

Arsenic exposure and pruritus: evidence from observational, interventional, and Mendelian randomization studies

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Abstract

Background: Pruritus has been reported as an adverse drug reaction to arsenic trioxide, but the association of arsenic exposure with pruritus has not been systematically investigated. To investigate the association of arsenic exposure with pruritus, we performed observational, interventional, and Mendelian randomization studies. **Methods:** A cross-sectional study was conducted in Shimen, China. A Mendelian randomization study was conducted to confirm the causal relationship between susceptibility to arsenic toxicity, in terms of genetically predicted percentages of monomethylated arsenic (MMA%) and dimethylated arsenic (DMA%) in urine, and chronic pruritus in the UK Biobank participants. Then, a case-control study in Shimen participants was conducted to determine the biomarker for pruritus, and arsenite-treated mice were used to confirm the biomarker. Last, a randomized, double-blind, placebo-controlled trial was conducted to test the efficacy of naloxone, a μ -opioid receptor antagonist, in arsenic-exposed patients with pruritus in Shimen. **Results:** Hair arsenic showed a dose-response relationship with the intensity of itch in 1092 participants. The Mendelian randomization analysis confirmed the causal relationship in the UK Biobank participants, with odds ratios of 1.043 for MMA% and 0.904 for DMA% above versus under median. Serum β -endorphin was identified as a significant biomarker associated with the intensity of itch. Consistently, treatment with arsenite in mice upregulated the level of β -endorphin. The randomized controlled trial showed that treatment with sublingual naloxone significantly relieved the intensity of itch in arsenic-exposed participants. **Conclusion:** Arsenic exposure is associated with pruritus, and β -endorphin serves as a biomarker of pruritus. Naloxone relieves pruritus in patients with [arseniasis](javascript:;).

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Author contributions: All authors participated in the field survey and collection of biological samples. X.H., Y.X., and D.J. performed the dermatological examinations. Senior dermatologists J.S., M.C., and X.C. were responsible for quality control for diagnosis. X.H., M.H., and Z.H. measured the biological samples. Y.H. performed the animal experiment. X.H., M.S., and S.Y. analyzed the data and drafted the manuscript. Z.H., M.H., J.S., M.C., X.C., and M.S. designed the study, critically reviewed and revised the manuscript. X.C. and M.S. obtained the funding and contributed the conceptualization of the study. All authors gave final approval to the version submitted for publication.

ABSTRACT

Background: Pruritus has been reported as an adverse drug reaction to arsenic trioxide, but the association of arsenic exposure with pruritus has not been systematically investigated. To investigate the association of arsenic exposure with pruritus, we performed observational, interventional, and Mendelian randomization studies.

Methods: A cross-sectional study was conducted in Shimen, China. A Mendelian randomization study was conducted to confirm the causal relationship between susceptibility to arsenic toxicity, in terms of genetically predicted percentages of monomethylated arsenic (MMA%) and dimethylated arsenic (DMA%) in urine, and chronic pruritus in the UK Biobank participants. Then, a case-control study in Shimen participants was conducted to determine the biomarker for pruritus, and arsenite-treated mice were used to confirm the biomarker. Last, a randomized, double-blind, placebo-controlled trial was conducted to test the efficacy of naloxone, a μ -opioid receptor antagonist, in arsenic-exposed patients with pruritus in Shimen.

Results: Hair arsenic showed a dose-response relationship with the intensity of itch in 1092 participants. The Mendelian randomization analysis confirmed the causal relationship in the UK Biobank participants, with odds ratios of 1.043 for MMA% and 0.904 for DMA% above versus under median. Serum β -endorphin was identified as a significant biomarker associated with the intensity of itch. Consistently, treatment with arsenite in mice upregulated the level of β -endorphin. The randomized controlled trial showed that treatment with sublingual naloxone significantly relieved the intensity of itch in arsenic-exposed participants.

Conclusion: Arsenic exposure is associated with pruritus, and β -endorphin serves as a biomarker of pruritus. Naloxone relieves pruritus in patients with arseniasis.

