_{adjusted}$ = 0.00357), ALT (OR: 0.5; 95% CI: 0.34–0.75; $p_{adjusted}$ = 0.036), GGT (OR: 0.55; 95% CI: 0.4–0.76; $p_{adjusted}$ = 0.00697). Bisphenol S (BPS), one of the substitute materials for bisphenol A (BPA), showed a negative association with ALT (OR: 0.77; 95% CI: 0.66–0.89; $p_{adjusted}$ = 0.0151). Frequent use of sunblock cream and level of GGT showed a negative association (OR: 0.25; 95% CI: 0.11–0.55; $p_{adjusted}$ = 0.036). The frequent use of sunblock cream group is defined as a group that applies sunblock cream more than once or twice a week. Finally, using the Manhattan plot for EWAS analysis, we identified that one factor, 3 factors, and 5 factors among the 72 environmental exposure variables showed statistically significant associations with AST, ALT, and GGT levels, respectively (**Fig. 2**).

DISCUSSION

This exploratory study aimed to identify the relationship between multiple exposures to chemical agents and human liver function. As mentioned earlier, exposure to chemicals in our daily life has the characteristics of chronic, low-concentration, simultaneous concurrent complicated exposure.[3,4] In this study, we borrowed the idea of the EWAS methodology to reflect these exposure characteristics. As a result, we identified 7 factors that could potentially influence liver function. Among these factors, 4 were identified as potential risk factors for the elevation of liver inflammatory indices.

Since this study is exploratory, matching and confirming our findings with other studies is crucial. Some factors showed a similar trend in association with previous studies; however, other factors showed different results. Plasma mercury level was positively associated with GGT level (OR: 1.45; 95% CI: 1.14–1.84; $p_{adjusted}$ = 0.0315). This trend has been confirmed in many other studies. Seo et al.[20] investigated 1,959 Korean subjects using data from the 2019 Korean National Health and Nutrition Examination Survey and found a positive association between blood mercury concentration and GGT level. In some studies, a high blood mercury level is associated with higher AST and ALT, which means that mercury could cause an overall inflammatory burden on the liver.[21] This elevation of liver inflammatory indices can be explained by mechanisms of oxidative stress, disruption of metabolism, and cell death. Exposure to mercury suppresses various antioxidant mechanisms by amplifying free radical production, resulting in increased protective enzymes such as GGT.[22,23] In addition to oxidative stress in the liver, mercury can potentially interrupt various endocrine mechanisms throughout the human body. A previous study by Hu et al.[24] reported a positive association between mercury and blood pressure. He et al.[25] conducted a prospective cohort study of 3,875 young adults in the US and found that high exposure to mercury in young adulthood may increase the risk of diabetes later in life. In general, mercury exposure is mostly due to organic dietary intake,[26,27] and most environmental exposure remains below the subclinical level, yet various health effects of mercury need to be identified.

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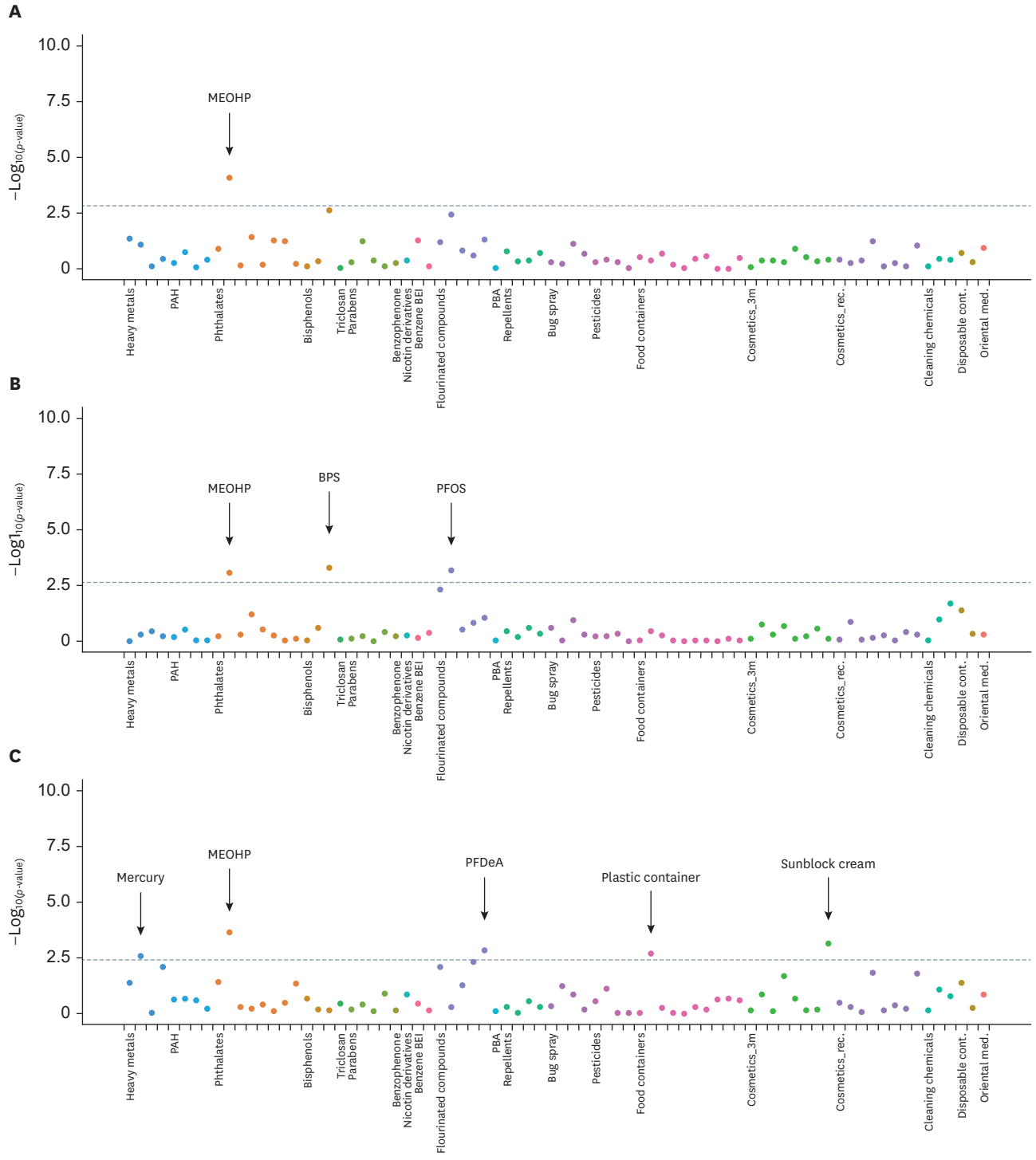

