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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Environmental factors, medical and family history, and comorbidities associated with primary biliary cholangitis in Japan: a multicenter case–control study

Kosuke Matsumoto[1] · Satoko Ohfuji[2] · Masanori Abe[3] · Atsumasa Komori[4] · Atsushi Takahashi[5] · Hideki Fujii[6,7] · Kazuhito Kawata[8] · Hidenao Noritake[8] · Tomoko Tadokoro[9] · Akira Honda[10] · Maiko Asami[11] · Tadashi Namisaki[12] · Masayuki Ueno[13,14] · Ken Sato[15] · Keisuke Kakisaka[16] · Mie Arakawa[17] · Takanori Ito[18] · Kazunari Tanaka[19] · Takeshi Matsui[19] · Toru Setsu[20] · Masaaki Takamura[20] · Satoshi Yasuda[21] · Tomohiro Katsumi[22] · Jun Itakura[23] · Tomoya Sano[24] · Yamato Tamura[1] · Ryo Miura[1] · Toshihiko Arizumi[1] · Yoshinari Asaoka[1] · Kiyoko Uno[25] · Ai Nishitani[25] · Yoshiyuki Ueno[22] · Shuji Terai[20] · Yasuhiro Takikawa[16] · Youichi Morimoto[13] · Hitoshi Yoshiji[12] · Satoshi Mochida[11] · Tadashi Ikegami[10] · Tsutomu Masaki[9] · Norifumi Kawada[7] · Hiromasa Ohira[5] · Atsushi Tanaka[1]

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Abstract

Background Primary biliary cholangitis (PBC) is considered to be caused by the interaction between genetic background and environmental triggers. Previous case–control studies have indicated the associations of environmental factors (tobacco smoking, a history of urinary tract infection, and hair dye) use with PBC. Therefore, we conducted a multicenter case–control study to identify the environmental factors associated with the development of PBC in Japan.

Methods From 21 participating centers in Japan, we prospectively enrolled 548 patients with PBC (male/female = 78/470, median age 66), and 548 age- and sex-matched controls. These participants completed a questionnaire comprising 121 items with respect to demographic, anthropometric, socioeconomic features, lifestyle, medical/familial history, and reproductive history in female individuals. The association was determined using conditional multivariate logistic regression analysis.

Results The identified factors were vault toilet at home in childhood [odds ratio (OR), 1.63; 95% confidence interval

✉ Kosuke Matsumoto
m-kosuke0716@med.teikyo-u.ac.jp

1 Department of Medicine, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo, Japan

2 Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan

3 Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Ehime, Japan

4 Clinical Research Center, National Hospital Organization (NHO) Nagasaki Medical Center, Nagasaki, Japan

5 Department of Gastroenterology, Fukushima Medical University School of Medicine, Fukushima, Japan

6 Department of Premier Preventive Medicine, Graduate School of Medicine, Osaka City University, Osaka, Japan

7 Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, Japan

8 Hepatology Division, Department of Internal Medicine II, Hamamatsu University School of Medicine, Shizuoka, Japan

9 Department of Gastroenterology and Neurology, Kagawa University School of Medicine, Kagawa, Japan

10 Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan

11 Department of Gastroenterology and Hepatology, Faculty of Medicine, Saitama Medical University, Saitama, Japan

12 Department of Gastroenterology, Nara Medical University, Nara, Japan

13 Department of Gastroenterology and Hepatology, Kurashiki Central Hospital, Okayama, Japan

(CI), 1.01–2.62], unpaved roads around the house in childhood (OR, 1.43; 95% CI, 1.07–1.92), ever smoking (OR, 1.70; 95% CI, 1.28–2.25), and hair dye use (OR, 1.57; 95% CI, 1.15–2.14) in the model for lifestyle factors, and a history of any type of autoimmune disease (OR, 8.74; 95% CI, 3.99–19.13), a history of Cesarean section (OR, 0.20; 95% CI, 0.077–0.53), and presence of PBC in first-degree relatives (OR, 21.1; 95% CI, 6.52–68.0) in the model for medical and familial factors.

Conclusions These results suggest that poor environmental hygiene in childhood (vault toilets and unpaved roads) and chronic exposure to chemicals (smoking and hair dye use) are likely to be risk factors for the development of PBC in Japan.