Key words: arsenic exposure; β -endorphin; Mendelian randomization; pruritus; randomized controlled trial.

INTRODUCTION

High arsenic is found in groundwater in parts of the United States, Chile, Mexico, China, Argentina, India, and Bangladesh¹, where inorganic arsenic exposure is reported to be associated with cancers, cardiovascular diseases, neurological diseases, and arsenic-related skin lesions (ArSL) including hyperkeratosis, skin cancers, and pigmentary changes²⁻⁴. Even in low-exposure populations (water arsenic $<1 \mu\text{g/L}$) in the US, rice consumption was associated with higher risk of squamous cell carcinoma of skin⁵. Arsenic-related pruritus was reported in very limited number of literatures, as an adverse drug reaction of arsenic trioxide (As_2O_3) therapy among patients with acute promyelocytic leukemia⁶ and patients with multiple myeloma⁷, as well as a highly frequent symptom among occupational workers with arseniasis⁸. Among the epidemiologic studies in arsenicosis regions worldwide, however, pruritus, a possible consequence of arsenic exposure, remained nearly unstated.

After decades of economic development in China, concerns about environmental consequences have been raised, and remarkable ecological improvements have been achieved in recent years. Shimen, a county located in central south China, had the largest realgar mineral in Asia. Mines and plants mushroomed in the realgar-rich area near Heshan village from the 1950s until 2011 when they were completely shut down by the government due to the pollution they caused. The local government embarked on a massive environment improvement project since 2012. In 1994, the arsenic concentration in river (the main source of drinking water before 1985) was reported to be $14531 \pm 1232 \mu\text{g/L}$ near Heshan⁹; while in 2014, the peak arsenic concentration in river was $765 \mu\text{g/L}$ ¹⁰. In spite of the successful environmental governance, adverse health outcomes after arsenic exposure continue to occur.

Arsenic-related cutaneous symptoms are underappreciated, and comprehensive identification of exposure and outcomes may improve patient prognosis. During the epidemiologic investigation in Shimen, we noticed that many of the participants claimed unrelieved chronic pruritus during the dermatological examination. Owing to a lack of evidence, we performed a series of studies to confirm the causal relationship between arsenic exposure and chronic pruritus.

METHODS

2.1 Cross-sectional study

A cross-sectional survey was conducted in Shimen county during November 2016. Residents from three villages in the mining region were recruited through cluster sampling. Details can be found in our previous publications^{11 12}. In brief, demographic information, behavioral characteristics with respect to bath and skin care, history of diseases and medications, and history of occupational arsenic exposure were inquired through a face-to-face questionnaire interview. Height, weight, and waist circumference were measured using a standardized procedure. Body mass index (BMI) was calculated as $\text{weight [kg]} / (\text{height [m]})^2$.

Skin examinations were performed by certificated dermatologists from Xiangya Hospital, Central South University. Skin disorders were diagnosed according to skin lesions and symptoms, and dermatoscope or skin biopsy when necessary. Participants were asked to record their current intensity of itch on a numerical

rating scale (NRS) from 0 to 10. Participants were grouped by NRS: no itch or mild itch (0–2), moderate itch (3–6), and severe itch (7–10) in data analysis¹³.

2.2 Mendelian randomization study

To confirm the causal relationship between arsenic exposure and pruritus, we conducted a Mendelian randomization (MR) analysis using external data from the UK Biobank, a prospective cohort that recruited 500,000 participants in the United Kingdom during 2007 and 2010. The percentages of monomethylated arsenic (MMA%) and dimethylated arsenic (DMA%) in total urinary arsenic are established indicators for the toxicity of arsenic, as high MMA% and low DMA% represent insufficient detoxication and vulnerability to arsenic exposure. Therefore, we constructed the polygenic risk scores (PRSs) for MMA% and DMA% according to a published genome-wide association study (GWAS) using data on urinary arsenic metabolite concentrations and genome-wide single nucleotide polymorphisms (SNPs) from 1,313 arsenic-exposed individuals¹⁴. They identified significant associations ($P < 5 \times 10^{-8}$) for both MMA% and DMA% near the *AS3MT* gene (arsenite methyltransferase; 10q24.32), with five SNPs showing independent associations. The number of alleles (0, 1, or 2) for each individual was then summed after multiplication with the β coefficients between the SNPs and MMA% or DMA% to derive the PRS_{MMA%} and PRS_{DMA%} for each participant in the UK Biobank. The outcome variable was the diagnosis of pruritus from either primary care or hospital admissions, based on 10th revision of the International Statistical Classification of Diseases and Related Health Problems (code: L29). To reduce heterogeneity, the MR analysis was restricted to individuals whose ethnic backgrounds were White (including British, Irish, and other White backgrounds), with genetic information available.

