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Fumonisin B₁ and Risk of Hepatocellular Carcinoma in Two Chinese Cohorts

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Abstract

Fumonisin B₁ (FB₁), a mycotoxin that contaminates corn in certain climates, has been demonstrated to cause hepatocellular cancer (HCC) in animal models. Whether a relationship between FB₁ and HCC exists in humans is not known. To examine the hypothesis, we conducted case-control studies nested within two large cohorts in China; the Haimen City Cohort and the General Population Study of the Nutritional Intervention Trials cohort in Linxian. In the Haimen City Cohort, nail FB₁ levels were determined in 271 HCC cases and 280 controls. In the General Population Nutritional Intervention Trial, nail FB₁ levels were determined in 72 HCC cases and 147 controls. In each population, odds ratios and 95% confidence intervals (95% CI) from logistic regression models estimated the association between measurable FB₁ and HCC, adjusting for hepatitis B virus infection and other factors. A meta-analysis that included both populations was also conducted. The analysis revealed no statistically significant association between FB₁ and HCC in either Haimen City (OR=1.10, 95% CI=0.64–1.89) or in Linxian (OR=1.47, 95% CI=0.70–3.07). Similarly, the pooled meta-analysis showed no statistically significant association between FB₁ exposure and HCC (OR=1.22, 95% CI=0.79–1.89). These findings, although somewhat preliminary, do not support an association between FB₁ and HCC.

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Keywords

fumonisin; hepatocellular carcinoma; cohort study; China; epidemiology

1. Introduction

Hepatocellular carcinoma (HCC) is the 6th most commonly occurring cancer in the world (Ferlay et al. 2010). In high-risk HCC areas, such as eastern Asia and sub-Saharan Africa, major risk factors include chronic infection with hepatitis B virus (HBV) and consumption of foods contaminated with the mycotoxin, aflatoxin B₁. Another mycotoxin, the secondary metabolite fumonisin B₁ (FB₁), is produced by the mold *Fusarium verticillioides* that preferentially grows on *Zea mays*. In addition to pleiotropic toxic actions (Norred et al. 1998), FB₁ induces tumors in laboratory animals (Stockmann-Juvala and Savolainen 2008). One study that added FB₁ at 50 ppm to the diet of male rats for 26 months, reported the induction of hepatocellular carcinomas, but no lesions in the esophagus or fore stomach (Gelderblom et al. 1991). In a two-year rodent bioassay testing the carcinogenicity of multiple doses of FB₁ in both sexes of rats and mice, dietary FB₁ at 50 ppm caused liver tumors in the female mice and kidney tumors in the male rats (Howard et al. 2001). The carcinogenic mechanism of FB₁ is a topic of continuing research (Gelderblom et al. 2008). Animals exposed to FB₁ have elevated serum sphingolipid levels, which might serve as a biomarker of exposure and reflect alternation that may be part of the mechanism of toxicity and carcinogenesis, namely inhibition of ceramide synthase (Desai et al. 2002). The inhibition of ceramide production could lead to reduced apoptosis and survival of DNA-damaged cells, stimulation of cell division and increased regeneration (Riley et al. 2001) and thereby, encourage carcinogenesis. Very little, however, is known about the effects of FB₁ in humans as most research has been conducted in rodents. Though FB₁ is poorly absorbed, small amounts have been measured in the liver and kidneys (Norred et al. 1993) and several studies have reported hepatotoxic effects, including toxic hepatitis (Gelderblom et al. 2008). Recently, the U.S. Food and Drug Administration published guidelines recommending that total fumonisins in human food be limited to 2 ppm (U.S. Food and Drug Administration 2001). Based on the animal data, FB₁ has been classified as a possible human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer (IARC) 2002). Ecologic studies have reported correlations between FB₁ in food and local rates of esophageal squamous cell carcinoma (Sydenham et al. 1990) and liver cancer (Sun et al. 2007; Ueno et al. 1997), but ecologic studies provide only a weak assessment of any putative carcinogen. A single epidemiologic study using a biomarker of individual exposure found no association with the risk of esophageal or stomach cancer in China (Abnet et al. 2001). In that study, exposure was assessed by serum sphingolipid concentrations, which may indicate only short-term differences in exposure. Fumonisin exposure through food has been and continues to be widespread in China (Chu and Li 1994; Gong et al. 2009). In Haimen City, China, a particularly high risk area for HCC, persistently elevated levels of FB₁ in corn have been reported (Ueno et al. 1997). In Linxian, a prior study suggested that FB₁ was likely to interact with other mycotoxins and might play a role in carcinogenesis in Linxian. Data from Linxian, however, have reported that levels of AFB₁ are low (Chu and Li 1994). Few studies of FB₁ in relationship to HCC in humans have been conducted, however, partially due to the lack of well-validated biomarkers for FB₁ and the short half-life of FB₁ in serum of approximately 128 min (DeLongchamp and Young 2001; Shephard et al. 2007). Hair FB₁ concentrations have been shown to reflect FB₁ intake in rodents and laboratory primates (Sewram et al. 2001) and may be useful for the assessment of exposure in humans (Sewram et al. 2003). While the actual half-life of FB₁ in nails has not yet been determined, residence time in the nail matrix is much longer than any of the other biological matrices (Sewram et al., 2001) because FB₁ becomes embedded within the