Keywords Risk factors · Environment · Autoimmunity · Smoking · Environmental hygiene

Abbreviations

PBC	Primary biliary cholangitis
OR	Odds ratio
CI	Confidence interval
AMA	Antimitochondrial autoantibodies
GWAS	Genome-wide association studies
UTI	Urinary tract infection
FDR	First-degree relative
BMI	Body mass index

Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease that can potentially progress to cirrhosis and liver failure in the absence of appropriate treatment [1]. Although the etiology of PBC has not been fully elucidated, robust evidence indicates that autoimmune reactions targeting intrahepatic biliary epithelial cells play a critical role in the pathogenesis of the disease [2]. Indeed, PBC is considered a model autoimmune disease because of the presence of disease-specific antimitochondrial autoantibodies (AMAs), an intense infiltration of mononuclear cells damaging the bile ducts, and a high prevalence of coincident autoimmune disorder [3].

As with other autoimmune diseases, PBC is a multifactorial disease and is considered to be caused by the interaction of both genetic background and environmental triggers [4, 5]. Epidemiological studies have provided genetic evidence based on familial clustering and they also suggest that PBC results from the combination of "bad genes and bad luck [6]". To determine the genetic basis of PBC, several genome-wide association studies (GWAS) conducted in North America, Europe, Japan, and China have identified numerous non-human leukocyte antigen risk loci contributing to the susceptibility of PBC [7–18], and a recent GWAS that globally combined samples from more than 10,000 patients with PBC revealed additional risk loci for PBC [19]. Furthermore, X chromosome-wide association studies including genotype data from Italy, the UK, Canada, China, and Japan identified significant loci on the X chromosome, which may be implicated in the female preponderance of PBC [20].

On the other hand, efforts have also been made to identify environmental factors that trigger the development of PBC in genetically susceptible individuals [21, 22]. While the number of studies is not ample; there were two studies from the UK [23, 24], two from the United States (US) [25, 26], one from France [27], and one from Korea that were carried out in the past [28]. All these were case–control studies, employing patients with PBC and sex- and age-matched controls, and they took advantage of ques-

[14] Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[15] Department of Gastroenterology and Hepatology, Gunma University Graduate School of Medicine, Gunma, Japan

[16] Division of Hepatology, Department of Internal Medicine, Iwate Medical University, Iwate, Japan

[17] Department of Gastroenterology, Faculty of Medicine, Oita University, Oita, Japan

[18] Department of Gastroenterology and Hepatology, Nagoya University Graduate School of Medicine, Aichi, Japan

[19] Center for Gastroenterology, Teine-Keijinkai Hospital, Hokkaido, Japan

[20] Division of Gastroenterology and Hepatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[21] Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, Gifu, Japan

[22] Department of Gastroenterology, Yamagata University Faculty of Medicine, Yamagata, Japan

[23] Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan

[24] Division of Gastroenterology, Department of Internal Medicine, Kurume University School of Medicine, Fukuoka, Japan

[25] Teikyo Academic Research Center, Teikyo University, Tokyo, Japan

tionnaires with respect to demographic, lifestyle, medical and familial factors, and reproductive history in women. All these studies have revealed associations between some constitutive and environmental factors. Among them, family history of PBC and a history of or current tobacco smoking were identified in all studies despite their geographic regions [23–28]. In addition, hair dye use and a history of recurrent urinary tract infection (UTI) have been repeatedly acknowledged as risk factors for PBC [24, 25, 27]. These results indicate that genetic predisposition (family history of PBC), chronic exposure to bacterial infection (a history of recurrent UTI), and chronic exposure to environmental chemicals (tobacco smoking and hair dye use) are likely to be involved in the development of PBC.

Nevertheless, environmental triggers may differ significantly depending on geographic regions, and no case–control studies to clarify environmental triggers have been conducted in Japan. Therefore, we conducted a case–control study of patients with PBC and sex- and age-matched controls accordingly.

Patients and methods

Study design and participants

A multicenter, case–control study was conducted at 21 collaborating centers in Japan between September 2020 and July 2021. The current case–control study was conducted in a similar manner as that of our previous studies [29–32]. Eligible cases were patients who were diagnosed with PBC aged $\geq$ 20 years. PBC was diagnosed according to the criteria established by the Japanese Intractable Hepatobiliary Disease Study Group [33]. The patients with PBC were consecutively asked to participate in this study at the outpatient clinic or during admission, and 548 patients with PBC completed and returned the questionnaires with respect to demographic and lifestyle factors and past and family history, and reproductive history in women, after giving their informed consent. Overlap cases with autoimmune hepatitis and cases with viral hepatitis as comorbidities were excluded from this study. However, PBC patients with other etiologies associated with lifestyle (alcoholic liver disease and non-alcoholic fatty liver disease) were included in the study.