2.3 Case-control study

To determine the potential biomarker of arsenic-related pruritus, we recruited 77 cases with severe itch, 95 cases with mild itch, and 57 controls with no itch from the participants in Shimen, and tested their serum levels of known biomarkers for itch, including β -endorphin, substance P, nerve growth factor, histamine, interleukin-31, IgE, endothelin-1, and 5-hydroxytryptamine. Participants of the three groups were roughly matched by age and sex. Participants with eczema, psoriasis or other conditions that may cause itch were excluded.

2.4 Animal study

To test whether chronic arsenic exposure upregulates β -endorphin, 18 C57BL/6J male mice (6–8 week) were purchased from the Department of Laboratory Animal Medicine of Central South University and raised under a controlled environment with constant temperature (22 ± 2 °C) and a 12-hour light-dark cycle. The mice were randomly assigned to vehicle control, 5 mg/L arsenite-treated group, or 15 mg/L arsenite-treated group, and each group contained 6 mice. The arsenite-treated mice were administered arsenite for 6 months by the drinking water. Mice in vehicle control group were fed with sterilized water correspondingly. Arsenic trioxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). After 6 months treatment, the mice were killed and blood sampled by eyeball extirpating for the detection of β -endorphin.

2.5 Randomized, double-blind, placebo-controlled trial

To test whether the inhibition of β -endorphin (μ -opioid) receptor alleviates itch in patients under chronic arsenic exposure, we conducted a randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov ID: NCT03751111). We randomly assigned patients to naloxone (0.4 mg/qd, sublingual) and placebo (0.4 mg/qd, sublingual) using a computer-generated sequence of random numbers during January 2019 to March 2019. The trial consisted of a one-week treatment period and a one-week withdrawal period. The primary outcome (severity of itch) was evaluated using the NRS through a face-to-face interview on baseline (Day 0) and the last day of the treatment period (Day 7), and a telephone interview on the last day of the withdrawal period (Day 14). The participants were also inquired about the possible adverse events of naloxone such as headache, sleep difficulty, sickness, and dizziness in each interview.

The inclusion criteria can be found in the protocol (supplementary file S1). In brief, the participants must

be above 18 years, be able to understand the study protocol and sign informed consent voluntarily, and report moderate-to-severe itch (NRS[?]₃). Participants were excluded if they used anti-histamines, anti-inflammatory, anti-pruritic, analgesic, antidepressant, or anti-epileptic agents within 2 weeks prior to the study, or had a history of pruritic skin diseases such as eczema and psoriasis.

2.6 Collection of biological samples and determination of biomarkers

Fasting blood, spot urine, and hair samples were collected from participants on the morning of recruitment day. Blood samples were saved on ice and transferred to the clinical laboratory of Shimen Central Hospital for routine measurement. Urine samples were collected into cleaned conical 50 ml polypropylene tubes, and packed into coolers with frozen ice packs. Hair samples were saved in envelopes and transferred to Shimen Center for Disease Control and Prevention for measurement. Blood arsenic and urine arsenic were analyzed in the laboratory of Tongji School of Public Health, Huazhong University of Science and Technology by inductively coupled plasma-mass spectrometry (Agilent 7700X, USA). Hair arsenic concentration was determined by nondispersive atomic fluorescence spectrometry (Ruiguang RGF-7800, China). Details could be found in our previous paper¹⁵.