keratin. Nails are an attractive matrix for other drug-related forensic investigations for this reason (Gaillard and Pepin, 1999) and have found their place in exposure assessment studies. In addition, the study participants were reluctant to provide hair samples, thus the hair method was adapted for use in human toenails with a similar collagen matrix. The development of assays to determine FB₁ levels in nails (Sewram et al. 2003) has somewhat circumvented these obstacles and enabled the current study to examine the association between HCC and FB₁ levels in toenails. In two case-control analyses nested within prospective studies in China and in a meta-analysis we tested the association between the measurable FB₁ in nails and HCC.

2. Material and Methods

2.1 Study populations

The current study includes two populations in China; the Haimen City Cohort in Jiangsu province and the Nutritional Intervention Trials cohort of Linxian in Henan province. The Haimen City Cohort study protocols and materials were approved by the Institutional Review Board of Fox Chase Cancer Center, the Medical Ethics Review Group of Haimen City and the Ethics Review Committee of Shanghai Medical University and the Institutional Review Boards of the U.S. National Cancer Institute. The Nutritional Intervention Trials study protocols were approved by the Institutional Review Boards of the Cancer Institute of the Chinese Academy of Medical Sciences and the U.S. National Cancer Institute. All study participants provided informed consent to participate.

2.1.1 The Haimen City Cohort—In early 1991, the Haimen City Cohort study was initiated to investigate the influence of environmental, viral and genetic risk factors on the risk of developing HCC (Evans et al. 2002). Briefly, between January 1992 and December 1993, 90,836 cohort participants were enrolled. All participants provided written consent, completed an epidemiologic questionnaire and donated a blood sample. The cohort, composed of healthy adults aged 25–64 years, included 58,454 men and 25,430 women. Participants were actively followed until September 2000 and then passively followed via death certificate determination. HCC diagnoses were ascertained by histology and/or liver imaging, α -fetoprotein elevation (>400 ng/ml), clinical criteria, or by death certificate in conjunction with postmortem interviews of family members. Study participants diagnosed with HCC less than 1 year after study enrollment were excluded from the current analysis to avoid misclassification of prevalent tumors. In addition, starting in January 1993, using ceramic nail clippers toenail specimens were collected from a subset of the cohort ($n=41,563$). For the current analysis, HCC cases ($n=271$) were frequency matched to controls ($n=280$) on sex and residential township using the criteria previously reported (Sakoda et al. 2005).