As a control, 548 age- and sex-matched individuals (range of 5 years) who visited the same center at the same time (within 2 months) for a disease other than PBC and any autoimmune disease were invited to participate in the study. Each cooperating center was asked to provide at least one pair of participants, the case and control. The current study was approved by the ethical committee of Teikyo University (#20-060).

Information collection

The patient's self-administered questionnaires included 121 items, as follows: (1) demographic, anthropometric, and socioeconomic features; (2) lifestyle; (3) past history of autoimmune diseases, non-autoimmune diseases, surgery, and vaccinations; (4) history of autoimmune diseases in first-degree relatives (FDRs); (5) reproductive history in female individuals. Most of these items were included in the questionnaires since previous studies had utilized these and identified some of them as environmental factors that were significantly associated with PBC. In addition, several items (vault toilet at home in childhood, Japanese-style toilet at home in childhood, ditches around the house in childhood, and unpaved roads around the house in childhood) were included in the current study to adjust with the Japanese environment. Details of the questionnaires are shown in Tables 1, 2, 3, 4, 5, and 6, and Supplementary Table 1.

Statistical analysis

Comparisons between cases and controls for potential associations were conducted using the nonparametric Wilcoxon test for continuous variables and the Chi-square test and Fisher's exact test for categorical variables. Factors with statistically significant differences in unadjusted bivariate analyses were entered into backward stepwise conditional multiple logistic regressions. We developed two different models as in a previous study [27]: the first was a model for lifestyle factors, and the second was a model for factors in terms of medical and familial history. Each model was adjusted for putative explanatory variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine significance.

All analyses were two-sided, and the level of significance was set at $P < 0.05$. Continuous variables are expressed as median [interquartile range (IQR)], and categorical variables are expressed as percentages. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0.

Results

Demographic, anthropometric, and socioeconomic features

The demographic, anthropometric, and socioeconomic features reported by the PBC cases and controls are shown

Table 1 Demographic, anthropometric, and socioeconomic features reported by PBC cases and controls

	Cases	Controls	*p* value
Sex (female)	470/548 (86%)	470/548 (86%)	NS
Age (year)	66 (14)	66 (14)	NS
20–	0	1	NS
30–	5	7	
40–	30	51	
50–	111	105	
60–	188	179	
70–	185	181	
80–	29	24	
Height (cm)	155 (10)	155 (9)	NS
Weight (kg)	53 (13)	56 (14.4)	< 0.001
BMI (kg/m^2)	22.3 (4.2)	23.3 (5.2)	< 0.001
Weight gain (kg)	3 (10)	5 (11.5)	< 0.001
Blood type (A/B/AB/O)	206/107/68/166	221/109/55/162	NS
Education level			NS
University or higher	49/542 (9%)	68/548 (12%)	
Others	493/542 (91%)	480/548 (88%)	
Annual household income			NS
< Average[a]	238/501 (48%)	240/503 (48%)	
≈ Average	146/501 (29%)	132/503 (26%)	
> Average	117,501 (23%)	131/503 (26%)	

Continuous and categorical variables are shown as medians (interquartile ranges) and numbers (percentages)

NS not significant, *BMI* body mass index

[a]Average of annual household income in 2021 is 4,410,000 Japanese Yen/year

in Table 1. No significant difference was observed in terms of sex (male/female = 78/478 in both groups) or age (median: 66 years in both groups). Although height was similar, body weight (53 kg vs. 56 kg, $p < 0.001$), body mass index (BMI) (22.3 vs. 23.3%, $p < 0.001$), and weight gain from the weight at 20 years old (3 kg vs. 5 kg, $p < 0.001$) were significantly lower in PBC patients than in controls. Blood type, education level (university or higher, or others), and annual household income were compared to the average in Japan (4,410,000 Japanese Yen/year) and they did not differ between cases and controls.