For human participants, serum levels of pruritic biomarkers were determined by ELISA, following the manufacturer’s instructions for commercial kits of β -endorphin (BSK00470, Bioss), substance P (BSK00600, Bioss), nerve growth factor (BSK00127, Bioss), histamine (BSK00598, Bioss), interleukin-31 (BSK00467, Bioss), IgE (BSK00568, Bioss), endothelin-1 (BSK00345, Bioss), and 5-hydroxytryptamine (BSK00601, Bioss). For mice, β -endorphin was measured using an ELISA kit (0557M1, Meimian). All standards and samples were run in duplicate, and the values were within the range of acceptable variability according to the kit’s standards.

2.7 Statistical analysis

Categorical variables were described by proportions, and intergroup differences were tested by chi-square test. Continuous variables of normal distribution were described by means and standard deviations (SD), and tested by one-way analysis (ANOVA) of variance. Continuous variables of skewed distribution, including the concentrations of arsenic in blood, urine, and hair and level of serum β -endorphin, were described by median and interquartile range (IQR), and tested by Kruskal-Wallis H test.

The dose-response relationship between hair arsenic and NRS was estimated by a generalized additive model with cubic spline, and presented in figures. To derive parametric estimates, a multiple linear model was used for continuous NRS score, and partial regression coefficient of hair arsenic was reported. For dichotomous outcomes (NRS[?]₃ or NRS[?]₇), logistic models were used, and odds ratios (ORs) with 95% confidence intervals (CIs) were reported. Potential risk factors¹⁶ for itch were introduced into the following models for the purpose of adjustment.

In the MR analysis, the associations of genetically predicted urinary MMA% and DMA% with pruritus in participants of the UK Biobank was estimated using logistic regression models, adjusting for age, sex, race, Townsend deprivation index, smoking status and alcohol drinking. PRSs were analyzed as either continuous or categorical (above vs. under median) variables.

In the randomized controlled trial, an intention-to-treat (ITT) analysis was performed to evaluate the efficacy of naloxone. The last observation carry-forward (LOCF) imputation method was used for unmeasured data in drop-out participants. Mixed effect models were used to estimate the efficacy, by constructing a model: $Y = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Time} + \beta_3 (\text{Group} \times \text{Time}) + e_i + v_{ij}$, where β_2 is the estimate for efficacy; e_i and v_{ij} refer to the errors between individuals and within an individual (repeated measurements), respectively.

Statistical analyses were performed in R Statistical Software 3.4.1. The significance level for all statistical tests was 0.05.

2.8 Ethics Statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving patients were approved by the institutional research ethics boards of Xiangya Hospital, Central

South University (No. 201603172). Written informed consent was obtained from all participants. All animal experiments were approved by the Animal Care Committee of Central South University and performed in accordance with the institutional guidelines.

RESULTS

3.1 Hair arsenic is associated with the intensity of itch in arsenic-exposed participants

In the cross-sectional study in Shimen, 1,097 participants were recruited, 5 missed key information, and 1,092 eligible cases were included. The mean age was 56.6 ± 12.7 years. A total of 390 (36%) participants reported different intensity of itch (NRS>0). Many of them claimed persistent and generalized pruritus during the interview. The demographic, behavioral, clinical characteristics and internal exposure assessment are presented in supplementary Table S1. The hair arsenic was dose-dependently associated with the intensity of itch ($P_{\text{trend}}=0.001$).

The associations of hair arsenic with pruritus are shown in Figure 1. Hair arsenic showed positive associations with NRS and the prevalence of moderate and severe itch. Table 1 shows the effect sizes of hair arsenic. Hair arsenic was identified a risk factor for the intensity of itch, and 1 $\mu\text{g/g}$ increment in hair arsenic was associated with 13% and 12% increased risks of moderate and severe itch, respectively, after adjustments.

3.2 Genetically predicted urinary MMA% and DMA% are associated with chronic pruritus

In the MR analysis, 408,823 participants of the UK Biobank were included (supplementary Table S2). The odds for chronic pruritus was 1.043 (95% CI: 1.003, 1.084; $P=0.036$) in participants with a $\text{PRS}_{\text{MMA}\%}$ above median versus under median. The odds for chronic pruritus was 0.904 (95% CI: 0.807, 1.011; $P=0.078$) in participants with a $\text{PRS}_{\text{DMA}\%}$ above versus under median (Figure 2). The results consistently support the causal relationship between genetic susceptibility to arsenic exposure and chronic pruritus.

