



# Relationships between acrylamide and glycidamide hemoglobin adduct levels and allergy-related outcomes in general US population, NHANES 2005–2006<sup>☆</sup>

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## ABSTRACT

**Background:** Acrylamide-induced immunotoxicity and allergic dermatitis have been reported in animal experiments and clinical reports, respectively. However, epidemiological evidence from the general population is limited.

**Objectives:** The purpose of the present study was to estimate the associations between acrylamide exposure and allergy-related outcomes in the general US population.

**Methods:** A total of 6982 subjects were selected from the National Health and Nutrition Examination Survey 2005–2006 (NHANES). Internal exposure was measured by the hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA). Allergy-related outcomes including asthma, hay fever, allergy, itchy rash, sneeze, wheeze and eczema were obtained by self-administered questionnaires. Allergic sensitization was assessed by the total immunoglobulin E (IgE) levels. The associations of HbAA and HbGA quartiles with allergy-related outcomes were calculated using logistic regression models with multivariable adjustments. Analyses were additionally stratified according to age, gender and serum cotinine levels.

**Results:** When setting quartile 1 of HbAA as reference, the odds ratios (ORs) [95% confidence intervals (CIs)] of quartile 2 to 4 for eczema were 1.18 (0.79–1.76), 1.14 (0.73–1.78) and 1.58 (1.14–2.18), respectively ( $p_{\text{trend}} = 0.002$ ). Individuals at the highest quartile of HbGA had significantly elevated likelihoods of itchy rash (OR = 1.37, 95% CI = 1.02–1.83,  $p_{\text{trend}} = 0.032$ ) and eczema (OR = 1.45, 95% CI = 1.06–1.97,  $p_{\text{trend}} = 0.044$ ). The stratification analyses indicated various results in different subgroups.

**Conclusions:** This study indicated significant associations between HbAA and HbGA levels and the likelihoods of allergy-related outcomes in the general US population, depending on age, gender and smoke exposure status. These findings suggested potential public health concerns for the widespread exposure to acrylamide.

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## 1. Instruction

Acrylamide is a biodegradable and water-soluble compound

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(Riboldi et al., 2014). As an important industrial chemical, acrylamide has been widely applied in water treatment, cosmetic formulations, molecular biological research and the textile and paper productions (Cosmetic Ingredient Review Expert Panel, 2005). It is estimated that about 100 thousands individuals may come in contact with acrylamide in the United States (Friedman, 2003). People are exposed to acrylamide through potential routes of ingestion, inhalation and dermal contact (Xu et al., 2014) during food intake, tobacco smoking, and occupational activities (Vesper et al., 2010).

In the human body, acrylamide is predominantly conjugated with glutathione, and nearly fifteen percent of acrylamide is metabolized into glycidamide by cytochrome P4502E1 (CYP2E1) (Fennell et al., 2005). The primary excretion route of both acrylamide and glycidamide is the by-products of mercapturic acid in urine (Boettcher et al., 2005). Hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA) are stable over the lifespan of erythrocytes (about 4 months), and thus, are well-established biomarkers of human internal exposure (Vesper et al., 2010).

The scientific interests in acrylamide did not increase until Swedish researchers reported its generation during food processing at temperature of  $>120$  °C, such as frying, toasting, baking or roasting foodstuffs which are rich of carbohydrates (Obon-Santacana et al., 2016; Tareke et al., 2002). The detrimental health effects of acrylamide and glycidamide have been extensively reviewed based on the experimental and epidemiological studies (Friedman, 2003; Xu et al., 2014).

Acrylamide is carcinogenic and genotoxic in mice (Johnson et al., 1986) and rats (Friedman et al., 1995), and regarded as a potential carcinogen (group 2A) to humans by the International Agency for Research on Cancer (IARC) (IARC, 1994). Besides, the cumulative neurotoxicity of acrylamide in workers exposed to acrylamide (He et al., 1989; Pennisi et al., 2013), to some extent, could be resorted to the covalent adducts between acrylamide and highly nucleophilic cysteine thiolate groups in presynaptic proteins and subsequent impaired neurotransmission (LoPachin and Gavin, 2012). Additionally, when mice or rats were treated with exogenous acrylamide, reproductive and developmental toxicity was observed (Shipp et al., 2006; Xu et al., 2014). Glycidamide forms DNA adducts (Von Tungeln et al., 2012) and is more cytotoxic and clastogenic than acrylamide in Chinese hamster V79H3 cells (Martins et al., 2007; Tsuda et al., 1993).

Although the health effects of acrylamide have been widely addressed before (Friedman, 2003; Xu et al., 2014), to date, information on immunotoxicity of acrylamide is limited (Stolevik et al., 2011). Zaidi et al. demonstrated the immunosuppressive effects of acrylamide administration in rats (Zaidi et al., 1994). Allergic contact dermatitis have been reported among laboratory technicians and workers exposed to acrylamide and its derivatives accidentally or occupationally in some case report studies (Dooms-Goossens et al., 1991; Garnier et al., 2003). However, the effects of acrylamide exposure on immunological health outcomes in the general population have been rarely investigated. To our knowledge, only one birth cohort study investigated the relationships between prenatal dietary intake of acrylamide, estimated by the food frequency questionnaires, and the risks of wheeze in infants (Stolevik et al., 2011).