Lifestyle factors

Comparisons between cases and controls in terms of various lifestyle factors potentially associated with PBC are shown in Table 2. The proportion of those with breast feeding tended to be higher in PBC; however, the difference was not significant (88% vs. 84%, $p = 0.060$). Among items regarding environmental hygiene during childhood, the proportion of participants with a vault toilet ("kumitori" type in Japanese; toilet with a hole below and not a flush type) at home in childhood was significantly higher in PBC cases than in controls (93% vs. 89%, $p = 0.021$). In addition, more individuals in cases had unpaved roads around the house in childhood than in controls (71% vs. 62%, $p = 0.003$). Alcohol consumption (14 drinks/week in men and 7 drinks/week in women) were similar between the two groups, while current drinkers were lower in cases (19% vs. 27%). Ever smokers (in the past and/or current) were significantly higher in cases than in controls (40% vs. 31%, $p = 0.005$), and number of those participants who were exposed to second-hand smoke did not differ between the groups. The proportion of those who reported as consuming coffee daily and to frequently use hair dye (2 times or more/year) was significantly higher in cases than in controls (78% vs. 71%, $p = 0.010$), for coffee, and though not significant, it tended to be higher in cases for hair dye (77% vs. 72%, $p = 0.071$), respectively. We failed to find any significant differences in the use of hair perm and nail polish.

Medical history

In Tables 3, 4, and 5, we demonstrated the results of comparisons between cases and controls: autoimmune

Table 2 Lifestyle factors reported by PBC cases and controls

	Cases	Controls	*p* value
Breast feeding	423/478 (88%)	402/477 (84%)	0.060
Vault toilet at home in childhood	502/541 (93%)	479/540 (89%)	0.021
Japanese-style toilet at home in childhood	520/544 (96%)	510/546 (93%)	NS
Ditches around the house in childhood	321/491 (65%)	324/502 (65%)	NS
Unpaved roads around the house in childhood	373/524 (71%)	328/526 (62%)	0.003
Alcohol			
Never	243/548 (44%)	236/548 (43%)	0.005
Past	199/548 (36%)	164/548 (30%)	
Current	106/548 (19%)	148/548 (27%)	
Alcohol, ever	305/548 (56%)	312/548 (57%)	NS
Smoking			
Never	331/548 (60%)	376/548 (69%)	0.011
Past	168/548 (31%)	126/548 (23%)	
Current	49/548 (9%)	46/548 (8%)	
Smoking, ever	217/548 (40%)	172/548 (31%)	0.005
Second-hand smoke exposure, ever	217/548 (40%)	172/548 (31%)	0.005
Coffee consumption	429/548 (78%)	391/548 (71%)	0.010
Hair dye use	423/548 (77%)	396/548 (72%)	0.071
Hair perm	164/548 (30%)	179/548 (33%)	NS
Nail polish	146/548 (27%)	145/548 (27%)	NS

Categorical variables are shown as number (percentage)

NS not significant

Table 3 History of autoimmune diseases reported by PBC cases and controls

	Cases	Controls	*p* value
Sjogren syndrome	69/520 (13%)	3/523 (0.6%)	< 0.001
Scleroderma	42/522 (8%)	0/529 (0%)	< 0.001
Hashimoto's disease	39/524 (7%)	15/532 (3%)	< 0.001
Raynaud syndrome	38/522 (7%)	1/525 (0.2%)	< 0.001
Rheumatoid arthritis	33/528 (6%)	19/539 (4%)	0.046
Multiple myositis	6/524 (1%)	0/529 (0%)	0.015
Systemic lupus erythematosus	4/511 (0.8%)	1/532 (0.2%)	NS
Multiple sclerosis	3/526 (0.6%)	1/529 (0.2%)	NS
Any type	158/548 (29%)	38/548 (7%)	< 0.001

Diseases are sorted in decreasing order of frequency in PBC group. Categorical variables are shown as number (percentage)

NS not significant

diseases (Table 3), non-autoimmune diseases (Table 4), surgery and vaccinations (Table 5). As expected, a history of common autoimmune diseases was found more frequently in cases than in controls, and any type of autoimmune disease was found in 29% of cases and 7% of controls ($p < 0.001$) (Table 3). Among various non-autoimmune diseases, we noticed a higher prevalence in cases than in controls in the history of herpes zoster (29% vs. 21%, $p = 0.002$), tuberculosis (3% vs. 1%, $p = 0.011$), diphtheria (1% vs. 0%, $p = 0.015$), and emphysema (2% and 0.4%, $p = 0.022$), and lower prevalence of malignant diseases in cases (12% vs. 21%, $p < 0.001$) (Table 4). We failed to find any significant difference in the reported frequency of UTI (30% vs. 28%, $p = 0.684$), which has been frequently identified as a risk factor for PBC in previous studies [24–27]. In terms of previous surgery, cesarean section was the only surgery differentially reported between the two groups, less frequently in cases than in controls (9% vs. 14%, $p = 0.022$) (Table 5). Finally, among the number of vaccinations, the percentages of