3.3 β -ενδορφιν ις α βιομαρκερ οφ ιτση ιν αρσενις-εξποσεδ παρτισιπαντς

The characteristics of the participants are shown in supplementary Table S3. Among the 8 mediators of itch, we identified serum β -endorphin as a significant biomarker that was dose-dependently associated with the intensity of itch in arsenic-exposed participants in Shimen (Figure 3). In contrast, the levels of substance P, nerve growth factor, histamine, interleukin-31, IgE, endothelin-1, and 5-hydroxytryptamine were not statistically different among the three groups.

3.4 Τρεατμεντ ωιτη αρσενιτε υπρεγυλατες τηε λεελ οφ β -ενδορφιν ιν μιςε

After a 6-month treatment with different concentrations of arsenite in drinking water (0, 5, 15 mg/L), the level of β -endorphin in mice increased in a dose-response manner (Figure 4). The difference of β -endorphin was significant between the control group and the 15 mg/L arsenite-treated group ($P=0.001$).

3.5 Naloxone relieves pruritus in arsenic-exposed participants

We initially planned to recruit 200 patients but finally enrolled 126 eligible participants. After random allocation, 64 were assigned to placebo control and 62 were assigned to treatment group (supplementary Figure S1). A total of 40 patients dropped out in week 1 (the treatment period) for various reasons, including lack of efficacy (n=35), adverse events (dizziness, n=2; cold, n=2), and worry of side effects (n=1). Four patients dropped out in week 2 (the withdrawal period) due to no efficacy. The baseline characteristics were comparable between the groups (supplementary Table S4). The changes in itch NRS are displayed in Figure 5. Compared to the control group, the NRS showed a significant reduction in the treatment group in week 2 ($\beta_{\text{Group} \times \text{Time}}=-0.98$, $P=0.040$) based on a mixed effect model (supplementary Table S5).

DISCUSSION

We performed a series of studies to evaluate the association of arsenic exposure with pruritus: (1) a cross-sectional study to uncover the positive association of hair arsenic with the intensity of pruritus in arsenic-exposed residents; (2) a Mendelian randomization study to confirm the causal relationship between genetic

susceptibility to arsenic exposure and chronic pruritus in the UK Biobank participants; (3) a case-control study to identify β -endorphin as a serum biomarker for arsenic-related pruritus; (4) an animal study to validate that chronic arsenic exposure upregulates the expression of β -endorphin; and (5) a randomized controlled trial to test the efficacy of naloxone on pruritus in patients with arseniasis.

During the field survey, many participants reported unrelieved itch during the dermatologic examination. Common causes for itch including dermatologic, systemic, neuropathic, and psychogenic factors were evaluated by physical examination as well as biochemical tests, and it was not likely to explain such a high prevalence of pruritus in the residents. Thus, we suspected the effect of arsenic exposure. The mechanisms underlying pruritus are quite complex. The itch signal is transmitted mainly by small, itch-selective C fibers originating in the skin. Histamine-triggered neurons and nonhistaminergic neurons may be involved. They form a synapse with secondary neurons that cross over to the contralateral spinothalamic tract and ascend to multiple brain areas involved in sensation, evaluative processes, emotion, reward, and memory¹⁷. In the peripheral nervous system, T-type calcium channels are prominent in C fibers. It was reported that peripheral T-type calcium channels are involved in histamine-dependent or histamine-independent itch processing. Pre-locally blocking T-type calcium channels in the peripheral afferents of skins yielded an inhibition in acute itch or pain behaviors^{10 18}. On the other hand, it has been reported that arsenic exposure could cause calcium influx in guinea pigs, human neuroblastoma SY-5Y, and embryonic kidney cells HEK 293^{19 20}. Steady-state calcium increases, transient calcium elevations, and calcium spikes were observed, indicating that both L-type and T-type voltage gated channels might be involved. However, direct evidences from *in vivo* and *in vitro* models are needed to support the hypothesis.

