Published in final edited form as:

Prostate. 2014 May ; 74(6): 637–646. doi:10.1002/pros.22781.

Case-Only Gene–Environment Interaction Between *ALAD* tagSNPs and Occupational Lead Exposure in Prostate Cancer

Christine Neslund-Dudas^{1,2,*}, Albert M. Levin^{1,2}, Andrew Rundle³, Jennifer Beebe-Dimmer², Cathryn H. Bock², Nora L. Nock⁴, Michelle Jankowski¹, Indrani Datta¹, Richard Krajenta¹, Q. Ping Dou^{5,6}, Bharati Mitra⁷, Deliang Tang⁸, and Benjamin A. Rybicki^{1,2}

¹Department of Public Health Sciences, Henry Ford Health System, Detroit, Michigan

²Population Studies and Prevention Programs, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan

³Department of Epidemiology, Columbia University Mailman School of Public Health, New York, New York

⁴Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio

⁵Developmental Therapeutics Programs, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan

⁶Department of Oncology, Pharmacology and Pathology, Wayne State University School of Medicine, Detroit, Michigan

⁷Department of Biochemistry & Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan

⁸Department of Environmental Health Sciences, Columbia University Mailman School of Public Health, New York, New York

Abstract

BACKGROUND—Black men have historically had higher blood lead levels than white men in the U.S. and have the highest incidence of prostate cancer in the world. Inorganic lead has been classified as a probable human carcinogen. Lead (Pb) inhibits delta-aminolevulinic acid dehydratase (*ALAD*), a gene recently implicated in other genitourinary cancers. The *ALAD* enzyme is involved in the second step of heme biosynthesis and is an endogenous inhibitor of the 26S proteasome, a master system for protein degradation and a current target of cancer therapy.

METHODS—Using a case-only study design, we assessed potential gene–environment ($G \times E$) interactions between lifetime occupational Pb exposure and 11 tagSNPs within *ALAD* in black ($N = 260$) and white ($N = 343$) prostate cancer cases.

RESULTS—Two *ALAD* tagSNPs in high linkage disequilibrium showed significant interaction with high Pb exposure among black cases (rs818684 interaction odds ratio or IOR = 2.73, 95% CI 1.43–5.22, $P = 0.002$; rs818689 IOR = 2.20, 95% CI 1.15–4.21, $P = 0.017$) and an additional tagSNP, rs2761016, showed G $\times$ E interaction with low Pb exposure (IOR = 2.08, 95% CI 1.13–3.84, $P = 0.019$). Further, the variant allele of rs818684 was associated with a higher Gleason grade in those with high Pb exposure among both blacks (OR 3.96, 95% CI 1.01–15.46, $P = 0.048$) and whites (OR 2.95, 95% CI 1.18–7.39, $P = 0.020$).

CONCLUSIONS—Genetic variation in *ALAD* may modify associations between Pb and prostate cancer. Additional studies of *ALAD*, Pb, and prostate cancer are warranted and should include black men.

Keywords

prostate cancer; *ALAD*; delta-aminolevulinic acid dehydratase; occupational exposure; lead; heavy metal; African American

INTRODUCTION

U.S. black men have the highest rate of prostate cancer in the world and are more likely to be diagnosed with the disease than white men in the U.S. [1]. To date, no environmental exposure has been consistently linked to prostate cancer. However, if there is a major environmental exposure for the disease black men in the U.S. may be at higher risk for exposure.

Historically, black men have had higher blood [2] and bone [3] Pb levels when compared to U.S. whites. The International Agency for Cancer Research (IARC) recognizes inorganic Pb and Pb compounds as probable human carcinogens based on animal studies and a limited number of human studies [4]. Only a few studies have specifically assessed Pb exposure and prostate cancer risk [5,6] and these studies have reported mixed findings, likely due to differences in not only duration and type (direct vs. indirect) of exposure assessment but also in accuracy of case ascertainment and availability of information on important confounders such as race and smoking history. Complicating these early studies, further, was the widespread exposure of the general public to leaded gasoline between 1940 and the mid-1990s, increasing opportunities for exposure misclassification. No study to date has included genetic factors that may modify the association between Pb and prostate cancer or included a large proportion of black men, who are disproportionately affected by the disease and have higher Pb burden.