Table 4 History of non-autoimmune diseases reported by PBC cases and controls

	Cases	Controls	*p* value
Infectious disease			
Measles	257/403 (64%)	253/400 (63%)	NS
Mumps	236/384 (62%)	226/386 (59%)	NS
Chickenpox	178/359 (50%)	212/387 (55%)	NS
Rubella	128/367 (35%)	148/375 (40%)	NS
Urinary tract infection	156/526 (30%)	149/526 (28%)	NS
Herpes Zoster	153/528 (29%)	110/536 (21%)	0.002
Vaginitis	38/451 (8%)	32/445 (7%)	NS
Pertussis	17/504 (3%)	11/495 (2%)	NS
Tuberculosis	15/526 (3%)	4/531 (1%)	0.011
Diphtheria	7/522 (1%)	0/515 (0%)	0.015
Shobeni fever	3/511 (0.6%)	3/505 (0.6%)	NS
Rheumatic fever	2/518 (0.4%)	3/519 (0.6%)	NS
Malaria	1/531 (0.2%)	0/538 (0%)	NS
Others			
Pollen allergy	176/517 (34%)	83/519 (35%)	NS
Malignant disease	60/514 (12%)	107/505 (21%)	< 0.001
Asthma	39/522 (8%)	47/532 (9%)	NS
Endometriosis	29/456 (6%)	35/452 (8%)	NS
Atopic dermatitis	25/521 (5%)	21/526 (4%)	NS
Chronic bronchitis	19/517 (4%)	22/517 (4%)	NS
Psoriasis	14/509 (3%)	10/515 (2%)	NS
Chronic heart failure	11/539 (2%)	9/537 (2%)	NS
Cerebral infarction	10/543 (2%)	19/539 (4%)	0.093
Emphysema	11/535 (2%)	2/534 (0.4%)	0.022
Cerebral hemorrhage	5/541 (1%)	7/540 (1%)	NS

Diseases are sorted in decreasing order of frequency in PBC group. Categorical variables are shown as number (percentage)

NS not significant

reported vaccinations against measles (38% vs. 46%, $p = 0.042$), polio (24% vs. 33%, $p = 0.010$), pertussis (11% vs. 18%, $p = 0.010$), and hepatitis B (4% vs. 9%, $p = 0.007$) were significantly lower in cases than in controls (Table 5).

History of autoimmune disease in FDRs

The history of autoimmune disease in FDRs is shown in Table 6. As expected, and in keeping with previous studies, PBC in FDRs was more frequently reported in PBC cases than in controls (13% vs. 1%, $p < 0.001$). Other autoimmune diseases that exhibited a significant difference between cases and controls were rheumatoid arthritis (7% and 3%, $p = 0.001$) and Sjogren syndrome (2% and 0%, $p < 0.001$).

Reproductive history

Finally, the reproductive histories of female PBC cases and controls are shown in Supplementary Table 1. We failed to detect any significant differences in menarche age, menopausal age, number of pregnancies, or any event or factor between cases and controls.

Multivariate logistic regression analysis

Factors identified as significant in the univariate analysis were entered into conditional backward stepwise multiple logistic regressions for adjustment of explanatory variables. We developed two different models: the first was a model for lifestyle factors adjusted for age, sex, BMI, breast feeding, alcohol consumption, second-hand smoke exposure, daily coffee consumption, hair perm, and nail polish. As shown in Table 7, identified factors after adjustment were vault toilet at home in childhood (OR,