The lifelong exposure of general population to acrylamide at low levels has raised public concerns for the potential health effects (Vesper et al., 2010). The goal of the present study was to assess the associations between circulating levels of HbAA and HbGA and allergy-related outcomes among the US general population based on a nationally-representative sample.

## 2. Methods

### 2.1. Study design and participants

The data of this study were extracted from the 2005–2006 National Health and Nutrition Examination Survey (NHANES) which was performed with complex multistage design to assess the health and nutritional status of the civilian noninstitutionalized population in the United States (CDC, 2014). Subjects who completed a health interview in the household were then invited to

participate in the health examination in specially-designed and equipped mobile centers. The written informed consent was obtained from all the participants, and the survey protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board.

NHANES 2005–2006 provided the population-based allergic component of total immunoglobulin E (IgE) levels to 19 allergens from blood, as well as the self-reported allergic symptoms and diseases. There were totally 10,348 participants investigated in NHANES 2005–2006, among them, 7727 individuals finished the HbAA and HbGA testing. We further excluded subjects with missing information on allergic conditions ( $n = 138$ ) and covariates ( $n = 607$ ). Finally, 6982 subjects with complete data were analyzed (Fig. 1).

### 2.2. Demographics and covariates

Information on the demographics and housing characteristics were obtained via the questionnaires. The demographics included age, gender, race/ethnicity (non-Hispanic black, non-Hispanic white, Mexican American and others), education levels (under high school, high school, above high school) and family poverty-income ratio (PIR,  $\leq 1$ ,  $>1$ ). The education status of children was replaced by that of household reference person who was the first subject on the screener module, aged over 18 years and owns or rents the family residence. The housing characteristics contained mildew smell (yes, no), furry animals (yes, no) and cockroaches in house (yes, no).

Body mass index (BMI,  $\text{kg/m}^2$ ) of adults was classified into normal weight of  $<25$ , overweight of  $25$  to  $<30$  and obesity of  $\geq 30$ . For children, BMI categories were defined according to the age- and gender-specific criteria from an international study (Cole et al., 2000). Status of tobacco smoke exposure was assessed by the levels of serum cotinine (ng/mL) which was tested by the isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS).

### 2.3. HbAA and HbGA measurement

HbAA and HbGA were tested in red blood cells which were isolated and washed from EDTA-whole blood immediately after specimen collection. The method of high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) was used to analyze the levels of HbAA and HbGA at the N-terminal valine of the hemoglobin protein chains (CDC, 2016).

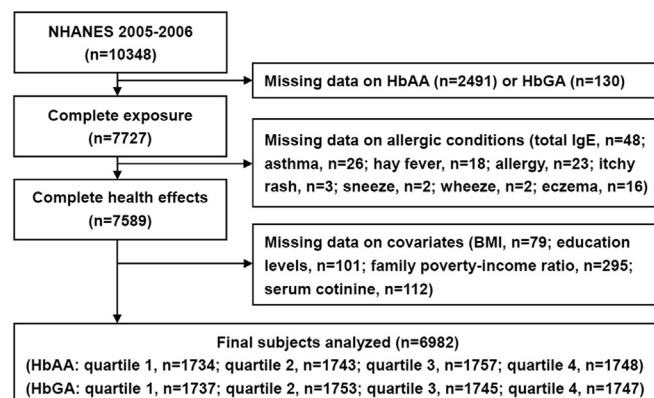


Fig. 1. Flow chart of the selection of eligible subjects.

## 2.4. Immunological health outcomes assessment

Both the allergic sensitization measured by total IgE and the self-reported allergic conditions were assessed. Briefly, after clotting and centrifugation, the serum specimens collected in the field were frozen, and then shipped to the laboratory on dry ice by overnight mail, and stored at  $< 70^{\circ}\text{C}$  until analysis. Total IgE levels were measured by the Pharmacia Diagnostics ImmunoCAP 1000 System (Kalamazoo, Michigan). Total IgE level of  $\geq 150$  kU/L was considered as allergic sensitization (Novak and Bieber, 2003).