The most provocative observations supporting continued inquiry into Pb and prostate cancer are reports that indicate Pb exposure affects levels of zinc (Zn) in the body [7–9]. The most relevant of these is a report by Telisman et al. [7] which found lower seminal fluid Zn levels in Pb exposed workers compared to non-exposed workers. Zn is essential for normal prostate development and the prostate is known for high levels of Zn compared to other soft tissues. However, Zn is low in prostate tumor tissue and prostatic fluid of prostate cancer cases [10,11] suggesting an important role for Zn in the development of the disease. In addition to modifying Zn levels, Pb can replace Zn in some Zn-containing proteins [12,13].

The best known example of this is the replacement of Zn by Pb in delta-aminolevulinic acid dehydratase (ALAD) which results in the inhibition of the enzyme [14] as ALAD contains eight identical subunits that each contain Zn. ALAD is responsible for catalyzing the second step in heme biosynthesis and is also an endogenous inhibitor of the 26S proteasome [15,16]. The proteasome has been implicated in several cancers and is a current target for cancer therapy [17], including therapies under development for prostate cancer [18,19]. An earlier report by our group found significant correlations between red blood cell Pb levels and proteasome activity in healthy controls but not in age and race matched prostate cancer cases [20]. Whether ALAD plays a role in the differences we observed in cases and controls is unknown at this time; however, recent studies in other genitourinary cancers [8,9] found genetic variants in *ALAD* to be associated with risk of disease.

Coupled together, these findings have led us to hypothesize that Pb exposure may be a risk factor for prostate cancer and that genetic variation in *ALAD*, may modify associations between Pb exposure and prostate cancer. Further U.S. black men may be at particular risk, due to the higher levels of Pb that have been reported in blacks. Since the biological interaction between *ALAD* and Pb is already established and there is no indication of dependence between the gene and Pb, as there is for other gene–exposure combinations such as genetic variants associated with addiction and tobacco use, the case-only design [17] is appropriate for this study. The case-only design is the most statistically efficient method to initially screen for potential gene $\times$ environment ($G \times E$) interactions in prostate cancer and eliminates potential problems with unmeasured confounders which may have been problematic in past cohort and case–control studies. Therefore, in this study we screened for potential $G \times E$ interaction between tagSNPs within *ALAD* and lifetime occupational Pb exposure in black and white prostate cancer cases. We also assessed associations between *ALAD* tagSNPs and prostate cancer aggressiveness taking Pb exposure status into account. Our findings are reported here.

METHODS

Study Population and Data Collection

The prostate cancer cases used in this study have been previously described [21]. In brief, cases were recruited from the patient population of a large vertically integrated health system serving Metropolitan Detroit. Detroit's major industry is automobile manufacturing and as such the health system provides care for a large population of current and retired autoworkers and individuals from industries which support automobile manufacturing. All cases were recruited for the study between 2001 and 2004 and had a pathological diagnosis of adenocarcinoma of the prostate. Those who agreed to participate were asked to complete a two-part interviewer-administered risk factor questionnaire, complete a food frequency questionnaire [22] and to donate a blood sample for DNA analysis. All study protocols were approved by the institution's Human Subjects Review Board. Age, race, smoking status, and job history information were all self-reported. Clinical data on disease aggressiveness was abstracted from pathology reports, medical records, and the health systems certified tumor registry. Address at the time of diagnosis was geocoded using MapInfo Professional® v7.0 and MapMarker® v.8.1 software programs (MapInfo Corporation, Troy, NY), in conjunction

with Spatial Re-Engineering Consultant's (SRC) Portfolio Desktop¹ (Orange, CA) to determine Census tract median household income and the Census tract proportion of housing built before 1950 using the U.S. Census 2000 Summary File 3. Both Census measures have been associated with higher blood lead levels [23].

Occupational Interview and Industrial Hygiene Assessment of Pb

As previously described [21], a face-to-face interview was conducted by trained interviewers to collect lifetime occupational history from age 18 for jobs held longer than six months. The job history data of all subjects were then reviewed by one of two industrial hygienists (IH) using semi-quantitative retrospective exposure assessment methodology previously described [24,25]. For each job, an IH assessed the probability of exposure, the years the exposure started and ended, the percent of work time exposure likely occurred, the route of exposure, and the frequency of the exposure during the work day.