Arsenic in drinking water is predominantly inorganic arsenic. In human body, inorganic arsenic is first methylated into MMA, which has high cytotoxicity and genotoxicity^{21 22}. MMA is then methylated to DMA in liver, which excretes through the kidney together with MMA and inorganic arsenic²³. Epidemiologic studies reported that a higher MMA% and a lower DMA% in urine are associated with increased risks of bladder, breast, lung, and skin cancers, as well as skin lesions²⁴⁻²⁶. Our MR analysis consistently showed that a genetically higher MMA% and lower DMA% were associated with an increased risk of chronic pruritus in a large external sample. This indicates that chronic pruritus may be attributable to unobserved environmental pollutants, even in regions under a low level of exposure.

There is increasing evidence that neuropeptides such as substance P, calcitonin gene-related protein (CGRP) or β -endorphin are involved in the pathogenesis of itch²⁷. β -endorphin is an endogenous agonist for μ -opioid receptor, which are both present in keratinocytes and free nerve endings²⁸. Chronic inflammatory skin disorders, such as atopic dermatitis and psoriasis have common features of increased β -endorphin expression and peptidergic nerve fibers^{29 30}. Furthermore, μ -opioid receptors antagonists (MORA) such as naloxone, naltrexone, and nalmefene are known to suppress pruritus in patients with chronic urticaria, atopic dermatitis, and psoriasis³¹. Our clinical trial provides evidence that naloxone may relieve pruritus in arsenic-exposed participants, which is deducible based on previous studies.

One possible mechanism underlying the pathogenesis of arsenic-related pruritus is that arsenic increases the expression of microRNA-21³² which promotes the secretion of interleukin-10³³, regulating the synthesis of β -endorphin³⁴. Chronic arsenic exposure leads to peripheral neuropathy characterized by symptoms like numbness, weakness, pain as well as paraesthesia in stocking and glove distributions³⁵. During the questionnaire interview, many participants reported symptoms of numbness, itch, and pain which might be caused by the peripheral neuropathy. Nerve conduction velocity test and electromyography test would be applied in further studies to identify the arsenic-related neuropathy.

Our findings are highly relevant to public health and clinical practice. First, if arsenic is confirmed to cause pruritus by more studies, the diagnosis criteria of endemic arsenicosis may need revisions. Second, for those receiving As₂O₃ therapy or occupationally exposed to arsenic, pruritus should be noticed as an adverse drug reaction or a symptom of arsenic poisoning. Third, when dealing with patients with pruritus of unknown reason in clinical settings, a history of exposure, including the place of residence, occupational exposure, and the use of traditional medications that contain arsenic, should be inquired, in addition to the detection for

hair arsenic. However, hair arsenic only reflects recent exposure (2 to 4 weeks). A national survey in 1996 showed that the hair arsenic in Chinese residents in non-pollution regions ranged from 0.004 to 9.999 $\mu\text{g/g}^{36}$. In our study, 83% of the participants had hair arsenic $<1 \mu\text{g/g}$, and 99% had hair arsenic $<10 \mu\text{g/g}$. This indicates that, patients with pruritus did not necessarily present elevated hair arsenic level.

Our study systematically investigated the association of arsenic with pruritus for the first time. It is possible that itch is a neglected but important symptom in correlation with arsenic exposure. Second, a series of methods were applied to test the hypothesis. We first observed the association in a cross-sectional study, and then verified the causal relationship using the MR analysis. We further performed a case-control study to identify the biomarker for itch, and validated this in mice and human.

The study also has limitations. A primary limitation is the lack of data on external exposure. The level of previous exposure could not be obtained owing to the lack of historical data, especially at the individual level. However, we measured the internal level of arsenic exposure in hair, serum, and urine samples, and consistently observed the association based on multiple methods. Second, the underlying mechanism has not been fully investigated, and more *in vivo* and *in vitro* experiments are warranted. Nevertheless, we clearly identified a genetic correlation between arsenic susceptibility and chronic pruritus in an external sample, providing new insights for mechanism studies in the future.