Information on the allergic symptoms and diseases was confirmed by the self-administered questionnaires. Interviews with individuals less than 16 years old were proxy-assisted. Seven immune-mediated conditions were ascertained with positive responses to the following questions:

- Current asthma: During the past 12 months, have you had an episode of asthma or an asthma attack? (MCQ040)
- Current hay fever: During the past 12 months, have you had an episode of hay fever? (AGQ030)
- Current allergy: During the past 12 months, have you had an allergy symptoms or an allergy attack? (AGQ060)
- Current itchy rash: Have you had this itchy rash at any time in the last 12 months? (AGQ140)
- Current sneeze: During the past 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? (AGQ100)
- Current wheeze: In the past 12 months have you had wheezing or whistling in your chest? (RDQ070)
- Ever eczema: Has a doctor or other health professional ever told you that you have eczema? (AGQ180)

## 2.5. Statistical analysis

The sampling weights for health examination (WTMEC2YR) and study design variables (SDMVPSU and SDMVSTRA) were applied in the data analyses. The lower limits of detection (LLOD) for HbAA, HbGA, serum cotinine and total IgE were 3.00 pmol/G Hb, 4.00 pmol/G Hb, 0.015 ng/mL and 2.0 kU/L, respectively. For samples below LLOD, levels of analytes were assigned as LLOD divided by the square root of 2. The demographic and housing characteristics were presented as frequency and proportion. Levels of HbAA and HbGA were calculated as geometric means and medians, and compared among different subgroups of age, gender and serum cotinine levels.

Logistic regression models were applied to estimate odds ratios (ORs) and confidence intervals (CIs) of both allergic sensitization and self-reported allergic conditions for each HbAA and HbGA quartile compared with the lowest quartile, with adjustment for demographic characteristics of age (year), gender (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), serum cotinine (ng/mL, ln-transformed), education level (under high school, high school or equivalent, above high school), BMI category (normal, overweight, obese), family PIR ( $\leq 1$ ,  $> 1$ ), housing characteristics of mildew (yes, no), animal (yes, no) and cockroaches (yes, no) in house.

Potential interactions were examined by adding a product term between quartiles of HbAA (or HbGA) and four variables of age, gender, race/ethnicity and serum cotinine, respectively, in above-mentioned models with allergic sensitization as dependent variable. The interaction effect between HbAA ( $p_{\text{interaction}} = 0.033$ ) [or HbGA ( $p_{\text{interaction}} = 0.011$ )] and gender was statistically significant. Furthermore, it has been reported that the prevalence of allergic

sensitization (Salo et al., 2014) was discrepant among children and adults. Compared with nonsmokers, levels of HbAA and HbGA are higher in smokers (Vesper et al., 2010). Therefore, stratification analyses were additionally conducted by age group (children, adults), gender (male, female) and serum cotinine levels ( $< \text{LLOD}$ ,  $\geq \text{LLOD}$ ).

## 3. Results

### 3.1. Study population characteristics

Among all the 6982 subjects included in the present study, 1740 (19.83%) cases with allergic sensitization, 620 (8.53%) with asthma, 304 (6.38%) with hay fever, 1282 (23.00%) with allergy, 476 (6.99%) with itchy rash, 2000 (33.33%) with sneeze, 903 (15.38%) with wheeze and 584 (8.79%) with eczema were identified.

As shown in Table 1, the weighted proportions of children, males, subjects of serum cotinine levels  $< \text{LLOD}$  were 19.54%, 48.80% and 17.40%, respectively. The majority of participants were non-Hispanic whites (70.51%), received education above high school (57.02%) and lived in the home with PIR  $> 1$  (87.06%). Nearly one third of the participants were obese. Subjects who lived in the house with mildew smell, animals and cockroaches accounted for 16.42%, 51.30% and 14.09%, respectively.

**Table 1**  
Characteristics of subjects analyzed, NHANES 2005–2006.

| Characteristics                | Frequency | Percentage (%) |
|--------------------------------|-----------|----------------|
| Age                            |           |                |
| Children (age $< 18$ years)    | 2473      | 19.54          |
| Adults (age $\geq 18$ years)   | 4509      | 80.46          |
| Gender                         |           |                |
| Male                           | 3395      | 48.80          |
| Female                         | 3587      | 51.20          |
| Race/ethnicity                 |           |                |
| Non-Hispanic white             | 2867      | 70.51          |
| Non-Hispanic black             | 1803      | 11.54          |
| Mexican American               | 1762      | 8.83           |
| Others                         | 550       | 9.12           |
| Serum cotinine                 |           |                |
| $< \text{LLOD}$                | 1235      | 17.40          |
| $\geq \text{LLOD}$             | 5747      | 82.60          |
| BMI <sup>a</sup>               |           |                |
| Normal                         | 3057      | 40.32          |
| Overweight                     | 1969      | 29.48          |
| Obese                          | 1956      | 30.20          |
| Education <sup>b</sup>         |           |                |
| Under high school              | 2045      | 17.85          |
| High school or equivalent      | 1697      | 25.13          |
| Above high school              | 3240      | 57.02          |
| PIR                            |           |                |
| $\leq 1$ (under poverty level) | 1629      | 12.94          |
| $> 1$ (above poverty level)    | 5353      | 87.06          |
| Mildew in house                |           |                |
| Yes                            | 1197      | 16.42          |
| No                             | 5785      | 83.58          |
| Animals in house               |           |                |
| Yes                            | 2840      | 51.30          |
| No                             | 4142      | 48.70          |
| Cockroaches in house           |           |                |
| Yes                            | 1419      | 14.09          |
| No                             | 5563      | 85.91          |

NHANES, National Health and Nutrition Examination Survey; LLOD, lower limit of detection; BMI, body mass index; PIR, family poverty-income ratio.