Genotyping

A total of 637 cases enrolled in the original study. DNA for 633 cases who were black or white and who approved use of their DNA were genotyped using a custom Illumina GoldenGate[®] panel of 1,469 tagSNPs for 128 candidate genes drawn from the phase II version of the International HapMap project (version 23, hapmap.org). SNPs were selected to tag common variation within genes. Genotypes from both European Caucasian CEPH (CEU) and West African Yoruba (YRI) samples were used to capture variation in the gene that would be present in white and black prostate cancer cases and controls. To optimally select a minimal number of tagSNPs that capture variation in both the CEU and YRI samples, we used the multiple population tagging method TAGster [26]. This method selects SNPs based on the pair-wise linkage disequilibrium (LD) measure r^2 and employs a modification of the greedy algorithm of Carlson et al. [27], where SNPs in high LD with a SNP selected as a tag in an earlier round of the tagging algorithm may still be considered as possible tags in subsequent rounds if they are found to tag other, as of yet untagged, variations. When more than a single population is used, the pair-wise r^2 LD structure is evaluated separately in each population, and tagSNPs are selected sequentially based on the number of SNPs that they tag across both populations. In the phase II HapMap (release 23), there were a total of 17 common (minor allele frequency ≥ 0.1) SNPs located within *ALAD* in the CEU and 13 in the YRI. Specifying the tagSNP selection threshold as $r^2 \geq 0.8$, TAGster selected 11 tagSNPs to capture a total of 14 (of the 17) and 12 (of the 13) common SNPs in the CEU and YRI, respectively. The SNPs left untagged in each sample resulted from the lack of a sufficiently validated Illumina GoldenGate SNP assay that would capture these variants. After genotyping was complete, a total of 603 cases were selected for study based on a threshold for percent missing data.

Statistical Analysis

Inhalation and ingestion of Pb are the principal routes of human exposure [28]. Therefore, to estimate respiratory cumulative lifetime occupational Pb exposure, a semi-quantitative exposure index was calculated for each study subject based on his job-specific IH exposure

assessment [21]. We further categorized respiratory occupational Pb exposure as none, low (<median) and high (≥ median) in this report.

Differences between blacks and whites for categorical variables were compared using chi-square tests. Evaluation of genotype frequencies for Hardy-Weinberg equilibrium (HWE) was also accomplished with the chi-square test ($P < 0.01$ used to determine SNPs deviating from HWE). In all of the following analyses, *ALAD* tagSNP genotypes are coded based on a dominant model, with the reference homozygous genotype determined by the reference allele for the SNP designated in the National Center for Biotechnology Information database dbSNP (build 32).

The case-only $G \times E$ interaction odds ratios (IOR) were calculated for each of the 11 *ALAD* tagSNPs and occupational respiratory Pb exposure (low vs. no exposure, high vs. no exposure) using unconditional logistic regression models, with genotype as the independent variable and Pb exposure as the dependent variable. Models were adjusted for age (continuous) and were computed separately for blacks and whites because of the differences in allele frequency by race. To evaluate the possible effect of population stratification, the proportion of African Ancestry previously estimated for black men in this study by Bock et al. [29], was evaluated as a possible confounder in models for black men. P values of < 0.05 were considered statistically significant.

We also evaluated the relationship of a subset of tagSNPs ($P < 0.10$ in case-only $G \times E$) with prostate cancer aggressiveness features (prostate specific antigen (PSA) > 10 , Gleason ≥ 7 with primary pattern ≥ 4 , and Stage $\geq T2C$) by Pb exposure strata using unconditional logistic regression. Each model included the other features of aggressiveness as covariates as well as age, Zn intake, smoking history, and median household income or proportion of housing built before 1950. Generalized estimating equation (GEE) models were used when Census tract level data was included as a potential confounder. Due to sample size limitations only one Census variable was used per model.