Table 5 History of surgery and vaccinations reported by PBC cases and controls

	Cases	Controls	*p* value
Surgery			
Appendectomy	124/540 (23%)	135/543 (25%)	NS
Cesarean section	41/466 (9%)	64/467 (14%)	0.022
Tonsillectomy	24/538 (5%)	18/538 (3%)	NS
Cholecystectomy	29/539 (5%)	37/542 (7%)	NS
Thyroidectomy	14/543 (3%)	18/544 (3%)	NS
Ulcer operation	8/536 (2%)	9/540 (2%)	NS
Vaccination			
Influenza	421/511 (82%)	443/523 (85%)	NS
BCG	392/492 (80%)	403/500 (81%)	NS
Measles	122/323 (38%)	160/351 (46%)	0.042
Rubella	103/343 (30%)	109/347 (31%)	NS
Polio	81/335 (24%)	112/336 (33%)	0.010
Mumps	65/324 (20%)	82/334 (25%)	NS
Diphtheria	54/337 (16%)	63,328 (%)	NS
Tetanus	57/00 (14%)	75/405 (19%)	NS
Pertussis	38/348 (11%)	64/361 (18%)	0.010
Hepatitis B	21/472 (4%)	44/493 (9%)	0.007
Typhoid fever	6/418 (1%)	8/424 (2%)	NS
Meningococcal	5/417 (1%)	8/416 (2%)	NS
Hepatitis A	3/471 (0.6%)	2/491 (0.4%)	NS
Yellow fever	2/423 (0.5%)	4/431 (0.9%)	NS
Cervical cancer	1/420 (0.2%)	3/433 (0.7%)	NS

Surgeries and vaccinations are sorted in decreasing order of frequency in PBC group. Categorical variables are shown as number (percentage)

NS not significant

1.63; 95% CI, 1.01–2.62, $p = 0.046$), unpaved roads around the house in childhood (OR, 1.43; 95% CI, 1.07–1.92, $p = 0.016$), ever smoking (OR, 1.70; 95% CI, 1.28–2.25, $p < 0.001$), and hair dye use (OR, 1.57; 95% CI, 1.15–2.14, $p = 0.004$) in the model for lifestyle factors. In the second model for medical and familial factors adjusted for age, sex, BMI, and other significant factors in medical/familial history, a history of any type of autoimmune disease (OR, 8.74; 95% CI, 3.99–19.13, $p < 0.001$), a history of cesarean section (OR, 0.20; 95% CI, 0.077–0.53, $p = 0.001$), and presence of PBC in FDRs (OR, 21.1; 95% CI, 6.52–68.0, $p < 0.001$) were identified as factors after adjustment that were significantly associated with PBC.

Subanalysis in participants under 55 years of age

Furthermore, since vault toilets and unpaved roads had almost disappeared in the environment around 1970 in Japan, we conducted a subanalysis in participants under 55 years of age ($n = 196$, 98 cases and 98 controls). As expected, the proportion of vault toilets at home and unpaved roads around the house in childhood greatly decreased in both cases and controls among participants under 55 years of age; the proportion of vault toilets at home in all participants and in participants under 55 years old sharply decreased from 93 to 75% in cases and from 89 to 66% in controls, respectively. The proportion of unpaved roads also decreased from 71 to 31% in cases and from 62 to 23% in controls. As a result, the proportion of lifestyle factors did not differ between cases and controls, except for those associated with smoking and alcohol

Table 6 History of autoimmune diseases in first-degree relatives reported by female PBC cases and controls

	Cases	Controls	*p* value
PBC	68/515 (13%)	4/519 (1%)	< 0.001
Rheumatoid arthritis	34/512 (7%)	13/527 (3%)	0.001
Hashimoto's disease	14/514 (3%)	10/523 (2%)	NS
Sjogren syndrome	10/99 (2%)	0/520 (0%)	< 0.001
Alcoholic liver disease	11/503 (2%)	20/527 (4%)	NS
Autoimmune hepatitis	5/499 (1%)	8/510 (2%)	NS
Systemic lupus erythematosus	4/505 (1%)	2/524 (0.4%)	NS
Raynaud syndrome	4/504 (1%)	3/521 (1%)	NS
Psoriasis	6/485 (1%)	3/503 (0.6%)	NS
Scleroderma	6/505 (1%)	1/523 (0.2%)	0.065
Multiple sclerosis	2/510 (0.4%)	1/525 (0.2%)	NS
Multiple myositis	0/508 (0%)	0/527 (0%)	NS

Diseases are sorted in decreasing order of frequency in PBC group. Categorical variables are shown as number (percentage)