2.1.2 The General Population of the Nutritional Intervention Trial of Linxian—

The Nutritional Intervention Trial cohort of Linxian has been previously described. In brief due to the high rates of esophageal and gastric cardia cancer in Linxian, an intervention trial was performed to test whether supplementation with micronutrients would reduce the rates of these cancers (Blot et al. 1993). The General Population Trial cohort included 29,584 healthy adults aged 40–69 years from four communes in Linxian County, Henan Province. In 1985, one year before the beginning of the trial intervention, each participant completed a questionnaire, received a brief physical examination and supplied a 10 mL blood sample. At the end of the trial period, in May 1991, all living subjects were asked to provide a toenail sample, and 71% of eligible subjects did so. Although the intervention trial ended in 1991, the cohort has remained under active follow-up with monthly contact between cohort members and village health workers. All newly diagnosed cancers are recorded and medical

records are collected for review by study personnel (Qu et al. 2007). Liver cancer diagnosis was based on pathologic review, biochemical assays, clinical examination, ultrasound, and computed tomography scan. All incident primary liver cancer cases (n=72) that occurred between May 1991 and May 2001 among subjects that provided a toenail sample were selected, as well as an age and sex frequency matched subset of the cohort as the referent group (n=147).

2.2 Laboratory analyses

FB₁ analyses were conducted at the Medical Research Council, South Africa. The study samples were analyzed by laboratory personnel blinded to case-control status. Hepatitis B virus (HBV) status was ascertained at the Haimen City Centers for Disease Control by determination of hepatitis B surface antigen (HBsAg) in serum by radioimmunoassay (Sakoda et al. 2005) and in Linxian by enzyme-linked immunosorbent assay (McGlynn et al. 2006).

2.2.1 Extraction of toenail samples—The median mass of the Haimen samples was 84 mg, while the median mass of the Linxian samples was 18.0 mg. A weighed quantity of each toenail sample, was placed into a stainless steel grinding jar (10 mL), cooled in liquid nitrogen for 2 minutes and milled for 12 seconds using a mixer mill (Retsch MM301, Haan, Germany) set at a frequency of 20 Hz. Test portions were quantitatively transferred into extraction flasks by repeatedly washing with methanol and refluxed in methanol (100 mL) for 5 hours. The extracts were subsequently filtered (Whatman No. 4 filter paper) and the methanol evaporated under reduced pressure. The filtrate was reconstituted in methanol-water (70:30, 20 mL) and clean up performed on BondElut LRC® strong anion exchange (SAX) solid phase extraction cartridges containing 500 mg of sorbent (Varian, Harbor City, CA, USA) according to the method of Sydenham *et al* (Sydenham et al. 1996). In brief, the filtrate was adjusted to pH 6.0 and passed through SAX sorbent, which had been preconditioned with methanol (5 mL), followed by methanol-water (5 mL, 7:3). The fumonisins were eluted with 1% acetic acid-methanol solution (10 mL). The eluate from the SAX cartridge was evaporated to dryness at 60 °C under nitrogen and the residue reconstituted into 300 uL of HPLC mobile phase prior to HPLC injection.

2.2.2 HPLC-MS-MS Analysis—Samples from the Linxian cohort were analyzed using a Spectra SERIES P2000 HPLC pump equipped with an AS 1000 autosampler (Thermo Separation Products Inc, Riviera Beach, FL, USA). On the Haimen City Cohort samples, HPLC was performed using an Agilent 1200 Series Binary Pump SL (Waldbronn, Germany) equipped with an Agilent 1200 series HiP-ALS SL auto sampler (Santa Clara, CA, USA). Chromatography on both the Linxian and Haimen City samples were undertaken in quadruplicate using binary gradient elution on a 150 × 4.6 mm I.D. Luna C₁₈ column (5 µm ODS-2, Phenomenex, Torrance, CA, USA) at 0.7 mL/min and a 50 × 4.6 mm Zorbax Eclipse XDB-C₁₈ column (5 µm ODS-2, Agilent Technologies, CA, USA) at 0.3 mL/min respectively. Both systems contained a 20 uL injection loop and the test samples were filtered through a 0.45 µm syringe filter (Millipore, Yonezawa, Japan) prior to injections. The binary elution mixture consisted of water-acetonitrile-formic acid in the ratios 90:10:0.1 (Solvent A) and 10:90:0.1 (Solvent B). The initial composition of 80% A and 20% B was adjusted linearly over a 6-minute period to 65% A and 35% B, held for two minutes, re-adjusted linearly over one minute to 80% A and 20% B and held for another one minute, resulting in a total run time of 10 minutes.