RESULTS

Case characteristics are reported in Table I by race. The average age of all participants was 62 years and did not differ between black and white cases. Dietary Zn intake was significantly lower in blacks than whites. Median household income and housing built before 1950 in the Census tract were significantly negatively correlated overall ($r = -0.61$, $P < 0.001$) and black cases had significantly lower median household income and a higher median proportion of housing built before 1950 than white cases. Industrial hygienists' review of in-depth interviews determined that nearly two-thirds of cases had a history of occupational Pb exposure. Although, a slightly higher proportion of black cases were exposed to Pb (66.9% vs. 64.4%, $P = 0.55$), whites had significantly longer exposure time (8.4 years vs. 11.9 years, $P = 0.02$). The median lifetime occupational (respiratory) Pb exposure within those exposed was 3.40 units and this did not differ significantly by race (black 3.02 vs. white 4.00, $P = 0.18$).

Figure 1 and Table II show the LD and minor allele frequencies of *ALAD* tagSNPs by race. SNPs rs818689 and rs818684 were in high LD in both races and there was minimal LD between the other *ALAD* tagSNPs. Among black and white cases, all but 1 of the 11 *ALAD* SNPs were in Hardy–Weinberg equilibrium ($P > 0.01$). In white cases, rs8177812 minor allele frequencies were higher than expected. This SNP was removed from further analysis in whites. Allele frequencies differed by race for more than half of the tagSNPs. Therefore, results are reported separately for black and white cases.

In case-only $G \times E$ analyses, significant interactions between three tagSNPs and respiratory Pb exposure were identified in black cases. As Table III shows, rs818689 and rs818684, two intron 1 SNPs in high LD, showed significant $G \times E$ interaction with high Pb exposure compared to no Pb exposure (rs818684 CT or TT vs. CC, IOR 2.73, 95% CI 1.43–5.22, $P = 0.002$; rs818689 CT or TT vs. CC, IOR 2.20, 95% CI 1.15–4.21, $P = 0.017$). rs2761016 (TC or CC vs. TT) showed a significant interaction among low respiratory Pb exposed men when compared to men with no Pb exposure (IOR 2.08, 95% CI 1.13–3.84, $P = 0.019$). There was no association between rs2761016 and high Pb exposure (IOR 1.04, 95% CI 0.56–1.95, $P = 0.90$). Unadjusted and age adjusted results were similar and inclusion of the proportion African Ancestry for black cases did not change the results. No significant $G \times E$ interactions were observed in white cases.

To further dissect the combined effects of *ALAD* genetic variation and Pb exposure, we next examined the association of the three *ALAD* tagSNPs, showing significant $G \times E$ in case-only analyses, and prostate cancer aggressiveness features stratified by occupational Pb exposure. The variant alleles of both rs818684 and rs818689 were associated with higher Gleason grade in both blacks and whites exposed to high levels of occupational Pb (Table IV). The strongest association was observed in black prostate cancer cases that carried the variant T allele of rs818684 as they were nearly four times more likely to have a high Gleason grade (OR = 3.96, 95% CI 1.01–15.46, $P = 0.05$) than those homozygous for C. Similarly, among whites exposed to high occupational Pb levels, carriers of the T allele had increased risk of high Gleason grade (OR = 2.95, 95% CI 1.18–7.39, $P = 0.02$) in comparison to non-carriers. Associations for both blacks (OR 3.72 vs. 3.96) and whites (OR 2.91 vs. 2.95) increased when models accounted for median household income. Models that included the proportion of housing built before 1950 (results not shown) were similar to those that included income. No significant findings were observed for PSA at diagnosis (< 10 ng/ml vs. ≥ 10 ng/ml) or stage of disease (T2c or higher vs. lower than T2c).

DISCUSSION

In this initial case-only screening for gene–environment interaction between Pb and *ALAD* genetic variants in prostate cancer, we identified potential interactions between three *ALAD* tagSNPs and occupational Pb exposure in black cases but not among whites. Although we did not observe higher lifetime occupational Pb exposure in blacks compared to whites, black cases with high Pb exposure were two to three times more likely to be carriers of the T allele for either rs818684 or rs818689 than black cases with no occupational Pb exposure. These SNPs, located in intron 1 of *ALAD*, are in high LD in both races (black $r^2 = 0.75$ and white $r^2 = 0.96$). Among black cases, intron 4 tagSNP rs2761016 C allele carriers exposed to