<sup>a</sup> BMI was categorized according to the age- and gender-specific criteria from an international study among children.

<sup>b</sup> The education level of household reference person was substituted for that of children.

### 3.2. HbAA and HbGA levels

HbAA and HbGA levels were compared between different sub-populations according to age, gender, and serum cotinine levels (Table 2). Among all the subjects, the geometric means of HbAA and HbGA were 61.90 and 50.56 pmol/G Hb, and the medians were 54.30 and 49.00 pmol/G Hb, respectively. HbAA levels were significantly higher among adults and males. Children had higher levels of HbGA. Compared with subjects of serum cotinine < LLOD, those of  $\geq$  LLOD were both exposed at increased levels of HbAA and HbGA.

### 3.3. Associations between HbAA and HbGA levels and allergy-related outcomes

We estimated adjusted ORs (95% CIs) for allergy-related outcomes across quartiles of HbAA (Table 3) and HbGA (Table 4), respectively. The data demonstrated that ORs (95% CIs) for eczema across HbAA quartiles were 1.18 (0.79–1.76), 1.14 (0.73–1.78) and 1.58 (1.14–2.18), respectively ( $p_{\text{trend}} = 0.002$ ). No statistically significant associations were found between HbAA levels and allergic sensitization, as well as other self-reported allergic conditions of asthma, hay fever, allergy, itchy rash, sneeze or wheeze.

Compared with subjects at the lowest quartile of HbGA, those at the highest quartile had significantly elevated likelihoods of itchy rash (OR = 1.37, 95% CI = 1.02–1.83,  $p_{\text{trend}} = 0.032$ ) and eczema (OR = 1.45, 95% CI = 1.06–1.97,  $p_{\text{trend}} = 0.044$ ), but not for other allergy-related outcomes.

### 3.4. Stratification analyses

Analyses on the effects of HbAA (Fig. 2, Table S1) and HbGA (Fig. 3, Table S2) on allergy-related outcomes were stratified by age, gender and serum cotinine levels, respectively. We found inverse associations between HbAA and HbGA levels and the likelihoods of self-reported allergic conditions of hay fever and asthma for children, but positive results of wheeze and eczema for adults. Among males, higher quartiles of HbAA and HbGA were related with increased risks of wheeze and itchy rash, respectively. While both increased and decreased risks of different allergic conditions were found among females. When subjects were stratified by smoke exposure status, those at the highest quartiles of HbAA had decreased risks of itchy rash in the subgroup of serum cotinine < LLOD. On the contrary, increased levels of HbAA and HbGA were associated with elevated risks of itchy rash, wheeze and eczema among individuals of serum cotinine  $\geq$  LLOD.

## 4. Discussion

Some investigations have been performed to address the relationships between the exposure of acrylamide and glycidamide and the risks of cancers in epidemiological and experimental studies (Xu et al., 2014). However, impacts of acrylamide and glycidamide on immunological health outcomes have been barely studied, especially for the general population. Based on a nationwide sample of the general US population, we explored whether the internal exposures of HbAA and HbGA are associated with allergy-related outcomes. We observed that the likelihoods of eczema increased with increasing levels of HbAA, and higher levels of HbGA were associated with elevated risks of eczema and itchy rash among the whole sample. Furthermore, results varied depending on age, gender and smoke exposure status. In the stratification analyses, generally, associations between HbAA and HbGA levels and the risks of specific self-reported allergic conditions were negative for children and individuals of serum cotinine levels < LLOD, whereas positive for adults, males and those of serum cotinine levels  $\geq$  LLOD.

Acrylamide administration to rats produced immunotoxicity including decreased weights of spleen, thymus and mesenteric lymph nodes, as well as suppressed humoral and cell mediated immunity (Zaidi et al., 1994). On one hand, acrylamide exposure is associated with both reduced levels of serum insulin and insulin resistance in adults (Lin et al., 2009). On the other, impaired immune responses were found in the mice model of type I diabetes, for which high glucose levels alter the lymphoid cell growth and leads to cell death (Rubinstein et al., 2008). Immune reactions are involved in the pathogenesis of diabetes and its complication, additionally, the diabetic condition itself can affect immune responses (Takeda et al., 2014). Therefore, we presumed a possible explanation that acrylamide exposure is related with the allergy-related outcomes through the influence on glucose homeostasis. The biological mechanism for the effects of acrylamide and glycidamide on immunological health outcomes has not been unraveled until now, which requires comprehensive data on in vitro and animal exposure experiments.