NS not significant

Table 7 Conditional multiple logistic regression analyses

Lifestyle factors	OR (95% CI)	*p* value
All subjects (*n* = 1096, 548 PBC and 548 controls)		
Vault toilet at home in childhood	1.63 (1.01–2.62)	0.046
Unpaved roads around the house in childhood	1.43 (1.07–1.92)	0.016
Smoking, ever	1.70 (1.28–2.25)	< 0.001
Hair dye use	1.57 (1.15–2.14)	0.004
Medical and family history		
Autoimmune disease, any type	8.74 (3.99–19.13)	< 0.001
Cesarean section	0.20 (0.077–0.53)	0.001
PBC in FDRs	21.1 (6.52–68.0)	< 0.001
Subjects under 55 years old (n = 196, 98 PBC and 98 controls)		
Smoking, ever	2.35 (1.21–4.58)	0.012

OR odds ratio, *CI* confidence interval

drinking (Supplementary Table 2). A multivariate logistic regression analysis for the first lifestyle model in subjects under 55 years old demonstrated that smoking was identified as significant (OR, 2.35; 95% CI, 1.21–4.58, $p = 0.012$), and other factors identified in all participants (vault toilet at home in childhood, unpaved roads around the house in childhood, hair dye use) were not significant (Table 7).

Discussion

In the current study, we conducted a multicenter case–control study of 548 patients with PBC and 548 controls from 21 centers in Japan to clarify environmental factors, medical and family history, and comorbidities associated with PBC. The results of this study revealed that vault toilet at home in childhood, unpaved roads around the house in childhood, ever smoking, and hair dye use, a history of any type of autoimmune disease, a history of cesarean section, and presence of PBC in FDRs were significantly associated with PBC. In particular, these results raised the possibility that poor environmental hygiene in childhood (vault toilets and unpaved roads) and chronic exposure to chemicals (smoking and hair dye use) are likely to be risk factors for developing PBC in genetically susceptible individuals.

We consecutively invited patients with PBC and controls in each participating center and obtained questionnaire data from 548 PBC cases and 548 controls. The sample size was fairly large, specifically it was the second one among previous studies, that is after the study by Gershwin et al. [25]. This large number was an advantage of this study. Furthermore, participating centers were dispersed throughout Japan, from Hokkaido to Kyushu. Since pairs of sex- and age-matched cases and controls were recruited from each center, geographical selection bias was minimized in the current study.

Anthropometric data showed that patients with PBC had significantly lower body weight and BMI than controls. This finding was also observed in a French study [27], although body height was similar between the two groups in our study. It is uncertain why patients with PBC had lower body weight, but this could be possibly attributable to chronic intestinal malabsorption due to reduced bile excretion [27] or better living practice of PBC patients with higher health consciousness. There was no significant difference in education level or annual household income between the cases and controls. Therefore, we decided to include BMI in addition to age and sex as adjusted covariates in the multivariate analyses.

One of the new findings of this study is the identification of poor environmental hygiene in childhood (vault toilet and unpaved roads) as risk factors for the development of PBC. The association of recurrent history of UTI with PBC has been frequently confirmed by previous case–control studies, despite variations in geographic area or case-finding methods [24–27]. *E. coli* is a predominant pathogen in most cases of UTI, and it is well known that *E. coli* infection is a key factor in breaking immunological tolerance against the mitochondria through molecular mimicry between the human and *E. coli* E2 subunit of the 2-oxo-acid dehydrogenase complexes, which are the main autoantigens of AMA, resulting in the production of AMA and disease-specific autoantibodies of PBC [34]. Although the frequency of UTI did not differ between cases and controls in the current study, both vault toilets and unpaved roads were likely to elicit chronic exposure to microbial pathogens, including *E. coli*. Therefore, the association of these two factors with PBC indicates an important role of microbial pathogens in the pathogenesis of the disease, such as its associations with UTI. Nevertheless, as lifestyle

has been rapidly westernized all over Japan since the 1960s, vault toilets and unpaved roads have disappeared in Japanese daily life. Based on this, we performed the comparison between cases and controls in participants who were under 55 years old in this study, and who were born after 1965. We found that the number of participants with vault toilets at home or unpaved roads around the houses in childhood sharply decreased, and a significant difference in terms of vault toilets and unpaved roads was not observed in participants under 55 years old in the univariate and multivariate analyses. Therefore, chronic exposure to microbial pathogens through poor environmental hygiene becomes important before, but not later on, along with improvement of environmental hygiene.