low levels of Pb also showed a potentially significant interaction with Pb compared to non-exposed blacks. While our findings do not achieve a Bonferroni level of significance, the results are similar to those reported by van Bemmelen et al. [30] for interaction between rs2761016 and Pb exposure in renal cell carcinoma. Further, rs818684 and rs818689 may be associated with higher Gleason grade in both blacks and whites exposed to high levels of Pb. A higher proportion of blacks carry the T alleles of rs818684 (46.5% vs. 32.4%, $P < 0.001$) and rs818689 (39.5% vs. 32.1%, $P = 0.05$) than whites. However, blacks carry a much lower proportion of the C allele of rs2761016 (55.2% vs. 73.0%, $P < 0.001$). To the best of our knowledge, this is the only study in the U.S. at present to have industrial hygiene coded data on lifetime occupational Pb exposure in black prostate cancer cases. These findings, although intriguing, should be viewed cautiously and need additional validation.

The National Health and Nutrition Examination Survey has historically shown black men to have higher blood Pb levels than white men in the U.S. [31] and U.S. black men are more likely to be diagnosed with and die from prostate cancer than white men [1]. Although the racial gap in blood Pb levels has been greatly reduced since the reduction and elimination of Pb in gasoline [3], Wilker et al. [32] have recently estimated that the half life of bone (tibia) Pb may be as much as 48.6 years, substantially longer than the 10 years previously reported [33,34]. This suggests that historical disparities in Pb exposure could have long-term effects on health and health disparities such as those observed in prostate cancer, if an association between Pb and prostate cancer is supported by future studies.

The interaction of *ALAD* and Pb in prostate cancer and in aggressiveness of disease, if validated, may be the result of either a difference in the binding of Pb to *ALAD* or in *ALAD* activity. rs1800435, a functional SNP in exon 4 of *ALAD* that was not included in this study because of low frequency in whites and reported absence in African populations [35], has been associated with differences in blood and bone Pb levels. Allele 1 of the polymorphism has been associated with higher cortical bone Pb levels and Allele 2 with higher blood Pb levels [36,37]. In the CEU population, rs1800435 does not appear to be in LD with any of the SNPs we identified. African populations may have other, as yet unidentified, functional *ALAD* SNPs.

Most of the evidence for Pb as a carcinogen suggests indirect mechanisms such as inhibition of DNA repair or production of free radicals with increased oxidative stress. It is also possible that *ALAD* activity may be more or less affected by Pb based on these variants. Studies reported by www.Oncomine.org [38] and data available on www.proteinatlas.org [39] indicate that *ALAD* mRNA expression is lower in prostate tumor than normal tissue and that *ALAD* protein expression occurs in all normal prostate tissue but in less than 20% of prostate tumor tissues studied. It should be noted that GWAS studies have identified a risk variant, *DAB2IP* (rs1571801), located downstream of *ALAD*. However, all of the 11 *ALAD* SNPs in this study were in weak LD with rs1571801 ($r^2 < 0.05$ for all 11 pairs), suggesting that the effects shown here for *ALAD* are likely to be independent of the *DAB2IP* locus.

ALAD and/or Pb may affect PSA levels and in turn detection of prostate cancer because of the role of *ALAD* plays in heme biosynthesis. Higher bone Pb level has been associated

with lower hemoglobin and hematocrit [40] and lower hematocrit has been linked to lower PSA levels as a result of hemodilution [41]. This would suggest the potential for reduced detection of prostate cancer through PSA in highly exposed men. Our case-only findings suggest an increased risk for prostate cancer with increasing Pb exposure and at least one study has shown blood Pb level to be positively associated with PSA [42]. Using existing blood chemistry data of 196 controls, we found no significant difference in hemoglobin, hematocrit or PSA by rs818684, rs818689, or rs2761016 genotype, by lifetime occupational respiratory Pb exposure or by genotype stratified by Pb exposure status (data not shown).

Significant $G \times E$ interactions were observed among black but not white cases overall although we did observe similarities in regards to $G \times E$ with higher Gleason grade. This could be due to differences in diet such as lower zinc intake in blacks. In addition, Rishi et al. [43] reported lower expression of two zinc transporters, hZIP1 and hZIP2, in normal prostate tissue of blacks compared to whites; lower zinc in prostate tissues of blacks could predispose these men to the effects of Pb especially since ALAD is zinc dependent. There may have also been less exposure misclassification among blacks. Although we evaluated occupational Pb exposure, there are other sources of environmental Pb. In Metropolitan Detroit most African Americans are residentially segregated to the City of Detroit and may have similar levels of nonoccupational exposures to Pb. Whites, however, live throughout the Metropolitan area and likely have a broader range of non-occupational Pb exposure. We did account for Census tract median household income and housing built before 1950 which may capture some level of non-occupational Pb exposure. However, these are crude measures of non-occupational exposure and our sample size limited our ability to fully evaluate these and other area measures that might be relevant to Pb exposure. Future studies should account more comprehensively for these nonoccupational Pb exposures and will need to account for these types of exposure over the life-course.