Tandem mass spectrometry with positive ion electrospray ionization was undertaken using a Finnigan MAT LCQ ion trap mass spectrometer (San Jose, CA, USA) and an Agilent 6530 Accurate-Mass Q-TOF LC/MS instrument (Santa Clara, CA, USA). MS parameters on the

LCQ system were optimized for FB₁ (5 ug/mL) by direct infusion into the source at a rate of 5 uL/min. Full scan MS-MS between m/z 330 and m/z 730 was undertaken using a collision energy of 34%. The resulting product ions were monitored as diagnostic indicators for the presence of FB₁ in the toenail samples. The HPLC eluate entered the mass spectrometer without splitting at a source voltage of 4.5 kV and a capillary voltage of 40 V, while the heated capillary temperature was maintained at 220 °C and the sheath to auxiliary gas ratio was set at 4:1. Optimizations of the MS parameters on the Agilent system were undertaken by repeatedly injecting the FB₁ standard (5 ug/mL) through the sample loop. A mass range of 100–1000 m/z in MS mode and 320–730 m/z in MS/MS mode with a scan rate of 3 spectra/s was selected. The source parameters were: gas temperature 300 °C, gas flow 6 L/min, nebulizer pressure 35 psi, sheath gas temperature 300 °C, sheath gas flow 10 L/min, VCap voltage 3500 V, nozzle voltage 1000 V and fragmentor voltage 150 V, collision energy 39 V. The device was operated by the software, MassHunter Acquisition B.02.01. The ion at m/z 704 was used as the quantifying ion.

2.2.3 Assay performance—Both analytical methods were validated using the ICH criteria and in a manner similar to that reported for the analysis of cocaine and its metabolites in nails (Valente-Campos et al. 2006). For the LCQ instrument, the limits of detection and quantitation for FB₁ were observed to be 25 and 60 picograms, respectively. The linearity of response for FB₁ was determined from the limit of quantitation up to the level of 250 ug/L with the calibration plot showing a coefficient of determination (R^2) >0.99. The precision of the measurements was determined by performing 15 injections under identical conditions and found to have a coefficient of variation of 4.86% at the 15 ug/L level. For the Agilent system, the on column Limit of Detection (LOD) and Limit of Quantitation (LOQ) determined to be 6 picograms and 20 pg respectively. The linearity of response from the LOQ to 50 ng showed a coefficient of determination (R^2) > 0.99. The recoveries from ground toenail samples (N=6) ranged between 60% and 77% with methanol extraction at the 15 ug/L level when determined using both instruments.

2.3 Statistical analyses

There were no statistically significant differences between the FB₁ nail concentration in cases and controls either in Linxian, (cases=1.96 ng/mg, controls=2.27 ng/mg, $p=0.16$), or in Haimen (cases=0.375 ng/mg, controls=0.143ng/mg, $p=0.50$) (data not shown). Due to the small number of individuals with measurable levels of FB₁ in either cohort, however, the subjects were categorized as having any or no FB₁ in their toenails. We used a two step analytic approach. First we calculated multivariate unconditional logistic regression models to estimate the population specific odds ratios (OR) and 95% confidence intervals (CIs) for the Haimen City and Linxian populations, separately. Both crude and adjusted logistic regression models were considered. For the adjusted models, sex, place of residence, age in years (<45, 46–57, ≥58), alcohol drinking (yes/no) and hepatitis B surface antigen (HBsAg) (positive/negative) were included. We present only the results from the adjusted models. In a second analysis, the study-population specific ORs were pooled using random-effects meta-analysis to generate a summary odds ratio. Heterogeneity between the study results was investigated using the I^2 statistic (Higgins et al. 2003). I^2 values approaching zero (0%) indicate little heterogeneity between studies while larger values are indicative of heterogeneity. The same methodology was used for sex-specific analysis. All statistical analyses were performed using Stata/SE 11.0 (StataCorp College Station, TX, USA).