Fig. 2. Manhattan plot of EWAS of environmentally hazardous chemicals for elevated liver enzymes. (A) Manhattan plot of EWAS for elevated aspartate aminotransferase. (B) Manhattan plot of EWAS for elevated alanine aminotransferase. (C) Manhattan plot of EWAS for elevated gamma glutamyl transferase. X-axis shows the groups of categorized chemicals. Y-axis shows $-\log_{10(p\text{-value})}$ of the generalized linear model analysis with each variables with elevated liver enzymes. Each categorized group was represented as same color of dots. The statistically significant cut-off values of false discovery rate < 0.05 were represented as grey dashed line.
MEOHP: 2-ethyl-5-oxohexyl phthalate; PAH: polycyclic aromatic hydrocarbon; PBA: 3-phenoxybenzoic acid; BPS: bisphenol S; PFOS: perfluorooctanesulfonate; PFDeA: perfluorodecanoic acid; EWAS: environment-wide association study; 3m: 3 months; rec.: recent; cont.: container; med.: medicine.

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In the perfluorinated compound category, PFOS showed a positive association with ALT (OR: 2.2; 95% CI: 1.39–3.46; $p_{adjusted}$ = 0.0147), and PFDeA also showed a positive association with GGT (OR: 2.73; 95% CI: 1.36–5.5; $p_{adjusted}$ = 0.0256). Perfluorinated compounds are widely used in industrial and household applications; they are used in various parts of human life, including coating materials for cookware, waterproof and stain-resistant fabrics, various packaging materials, and firefighting materials.[28] Due to its universal use in daily life, its potential health effects on human body have attracted attention. As a result, many studies have been performed on perfluorinated compounds. According to a systematic review and meta-analysis by Costello et al.,[29] which yielded 85 rodent studies and 24 epidemiological studies of humans, the legacy of PFAS showed liver toxicity in humans and rodents. This study showed a positive association between ALT and exposure to various perfluorinated compounds, including perfluorooctanoic acid (PFOA) and PFOS. In animal studies, PFAS showed a disruption of lipid metabolism, resulting in consistent accumulation of lipids and induction of steatosis.[30,31] It is also known that PFOS and PFOA, subfamilies of PFAS, can cause liver enlargement in rodents and hepatocellular adenomas in rats.[32] The peroxisome proliferator-activated receptor-alpha, a major regulator of lipid metabolism in the liver, plays a role in these metabolic disruptions.[33-35] People who used plastic containers for food storage in refrigerators showed higher levels of GGT than those who used glass containers for storage (OR: 1.51; 95% CI: 1.16–1.95; $p_{adjusted}$ = 0.0153). Studies on the direct association between the use of plastic containers and human liver function are difficult to find. However, plastic containers usually contain hazardous substances such as bisphenols, phthalate metabolites, polyvinyl chloride, and perfluorinated alkylated substances.[36,37] Since these chemicals are proven to be hepatotoxic, the use of plastic containers in daily life could affect liver function in this manner.