Our results confirm the relationship between PBC and smoking, as previously demonstrated by studies in the US, UK, France, and Korea [24, 25, 27, 28]. The validation of the link between PBC and smoking across different ethnicities and geographical areas is robust evidence to strongly support the hypothesis that inhaled chemical substances from tobacco may provoke breaking immunological tolerance. Indeed, this role of tobacco has been suggested in other autoimmune diseases [35–37]. In addition, we also confirmed the association of hair dye use with PBC, as shown in the US and UK studies [24, 25]. These observations prompted researchers to examine environmental mimotopes in the form of xenobiotics, and had led to the hypothesis that xenobiotic modification of pyruvate dehydrogenase complex-E2, a major autoantigen of AMA, with chemicals abundantly found in daily life, plays a role in generating immunogenic neoantigens and breaking of tolerance in PBC [22].

It is of note that multiple logistic regression analyses in participants under 55 years of age in the current study indicated only ever smoking as significant, neither environmentally poor hygiene nor hair dye use. We conducted a nationwide epidemiological study in 2016, that revealed an increase in the prevalence of PBC as well as the male-to-female ratio [38]. It is reasonable to assume that environmental risk factors triggering PBC may have changed over time, resulting in epidemiological alterations in Japan. We successfully demonstrated that environmental factors regarding poor hygiene have disappeared in patients under 55 years, but failed to identify newly emerging environment factors in this population except for smoking, such as xenobiotics suggested in the recent studies from the UK [39, 40]. Indeed, Probert et al. demonstrate a man-made chemical present in soils around a waste site is capable of breaking immunological tolerance through molecular mimicry and elicit autoimmunity and hepatocellular damage [40]. As the number and types of chemicals in our daily life greatly increased in these days, environmental xenobiotics definitely have become more important as triggering factors for PBC, instead of poor hygiene, and contributing to recent increase of the prevalence of PBC. Both sexes are equally exposed to these xenobiotics, presumably explaining relative increase of male patients with PBC.

In terms of medical, familial, and reproductive history, we observed a history of autoimmune disease and cesarean section, and the presence of PBC in FDRs. It is not surprising that autoimmune disease in the past and PBC in FDRs are significantly associated with PBC. Frequent occurrence of other autoimmune diseases, such as comorbidities and familial clustering, is well known in patients with PBC. The overall prevalence of PBC in FDRs was 13% in the current study, the highest among previous studies from the US, France, and Korea, in which the prevalence of PBC in FDR ranged from 3 to 6% [25–28]. It is unknown whether this high proportion in Japan is attributable to "real" more intensive clustering or better recognition and examination of PBC in FDRs.

This study has several limitations. First, the participants were asked to fill out self-administered questionnaires; hence, a recall bias was inevitably present in the study. However, a relatively large sample size may compensate for this limitation. Second, since all collaborating centers were tertiary and referral centers, participants may not be representative of patients with PBC in general. However, PBC is a relatively rare disease in Japan, and hence, patients tend to be introduced to these referral centers. We can thus assume that 548 patients with PBC in the current study are likely to reflect the whole figure of PBC in Japan. Third, we do not know the onset or development (not diagnosis date) of PBC, and therefore, it is extremely difficult to determine the temporal relationship and causality of environmental factors indicated as significant in this study with the onset of PBC. Nevertheless, poor environmental hygiene in childhood (vault toilets and unpaved roads) is likely to lead to the onset of PBC, and we can assume these factors as risk factors accordingly. Furthermore, in terms of smoking and hair dye use, we can say that these two are risk factors for the development of PBC, since it is common to start smoking or hair dye use long before the age of 50–60 years, which is the mean age of PBC diagnosis in Japan.

In conclusion, we demonstrated that environmental poor hygiene (vault toilets and unpaved roads) in childhood and chronic exposure to chemicals (smoking and hair dye use) are likely to be risk factors for developing PBC These results were sound and coincident with previous case–control studies conducted in different ethnicities and geographic areas, thus strongly supporting the importance of these findings. Further studies are warranted to clarify the pathogenesis of PBC, based on the observation that these

factors are significantly involved in the development of PBC.

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Declarations

Conflict of interest The authors have no conflicts of interests to declare.

Ethical approval All the 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. The study protocol was approved by the Institutional Ethics Committee of Teikyo University, School of Medicine (20-602).

Informed consent Informed consent was obtained from all individual participants included in the study.

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