3. Results

The characteristics of the study populations are shown in Table 1. A total of 343 primary liver cancer cases and 427 controls were included. The Haimen City population included

more men (92%) than the Linxian population (75%) and had a younger mean age at diagnosis (45 years vs. 56 years). Alcohol consumption was more common in Haimen City, with more than half of the study participants drinking ≥ 4 drinks/week, while in Linxian only 26% reported that they have had alcohol the last 12 months. Further, in the Haimen City study, 67% of the cases were HBsAg (+) while only 26% of the Linxian cases were HBsAg (+). FB₁ was detected in a similar percentage of cases and controls in both studies (19–25%).

As shown in Table 2, nail levels of FB₁ in the cases and controls did not differ significantly in either the crude or the adjusted models. The risks were slightly higher in the Linxian population OR=1.47 (95% CI: 0.70–3.07), than the Haimen City Cohort OR=1.10 (95% CI: 0.64–1.89), though neither reached statistical significance.

In the pooled analysis of FB₁ exposure we observed no statistically significant associations with HCC (overall OR=1.22, 95% CI: 0.79–1.89), Fig. 1. Again, there was no difference in the results of the crude or adjusted models. The I²-value from the random effects meta-analysis of FB₁ exposure indicated very low levels of heterogeneity for liver cancer (I²=0.0%). A meta-analysis restricted to males found no significant association between FB₁ and HCC (OR=0.99, 95% CI: 0.61–1.61) and low levels of heterogeneity (I²=0.0%), Fig. 2. Similarly, among women in a combined analysis, there was no statistically significant association between FB₁ and HCC (OR=3.52, 95% CI: 0.38–32.96). While there was a moderate levels of heterogeneity in the female data (I²=66.2%), it was not statistically significant.

4. Discussion

It has been estimated that 30% of HCC, worldwide, may be attributable to AFB₁ exposure (Liu and Wu 2010). Whether FB₁ could also be a risk factor for HCC is not clear although previous dietary studies (Li et al. 2001; Sun et al. 2007; Ueno et al. 1997) have reported the co-occurrence of FB₁ and AFB₁ in corn in high-risk HCC areas. In a recent report, FB₁ and AFB₁ were very likely to co-contaminate corn in both a HCC high risk area (Fusui County, Guangxi Zhuang Autonomous Region) and in an esophageal high risk area (Chuzhou region, Huaian City, Jiangsu Province) (Sun et al. 2011). Both AFB₁ and FB₁ concentrations were higher in the esophageal high risk area, suggesting that attempts to mediate the mycotoxin problem in high rate HCC areas may have had an effect. The synergistic effect of AFB₁ and HBV infection on the risk of HCC, has heightened awareness that mycotoxin eradication could have a large effect on lowering HCC rates in high-rate areas (Chu and Li 1994).

In the current meta-analysis, we investigated the relationship between toenail levels of FB₁ and liver cancer within Chinese adults from two study populations; the Haimen City Cohort and the General Population Nutritional Intervention Trial Cohort. In Haimen the incidence of liver cancer is among the highest in the world while the Linxian population is considered a high risk population for gastric and esophageal cancers. We found no statistically significant association between FB₁ and HCC in either population. Despite a previous report that corn in Haimen City was highly contaminated (37 out of 40 corn samples) with FB₁ (Ueno et al. 1997), FB₁ was undetectable in the nail samples of 76% of the study participants from Haimen City Cohort. When the meta-analysis was stratified by sex, there were no statistically significant associations among either men or women. In Linxian, while there was indication of a significant association between FB₁ and HCC among women, these results were not confirmed in Haimen and were based on a small sample size.

We used a simple classification in this study: measurable FB₁ in the toenail. Previous work suggests that the concentrations of FB₁ in hair are proportional to intake of FB₁ (Sewram et al. 2003; Sewram et al. 2001) but we did not test this directly in this study. Measuring a parent compound in a biological sample integrates both intake, loss through metabolism, and propensity to retain the parent compound rather than direct excretion without metabolism. Future studies will be required to understand the relationship of FB₁ intake to FB₁ toenail concentration. We note that all subjects were cancer-free at the time of toenail collection and the liver cancers cases occurred up to 10 years after toenail collection, so reverse causation is unlikely.