MEOHP, a phthalate metabolite, showed a negative association with AST (OR: 0.54; 95% CI: 0.39–0.73; $p_{adjusted}$ = 0.00357), ALT (OR: 0.5; 95% CI: 0.34–0.75; $p_{adjusted}$ = 0.036), and GGT (OR: 0.55; 95% CI: 0.4–0.76; $p_{adjusted}$ = 0.00697). Phthalates are widely used as plasticizers and additives in cosmetics.[38] Since phthalates are used in almost all modern industries, studies to determine their health effects on humans have been widely performed. Phthalates are well known for their endocrine-disrupting mechanisms, including suppression of the reproductive tract, childhood obesity, early menarche,[38,39] and other adverse effects in various organs are actively revealed. MEOHP is a metabolite of di-2-ethylhexyl phthalate (DEHP). In general, DEHP has various adverse effects on multiple organs, including the liver.[40] The negative association of MEOHP and liver function indices in this study could be thought to have inconsistent outcomes compared to previous studies. However, some studies showed similar trends in the relationship between GGT and MEOHP.[41] MEOHP is a relatively stable form of DEHP derivatives. Among the metabolites of DEHP, mono (2-ethylhexyl) phthalate (MEHP), which can be converted to MEOHP through hydroxylation and oxidation, has the most bioactive traits.[42] Therefore, higher levels of MEOHP could indicate that an individual's ability to convert more bioactive substances (MEHP) to relatively less toxic substances (MEOHP) is high, resulting in a lower inflammatory index of the liver (GGT).[41,43,44]

BPS showed a negative association with the level of ALT (OR: 0.77; 95% CI: 0.66–0.89; $p_{adjusted}$ = 0.0151). As BPA is well known for its toxic effects on human health, BPS was introduced as a substitute. However, many in vitro studies have recently shown that BPS has toxic effects on various organs, including the liver.[45] Our study showed different trends from these studies; we could not find solid evidence that could support our results. This might be because there are always possibilities of inconsistency in the trends of the results due to differences in study

design or populations or methods to evaluate the level of exposure.[41] Therefore, more studies are crucial. The frequency of sunblock cream use over the last 3 months showed a negative relationship with the level of GGT (OR: 0.25; 95% CI: 0.11–0.55; $p_{adjusted}$ = 0.036). Studies investigating the association between sunblock cream use and human liver function are not readily available. There has been a report that the use of sunscreens under sun radiation shows elevated ALT and AST in the animal study.[46] In general, sunlight is believed to have anti-inflammatory effects on the liver by the production of active vitamin D (1,25(OH)2D).[47] This trend could be partially inconsistent with our results. However, in our study, we defined the high-exposure group to sunblock cream based on a questionnaire asking, "How often did you wear sunblock cream for the last 3 months?" We could not quantify the actual use of chemicals or the actual amount of sunlight that is exposed by these questions. This could be why our result was somewhat inconsistent with previous literature and, at the same time, could be one of the limitations of this study.

One of the strengths of this study, in EWAS, is that we could investigate global and comprehensive patterns of association between various factors and certain diseases. Traditional epidemiologic approaches to diseases have focused on the relationship between specific factors or a very limited number of factors and diseases. The EWAS methodology could provide a global view of these associations.[16] Furthermore, visualization of the result with a Manhattan plot could strengthen this study by comprehensively skimming the relationships between factors. For example, in **Fig. 2C**, the *p*-values of the heavy metals and fluorinated compounds are distributed near the set point of FDR of 0.05. These variables did not reach the cut-off value of statistical significance (FDR < 0.05), but we still have an overall understanding that the components of these groups tend to have a higher association with liver function.

This study has some limitations. First, since this was a cross-sectional study, the causal inference of the results would be based on an assumption. Second, many variables in this study were based on questionnaires. We tried to indirectly determine individuals' life patterns or habits, implying their exposure to chemicals. Questionnaires are the only way to evaluate the life patterns and habits of individuals. However, questionnaires have the possibility of recall bias. Third, again from the point of questionnaires, although we designed the variables into a binary classification based on frequency, the responses to the questionnaires cannot exactly reflect the actual amount of exposure. Therefore, there is the possibility of an unclear or partial disruption in the analysis. EWAS studies usually require a large number of variables, our study included 72 variables, of which only 34 were data from blood and urine analyses. This is a relatively low number of variables compared to large data sets such as the National Health and Nutrition Examination Survey in the US. However, we attempted to perform an exploratory analysis based on the Korean population; therefore, the KoNEHS was the best survey data we could use. Finally, as mentioned earlier, our dataset does not contain a sufficient number of study subjects to perform a validation process. The absence of a validation step could show limitations in securing the replicability of the analysis.

CONCLUSIONS

In conclusion, we applied the EWAS methodology to investigate the association of various environmentally hazardous chemicals with human liver function in the Korean population using the 4th KoNEHS data. Seven potential risk factors were identified. Exploratory studies on various diseases can be carried out by applying the EWAS frame.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

List of environmentally hazardous substances analyzed with blood and urine samples

Click here to view

Supplementary Table 2

List of questionnaires

Click here to view

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