CONCLUSIONS

Our data suggest that chronic arsenic exposure is associated with pruritus, and β -endorphin may mediate this association. The treatment with naloxone relieves the intensity of pruritus in patients with arseniasis. However, the mechanism underlying arsenic-related itch is not clear yet.

Table 1. Estimation of the effect size of hair arsenic on pruritus

Model	Covariates for adjustment	NRS (<i>continuous</i>) β	NRS (<i>continuous</i>) 95% CI	NRS (<i>continuous</i>) <i>P</i>	NRS[?]3 vs. NRS<3 OR
I	Crude model	0.24	(0.14, 0.35)	<0.001	1.15
II	Demographic factors ^a	0.21	(0.11, 0.32)	<0.001	1.13
III	Proxy variables of exposure ^b	0.18	(0.08, 0.28)	<0.001	1.12
IV	Behavioral factors ^c	0.20	(0.10, 0.30)	<0.001	1.13
V	Allergy-related conditions ^d	0.20	(0.10, 0.30)	<0.001	1.13
VI	Integrative model ^e	0.17	(0.07, 0.26)	0.001	1.13

NRS: numerical rating scale. β : partial regression coefficient of hair arsenic ($\mu\text{g/g}$). OR: odds ratio. CI: confidence interval.

^a Adjusted for age and education.

^b Adjusted for age, village, occupational exposure history, and arsenic-related skin lesions.

^c Adjusted for age, frequency of bath, temperature of bath water, and use of cleansing product.

^d Adjusted for age, allergy to certain substances and cough.

^e Adjusted for significant covariates including age, village, occupational exposure history, arsenic-related skin lesions, frequency of bath, temperature of bath water, use of cleansing product, allergy to certain substances, cough.

FIGURE LEGENDS

Figure 1. Associations of hair arsenic concentration with intensity of itch. (A) Continuous NRS for itch; (B) moderate itch, NRS[?]3; (C) severe itch, NRS[?]7.

Figure 2. Continuous and categorical models of PRS_{MMA%} and PRS_{DMA%} with the prevalence of pruritus in participants of the UK Biobank. DMA, dimethylated arsenic; MMA, monomethylated arsenic; PRS, polygenic risk score.

Figure 3. Serum concentrations of itch biomarkers in controls and patients with mild and severe itch. (A) β -endorphin; (B) substance P; (C) nerve growth factor; (D) histamine; (E) interleukin-31; (F) IgE; (G) endothelin-1; (H) 5-hydroxytryptamine.

Figure 4. The concentrations of β -endorphin in the arsenite-treated mice.

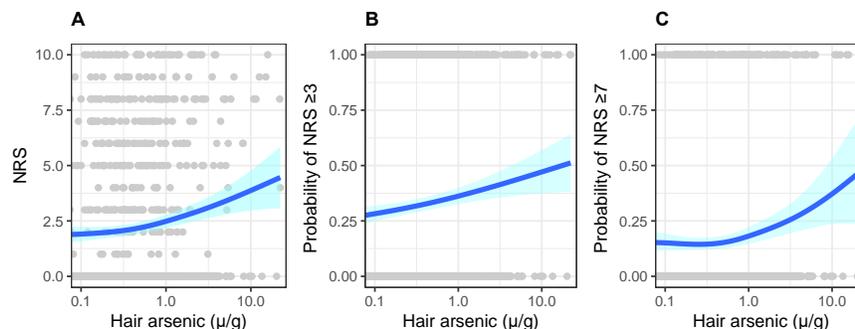
Figure 5. Intensity of itch in naloxone versus placebo groups in the baseline and follow-up periods. The bars signify 95% confidence intervals of the means.

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Exposure	Model	OR (95% CI)	P
PRS of MMA%	Continuous	1.027 (1.007, 1.048)	0.009
	Above vs. under median	1.043 (1.003, 1.084)	0.036
PRS of DMA%	Continuous	0.989 (0.979, 1.000)	0.046
	Above vs. under median	0.904 (0.807, 1.011)	0.078