In the present study, the likelihoods of allergic conditions decreased for children but increased for adults with increasing HbAA and HbGA levels, suggesting that the effect of HbAA and HbGA on allergic reaction may be age-related. Sensitization to allergens was regarded as a dynamic process and affected by age in a European longitudinal study (Jarvis et al., 2005). Similarly, there exists evidence that exposure to endotoxin is protective against atopy (Celedon et al., 2007) and asthma development (Carlsten

**Table 2**  
HbAA and HbGA levels by age, gender and serum cotinine status, NHANES 2005–2006.

| Stratifications | HbAA (pmol/G Hb)    |               |        | HbGA (pmol/G Hb)    |               |       |
|-----------------|---------------------|---------------|--------|---------------------|---------------|-------|
|                 | GM (95% CI)         | Median (IQR)  | $p^a$  | GM (95% CI)         | Median (IQR)  | $p^b$ |
| All             | 61.90 (58.47–65.54) | 54.30 (31.30) | –      | 50.56 (47.17–54.20) | 49.00 (32.20) | –     |
| Age             |                     |               |        |                     |               |       |
| Children        | 55.33 (52.89–57.89) | 55.50 (22.90) | <0.001 | 53.11 (50.41–55.96) | 52.20 (28.20) | 0.007 |
| Adults          | 63.61 (59.79–67.67) | 53.30 (40.40) |        | 49.96 (46.31–53.89) | 46.70 (33.60) |       |
| Gender          |                     |               |        |                     |               |       |
| Male            | 65.44 (61.30–69.86) | 56.30 (35.90) | <0.001 | 50.55 (46.70–54.70) | 48.40 (34.70) | 0.977 |
| Female          | 58.71 (55.39–62.22) | 52.80 (27.90) |        | 50.58 (47.35–54.02) | 49.50 (29.40) |       |
| Serum cotinine  |                     |               |        |                     |               |       |
| < LLOD          | 47.55 (44.98–50.28) | 48.60 (22.00) | <0.001 | 42.89 (40.24–45.72) | 46.30 (25.60) | 0.001 |
| $\geq$ LLOD     | 65.44 (61.42–69.72) | 55.70 (34.80) |        | 52.34 (48.23–56.81) | 49.60 (33.60) |       |

HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide; GM, geometric mean; CI, confidence interval; IQR, interquartile range; NHANES, National Health and Nutrition Examination Survey; LLOD, lower limit of detection.

<sup>a</sup> A linear model was established with In-transformed HbAA as dependent variable and stratifications as independent variable.

<sup>b</sup> A linear model was established with In-transformed HbGA as dependent variable and stratifications as independent variable.

**Table 3**ORs (95% CIs)<sup>a</sup> for allergic conditions by quartiles of HbAA levels, NHANES 2005–2006.

| Immunological health outcomes       | Quartiles of HbAA levels (pmol/G Hb) |                          |                          |                          | <i>p</i> trend <sup>b</sup> |
|-------------------------------------|--------------------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
|                                     | Quartile 1<br>(N = 1734)             | Quartile 2<br>(N = 1743) | Quartile 3<br>(N = 1757) | Quartile 4<br>(N = 1748) |                             |
| Allergic sensitization <sup>c</sup> | Reference                            | 0.91 (0.78–1.07)         | 0.93 (0.72–1.19)         | 0.91 (0.75–1.10)         | 0.406                       |
| Self-reported allergic conditions   |                                      |                          |                          |                          |                             |
| Asthma                              | Reference                            | 1.21 (0.84–1.75)         | 1.27 (0.91–1.79)         | 1.04 (0.70–1.56)         | 0.949                       |
| Hay fever                           | Reference                            | 0.84 (0.58–1.22)         | 0.74 (0.51–1.07)         | 0.98 (0.56–1.71)         | 0.999                       |
| Allergy                             | Reference                            | 0.91 (0.71–1.16)         | 1.13 (0.88–1.46)         | 1.14 (0.81–1.60)         | 0.252                       |
| Itchy rash                          | Reference                            | 1.20 (0.87–1.67)         | 1.02 (0.61–1.72)         | 1.22 (0.88–1.70)         | 0.345                       |
| Sneeze                              | Reference                            | 0.99 (0.79–1.23)         | 0.92 (0.74–1.14)         | 0.94 (0.75–1.19)         | 0.602                       |
| Wheeze                              | Reference                            | 1.03 (0.70–1.53)         | 0.91 (0.67–1.22)         | 1.45 (0.98–2.16)         | 0.027                       |
| Eczema                              | Reference                            | 1.18 (0.79–1.76)         | 1.14 (0.73–1.78)         | <b>1.58 (1.14–2.18)</b>  | 0.002                       |

OR, odds ratio; CI, confidence interval; HbAA, hemoglobin adducts of acrylamide; NHANES, National Health and Nutrition Examination Survey; IgE, immunoglobulin E; Quartile 1, <42.20 pmol/G Hb; Quartile 2, ≥42.20–54.30 pmol/G Hb; Quartile 3, ≥54.30–73.50 pmol/G Hb; Quartile 4, ≥73.50 pmol/G Hb.

Values in boldface mean statistically significant (*p*-value < 0.05).