A major strength of this analysis is that it was conducted using two large, prospectively designed cohort studies where FB₁ levels were measured in pre-diagnostic toenail clippings and questionnaire information was collected prior to the development of cancer. Previous reports have found an association between urinary aflatoxin-nucleic acid adducts and liver cancer in China (Qian et al. 1994). In that study, the adjusted OR (95% CI) for aflatoxin adducts was 5.0 (2.1–11.8), but there was a significant interaction with hepatitis such that the OR was 3.4 (95%CI=1.1–10.0) in hepatitis-negative subjects and 59.4 (95%CI=16.6–212.0) in hepatitis-positive subjects (Qian et al. 1994). We had insufficient power to formally test for an interaction between FB₁ exposure and HBV in our study.

Despite its strengths, our study has several limitations. We had no assessment of aflatoxin B₁ (AFB₁) exposure and AFB₁ is a known hepatocarcinogen in China. FB₁ and AFB₁ producing molds can co-occur in both Linxian (Chu and Li 1994) and Haimen and therefore AFB₁ exposure could confound or interact with (McKean et al. 2006) the association between FB₁ and HCC. In addition, despite prior reports of FB₁ in the food-supply, only a minority of nail samples had detectable FB₁ levels. The modest number and low exposure prevalence limited the analysis capability to look at interesting factors related to the disease. In addition, nail specimens reflect exposure only over the time period of nail growth so FB₁ exposure in the remote past would not be well captured. The inability to measure past FB₁ exposure might have biased the study toward a null finding. The current study also did not measure FB₁ levels in foods, thus it is not possible to determine how well nail FB₁ levels correlate with dietary intake levels. Also, the current results might not be consistent with fumonisin contamination of the food supply because absorption after ingestion is reportedly low; in addition, the half-life of fumonisin in the body is not long (Shephard et al. 2007) (DeLongchamp and Young 2001). Further FB₁ exposure in China is likely to fall as consumption of high exposure products such as corn decline.

In conclusion, in these preliminary studies, we found little evidence to support an association between toenail FB₁ levels and HCC in Chinese adults. Our results suggest that FB₁ exposure may not be related to the development of liver cancer in humans, however further studies are needed to confirm this.

References

- Abnet CC, Borkowf CB, Qiao YL, Albert PS, Wang E, Merrill AH Jr, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Sphingolipids as biomarkers of fumonisin exposure and risk of esophageal squamous cell carcinoma in china. *Cancer Causes Control*. 2001; 12:821–828. [PubMed: 11714110]
- Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl. Cancer Inst*. 1993; 85:1483–1492. [PubMed: 8360931]
- Chu FS, Li GY. Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol*. 1994; 60:847–852. [PubMed: 8161178]