<sup>a</sup> Adjusted for demographic characteristics of age (year), gender (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), serum cotinine (ng/mL, ln-transformed), education level (under high school, high school or equivalent, above high school), BMI category (normal, overweight, obese), family poverty-income ratio (≤1, >1), housing characteristics of mildew (yes, no), animal (yes, no) and cockroaches (yes, no) in house.

<sup>b</sup> *p* values for the medians of each quartile of HbAA levels included in the logistic regression models.

<sup>c</sup> Total IgE ≥150 kU/L.

**Table 4**ORs (95% CIs)<sup>a</sup> for allergic conditions by quartiles of HbGA levels, NHANES 2005–2006.

| Immunological health outcomes       | Quartiles of HbGA levels (pmol/G Hb) |                          |                          |                          | <i>p</i> trend <sup>b</sup> |
|-------------------------------------|--------------------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
|                                     | Quartile 1<br>(N = 1737)             | Quartile 2<br>(N = 1753) | Quartile 3<br>(N = 1745) | Quartile 4<br>(N = 1747) |                             |
| Allergic sensitization <sup>c</sup> | Reference                            | 1.02 (0.82–1.26)         | 0.86 (0.74–1.00)         | 0.88 (0.68–1.13)         | 0.169                       |
| Self-reported allergic conditions   |                                      |                          |                          |                          |                             |
| Asthma                              | Reference                            | 1.11 (0.75–1.66)         | 1.19 (0.82–1.73)         | 1.13 (0.76–1.68)         | 0.581                       |
| Hay fever                           | Reference                            | 0.81 (0.56–1.16)         | 0.81 (0.55–1.21)         | 1.16 (0.68–1.97)         | 0.508                       |
| Allergy                             | Reference                            | 0.96 (0.70–1.32)         | 1.00 (0.76–1.30)         | 1.21 (0.89–1.63)         | 0.120                       |
| Itchy rash                          | Reference                            | 1.17 (0.75–1.81)         | 1.09 (0.72–1.65)         | <b>1.37 (1.02–1.83)</b>  | 0.032                       |
| Sneeze                              | Reference                            | 1.10 (0.88–1.38)         | 0.86 (0.68–1.10)         | 1.06 (0.83–1.36)         | 0.923                       |
| Wheeze                              | Reference                            | 0.96 (0.64–1.44)         | 0.99 (0.65–1.51)         | 1.50 (0.96–2.36)         | 0.024                       |
| Eczema                              | Reference                            | 1.22 (0.87–1.71)         | 1.11 (0.65–1.90)         | <b>1.45 (1.06–1.97)</b>  | 0.044                       |

OR, odds ratio; CI, confidence interval; HbGA, hemoglobin adducts of glycidamide; NHANES, National Health and Nutrition Examination Survey; IgE, immunoglobulin E; Quartile 1, <35.80 pmol/G Hb; Quartile 2, ≥35.80–49.00 pmol/G Hb; Quartile 3, ≥49.00–68.00 pmol/G Hb; Quartile 4, ≥68.00 pmol/G Hb.

Values in boldface mean statistically significant (*p*-value < 0.05).

<sup>a</sup> Adjusted for demographic characteristics of age (year), gender (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), serum cotinine (ng/mL, ln-transformed), education level (under high school, high school or equivalent, above high school), BMI category (normal, overweight, obese), family poverty-income ratio (≤1, >1), housing characteristics of mildew (yes, no), animal (yes, no) and cockroaches (yes, no) in house.

<sup>b</sup> *p* values for the medians of each quartile of HbGA levels included in the logistic regression models.

<sup>c</sup> Total IgE ≥150 kU/L.

et al., 2011) among children, whereas detrimental among adults (Min and Min, 2015).

Three mechanisms have been proposed as a possible explanation for the discrepancy between children and adults (Min and Min, 2015). Firstly, the ‘hygiene hypothesis’ posits that exposure to infectious agents early in life protects against the development of allergic diseases during adulthood, although a consensus has yet to be reached (Strachan, 2000). Secondly, advanced age is accompanied with immunosenescence which corresponds to age-related changes in the number and function of immune cells (Sansoni et al., 2008), inducing dysregulated immune function and consequent impaired adaptability and homeostasis (Grimley Evans, 2000). Thus, older subjects are more susceptible to immunotoxic xenobiotics, as a result of attenuated immunocompetence (Min and Min, 2015). Thirdly, the contrary results may be interpreted by the properties of exposed compounds, as well as the differences of environmental fate and metabolism across age stages (Meng et al., 2016).

Our findings showed significant interactions between gender and the levels of HbAA and HbGA. We observed adverse impacts for males, and both adverse and protective effects for females. A few

studies have been conducted to explore the gender-specific effects of environmental exposures and allergic-related outcomes, however obtaining inconsistent results (Chen et al., 2002; Savage et al., 2012).

For instance, compared with females, associations between urinary triclosan levels and risks of food sensitization were more pronounced in males (Savage et al., 2012). It was presumed that males might be more susceptible to the harmful influence of a second risk factor, as a result leading to gender interactions (Savage et al., 2012). In contrast, the asthma morbidity could be partly explained by the household pets and smoking status among female but not male Canadians (Chen et al., 2002). And a greater susceptibility of females to the environmental pollutants was speculated (Almqvist et al., 2008).

Some studies reported that asthma and wheeze are more prevalent in males than that in females during the childhood, whereas the pattern reverses after adolescent onsets (Almqvist et al., 2008). The switch in prevalence and severity of asthma around puberty were resorted to the hormonal changes and gender-specific differences in environmental exposures (Almqvist et al., 2008). Sex hormones were deemed to play critical roles in

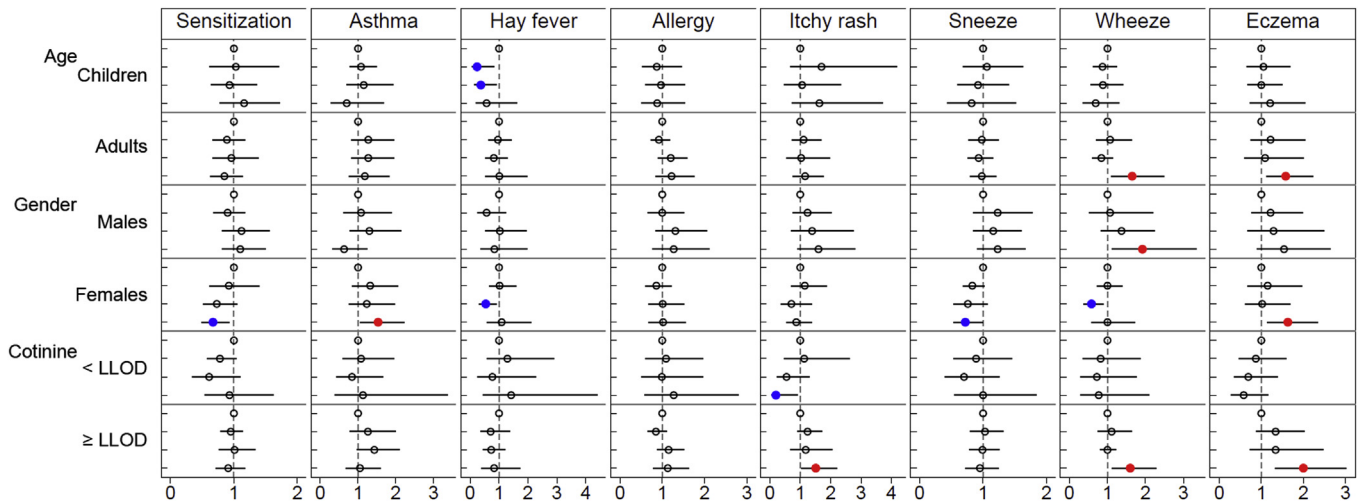


**Fig. 2.** Stratification analyses on ORs (95% CIs) for allergic conditions by quartiles of HbAA levels. Analyses were stratified by age (children, adults), gender (males, females) and serum cotinine levels (<LLOD,  $\geq$  LLOD). The dots and horizontal bars represent the ORs and corresponding 95% CIs, respectively. Solid dots in red and blue mean positive and negative associations with statistical significance ( $p$ -value < 0.05), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