- Delongchamp RR, Young JF. Tissue sphinganine as a biomarker of fumonisin-induced apoptosis. *Food Addit. Contam.* 2001; 18:255–261. [PubMed: 11304034]
- Desai K, Sullards MC, Allegood J, Wang E, Schmelz EM, Hartl M, Humpf HU, Liotta DC, Peng Q, Merrill AH Jr. Fumonisin and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. *Biochim. Biophys. Acta.* 2002; 1585:188–192. [PubMed: 12531553]
- Evans AA, Chen G, Ross EA, Shen FM, Lin WY, London WT. Eight-year follow-up of the 90,000-person Haimen City cohort: I. Hepatocellular carcinoma mortality, risk factors, and gender differences. *Cancer Epidemiol. Biomarkers Prev.* 2002; 11:369–376. [PubMed: 11927497]
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer.* 2010
- Gelderblom WC, Kriek NP, Marasas WF, Thiel PG. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B1, in rats. *Carcinogenesis.* 1991; 12:1247–1251. [PubMed: 1649015]
- Gelderblom WC, Marasas WF, Lebepe-Mazur S, Swanevelder S, Abel S. Cancer initiating properties of fumonisin B1 in a short-term rat liver carcinogenesis assay. *Toxicology.* 2008; 250:89–95. [PubMed: 18616970]
- Gong HZ, Ji R, Li YX, Zhang HY, Li B, Zhao Y, Sun L, Yu F, Yang J. Occurrence of fumonisin B(1) in corn from the main corn-producing areas of China. *Mycopathologia.* 2009; 167:31–36. [PubMed: 18636342]
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003; 327:557–560. [PubMed: 12958120]
- Howard PC, Eppley RM, Stack ME, Warbritton A, Voss KA, Lorentzen RJ, Kovach RM, Bucci TJ. Fumonisin b1 carcinogenicity in a two-year feeding study using F344 rats and B6C3F1 mice. *Environ. Health Perspect.* 2001; 109(Suppl 2):277–282. [PubMed: 11359696]
- International Agency for Research on Cancer (IARC). International Agency for Research on Cancer (IARC). 2002. Summaries & Evaluations; p. 301
- Li FQ, Yoshizawa T, Kawamura O, Luo XY, Li YW. Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. *J. Agric. Food Chem.* 2001; 49:4122–4126. [PubMed: 11513719]
- Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ. Health Perspect.* 2010; 118:818–824. [PubMed: 20172840]
- McGlynn KA, Abnet CC, Zhang M, Sun XD, Fan JH, O'Brien TR, Wei WQ, Ortiz-Conde BA, Dawsey SM, Weber JP, Taylor PR, Katki H, Mark SD, Qiao YL. Serum concentrations of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and risk of primary liver cancer. *J Natl. Cancer Inst.* 2006; 98:1005–1010. [PubMed: 16849683]
- McKean C, Tang L, Tang M, Billam M, Wang Z, Theodorakis CW, Kendall RJ, Wang JS. Comparative acute and combinative toxicity of aflatoxin B1 and fumonisin B1 in animals and human cells. *Food Chem. Toxicol.* 2006; 44:868–876. [PubMed: 16427177]
- Norred WP, Plattner RD, Chamberlain WJ. Distribution and excretion of [¹⁴C]fumonisin B1 in male Sprague-Dawley rats. *Nat. Toxins.* 1993; 1:341–346. [PubMed: 8167955]
- Norred WP, Voss KA, Riley RT, Meredith FI, Bacon CW, Merrill AH Jr. Mycotoxins and health hazards: toxicological aspects and mechanism of action of fumonisins. *J Toxicol. Sci.* 1998; 23(Suppl 2):160–164. [PubMed: 9760455]
- Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol. Biomarkers Prev.* 1994; 3:3–10. [PubMed: 8118382]
- Qu CX, Kamangar F, Fan JH, Yu B, Sun XD, Taylor PR, Chen BE, Abnet CC, Qiao YL, Mark SD, Dawsey SM. Chemoprevention of primary liver cancer: a randomized, double-blind trial in Linxian, China. *J Natl. Cancer Inst.* 2007; 99:1240–1247. [PubMed: 17686823]
- Riley RT, Enongene E, Voss KA, Norred WP, Meredith FI, Sharma RP, Spitsbergen J, Williams DE, Carlson DB, Merrill AH Jr. Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environ. Health Perspect.* 2001; 109(Suppl 2):301–308. [PubMed: 11359699]

- Sakoda LC, Graubard BI, Evans AA, London WT, Lin WY, Shen FM, McGlynn KA. Toenail selenium and risk of hepatocellular carcinoma mortality in Haimen City, China. *Int. J. Cancer*. 2005; 115:618–624. [PubMed: 15704105]
- Sewram V, Mshicileli N, Shephard GS, Marasas WF. Fumonisin mycotoxins in human hair. *Biomarkers*. 2003; 8:110–118. [PubMed: 12775496]
- Sewram V, Nair JJ, Nieuwoudt TW, Gelderblom WC, Marasas WF, Shephard GS. Assessing chronic exposure to fumonisin mycotoxins: the use of hair as a suitable noninvasive matrix. *J. Anal. Toxicol.* 2001; 25:450–455. [PubMed: 11550819]
- Shephard GS, Van Der Westhuizen L, Sewram V. Biomarkers of exposure to fumonisin mycotoxins: a review. *Food Addit. Contam.* 2007; 24:1196–1201. [PubMed: 17886192]
- Stockmann-Juvala H, Savolainen K. A review of the toxic effects and mechanisms of action of fumonisin B1. *Hum. Exp. Toxicol.* 2008; 27:799–809. [PubMed: 19244287]
- Sun G, Wang S, Hu X, Su J, Huang T, Yu J, Tang L, Gao W, Wang JS. Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Addit. Contam.* 2007; 24:181–185. [PubMed: 17364919]
- Sun G, Wang S, Hu X, Su J, Zhang Y, Xie Y, Zhang H, Tang L, Wang JS. Co-contamination of aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. *Food Addit. Contam Part A Chem. Anal. Control Expo. Risk Assess.* 2011; 28:461–470. [PubMed: 21259142]
- Sydenham EW, Shephard GS, Thiel PG, Stockenstrom S, Snijman PW, Van Schalkwyk DJ. Liquid chromatographic determination of fumonisins B1, B2, and B3 in corn: AOAC-IUPAC Collaborative Study. *J AOAC Int.* 1996; 79:688–696. [PubMed: 8634538]
- Sydenham EW, Thiel PG, Marasas WFO, Shephard GS, Van Schalkwyk DJ, Kock KR. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. *J. Agric. Food Chem.* 1990; 38:1900–1903.
- U.S. Food and Drug Administration. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds. 2001.
<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm109231.htm>
- Ueno Y, Iijima K, Wang SD, Sugiura Y, Sekijima M, Tanaka T, Chen C, Yu SZ. Fumonisin as a possible contributory risk factor for primary liver cancer: a 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. *Food Chem. Toxicol.* 1997; 35:1143–1150. [PubMed: 9449219]
- Valente-Campos S, Yonamine M, de Moraes Moreau RL, Silva OA. Validation of a method to detect cocaine and its metabolites in nails by gas chromatography-mass spectrometry. *Forensic Sci. Int.* 2006; 159:218–222. [PubMed: 16183230]
- Gaillard Y, Pepin G. Testing hair for pharmaceuticals. *J Chromatogr. B Biomed. Sci. Appl.* 1999; 733:231–246. [PubMed: 10572983]

Highlights

- Fumonisin B₁, a mycotoxin contaminates corn in certain climates and causes hepatocellular cancer (HCC) in some animal models.
- The limitation of FB₁ short half-life of in serum, in this study therefore FB₁ levels were measured in toenails using HPLC.
- The association between FB₁ in toenails and HCC was investigated separately and in a meta-analysis for the two cohorts.
- No association was found between FB₁ and HCC in either Haimen or in Linxian population or in the pooled meta-analysis.

These findings do not support an association between FB₁ and HCC.

Fig.1.
Meta-analysis of FB₁ and HCC stratified by study population showing odds ratios and 95% confidence intervals on a log scale.

Fig.2. Meta-analysis of FB₁ and HCC stratified by sex and study population showing odds ratios and 95% confidence intervals on a log scale.

Table 1

Characteristics of the study populations by study

Characteristics	Haimen City ^a		Linxian ^b	
	Cases	Controls	Cases	Controls
Study samples collected, year	1993–2000		1991–2001	
Toenail samples, n	271	280	72	147
Men, n (%)	248 (92)	258 (92)	54 (75)	109 (74)
Age, mean (SD)	45 (8.7)	45 (8.8)	56 (7.8)	56 (7.8)
HBsAg (+), n (%)	182 (67)	31 (11)	19 (26)	10 (7)
Consumed alcohol, n (%)	151 (56)	156 (56)	13 (18)	44 (30)
Smoked cigarettes, n (%)	181 (67)	169 (60)	39 (54)	71 (48)
FB ₁ detected, n (%)	66 (25)	62 (22)	17 (24)	28 (19)

^aThe Haimen City Cohort^bThe General Population Nutritional Intervention Trial Cohort

Table 2Fumonisin B₁ Exposure and Risk of HCC by Study Population

Population	Cases	Controls	OR _{crude} (95%CI)	OR _{adjusted} ^a (95%CI)
Haimen City Cohort	271	280	1.14 (0.77–1.70)	1.10 (0.64–1.89)
Linxian	72	147	1.31 (0.66–2.60)	1.47 (0.70–3.07)

OR; Odds ratios, CI; confidence intervals

^a ORs adjusted by sex, age, residence area, alcohol drinking, and HBsAg