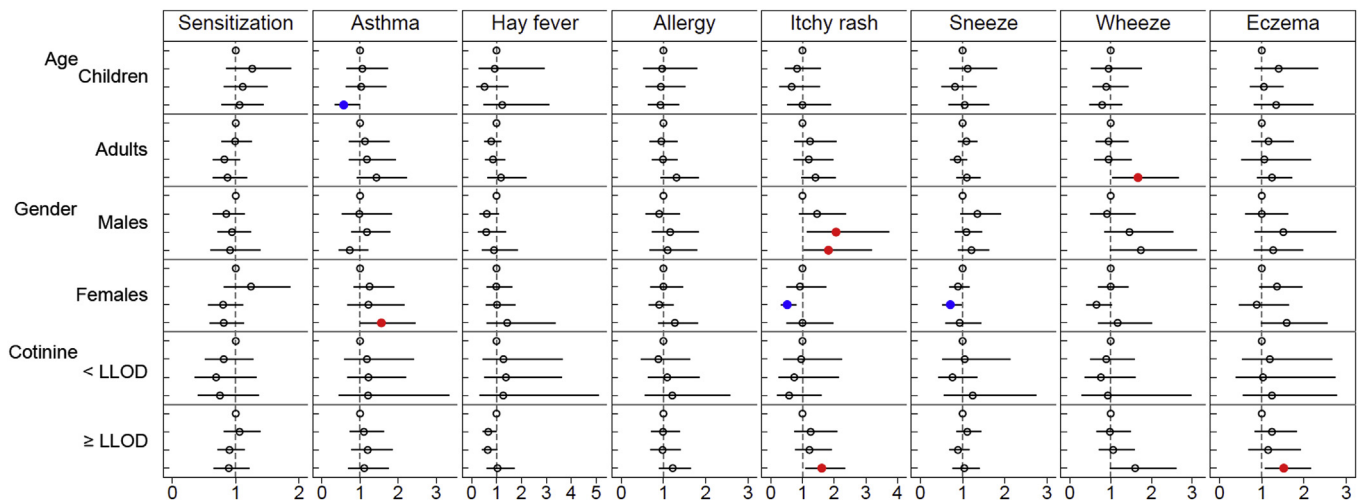


**Fig. 3.** Stratification analyses on ORs (95% CIs) for allergic conditions by quartiles of HbGA levels. Analyses were stratified by age (children, adults), gender (males, females) and serum cotinine levels (<LLOD,  $\geq$  LLOD). The dots and horizontal bars represent the ORs and corresponding 95% CIs, respectively. Solid dots in red and blue mean positive and negative associations with statistical significance ( $p$ -value < 0.05), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the development and outcome of the allergic immune response (Almqvist et al., 2008).

Smoking is a risk factor for allergy (Saulyte et al., 2014) and a major source of acrylamide exposure (Vesper et al., 2010). Therefore, smoking is a possible confounding or modifying effect on the associations between acrylamide and allergy. In the stratification analyses, all the participants were categorized into two groups by the LLOD of serum cotinine (Weitzman et al., 2005), which is a proximate metabolite of nicotine and is regarded as an excellent biomarker for tobacco smoke exposure including both active smokers and nonsmokers exposed to second smoke (Vesper et al., 2007).

In the subgroup of cotinine levels < LLOD, individuals at the fourth quartile of HbAA levels had decreased risks of itchy rash compared with those at the first quartile (OR = 0.20, 95% CI = 0.05–0.91, Fig. 2, Table S1), for which the findings should be interpreted with cautions because of the limited sample size

( $n = 134$ ) at the highest quartile. Our data indicated that HbAA and HbGA levels were positively related with itchy rash, wheeze and eczema among subjects with cotinine levels  $\geq$  LLOD. The interactions between cotinine levels and HbAA and HbGA exposure were not statistically significant for allergic sensitization in the present study. Furthermore, all the odds ratios were estimated with the adjustment of cotinine levels. It is not clear whether or how the tobacco smoke exposure is involved in the biological process of acrylamide-induced allergy-related outcomes, which requires further studies.

There are several strengths for the present study. The NHANES study collected a large-scaled, nationally-representative sample of the general US population. Particularly, NHANES 2005–2006 provided comprehensive information on the demographics, housing characteristics and allergic components of IgE testing and self-reported allergic conditions. In some previous studies, dietary intake of acrylamide was assessed by the method of food frequency

questionnaires, which might lead to inaccuracy because of the various dietary habits and ignored exposure routes such as smoking (Freisling et al., 2013; Xu et al., 2014). The quantification of HbAA and HbGA are stable over the lifespan of erythrocytes and have been widely applied as biomarkers of human internal exposure to acrylamide (Vesper et al., 2010). A review on levels of HbAA and HbGA was conducted to comprehensively compare our results with previous studies (Table S3). That inconsistent levels of hemoglobin adducts across studies were observed may be explained by differences in demographics, acrylamide exposures and sample size of the participants, as well as different analytical methods (Vesper et al., 2010). Despite of the nationwide sample in US, the generalization of our findings should be treated with cautions.

Limitations have to be addressed at the same time. Firstly, the cross-sectional design precluded the confirmation of causality between acrylamide exposure and allergy-related outcomes. Secondly, the self-reported allergic conditions might be biased, especially among children whose questionnaires were completed with the help from adult proxies. Thirdly, a single measurement of HbAA and HbGA may not capture the long-term average exposure and intra-individual variability (Obon-Santacana et al., 2016). Additionally, levels of HbAA and HbGA were standardized by hemoglobin concentration in the blood and expressed as “pmol/G Hb”. Actual levels of acrylamide exposure may be estimated with bias because of the hemoglobin status, which is affected by factors of age, gender, smoking, drinking, diet and physical activity (Milman and Pedersen, 2009; Xu et al., 2014). Therefore, misclassification of cases may take place when HbAA and HbGA are used as internal exposure estimates. Furthermore, the possibility of residual confounding factors could not be excluded although the demographics and housing characteristics were adjusted in the models.

## 5. Conclusion

In conclusion, this study demonstrated that HbAA and HbGA levels were associated with the likelihoods of allergy-related outcomes among the general US population, depending on age, gender and smoke exposure status. Future longitudinal studies with repeated measurements are recommended to establish correct temporality of exposure and health effects. Laboratorial explorations on the immunotoxicity of acrylamide and its metabolites are warranted to elucidate the biological mechanisms. Because of the ubiquitous exposure to acrylamide and its metabolites in the general population, the potential public health applications of the findings are substantial.

## Conflicts of interests

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2017.03.016>.

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