It should be noted that other metals can affect ALAD activity. Arsenic can inhibit ALAD [44–46] and sometimes co-occurs with Pb. Cadmium has varying effects on ALAD that appear to be tissue specific [14,47,48]. Whittaker et al. [49] recently showed that different mixtures of Pb, arsenic, and cadmium modified ALAD activity in rats and depended on the combination of metals, tissue type, and duration of exposure.

Although, this study had many strengths including location in a major manufacturing city with a diverse population and pathologically confirmed prostate cancer cases, we had to rely on semi-quantitative occupational interviews to assess Pb exposure history. We had no biologic measure of ALAD activity or of Pb burden in bone or blood in the overall study. We also could not account for non-occupational exposures to Pb which could be substantial in Metropolitan Detroit because of the industrial setting and overwhelming use of personal automobiles which used leaded gasoline until shortly before this study was conducted.

Future research should continue to explore Pb and *ALAD* in prostate cancer and prostate cancer disparities and ultimately, the functional significance of *ALAD* common genetic variation needs to be established in both blacks and whites in order to validate these findings.

Acknowledgments

The authors would like to thank the staff of the GECAP study including Industrial Hygienists, Drs. Ludmilla Ecklund and James Rosbolt.

This work was financially supported by NIEHS 5R01 ES011126 (PI, B.R.), CDMRP W81XWH-07-1-0252 (PI, C.N.-D.), CDMRP W81XWH-06-1-0181 (PI, C.H. B.), and Henry Ford Health System Research Fund.

Grant sponsor: NIEHS; Grant number: 5R01 ES011126; Grant sponsor: CDMRP; Grant number: W81XWH-07-1-0252; Grant sponsor: CDMRP; Grant number: W81XWH-06-1-0181; Grant sponsor: Henry Ford Health System Research Fund.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2013. Atlanta: American Cancer Society; 2013.
2. Muntner P, Menke A, DeSalvo KB, Rabito FA, Batuman V. Continued decline in blood lead levels among adults in the United States: The National Health and Nutrition Examination Surveys. *Arch Intern Med*. 2005; 165(18):2155–2161. [PubMed: 16217007]
3. Theppeang K, Glass TA, Bandeen-Roche K, Todd AC, Rohde CA, Schwartz BS. Gender and race/ethnicity differences in lead dose biomarkers. *Am J Public Health*. 2008; 98(7):1248–1255. [PubMed: 18511728]
4. International Agency For Research On C. Inorganic and organic lead compounds: Summary of data reported and evaluation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2006:87.
5. Lam TV, Agovino P, Niu X, Roche L. Linkage study of cancer risk among lead-exposed workers in New Jersey. *Sci Total Environ*. 2007; 372(2–3):455–462. [PubMed: 17129599]
6. Rousseau MC, Parent ME, Nadon L, Latreille B, Siemiatycki J. Occupational exposure to lead compounds and risk of cancer among men: A population-based case–control study. *Am J Epidemiol*. 2007; 166(9):1005–1014. [PubMed: 17690218]
7. Telisman S, Cvitkovic P, Jurasovic J, Pizent A, Gavella M, Rocic B. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environ Health Perspect*. 2000; 108(1):45–53. [PubMed: 10620523]
8. Victory W, Thomas D, Schoeps P, Vander AJ. Lead increases urinary zinc excretion in rats. *Biol Trace Elem Res*. 1982; 4(2–3):211–219. [PubMed: 24271992]
9. Victory W, Miller CR, Zhu SY, Goyer RA. Effect of different levels and periods of lead exposure on tissue levels and excretion of lead, zinc, and calcium in the rat. *Fundam Appl Toxicol*. 1987; 8(4):506–516. [PubMed: 3609537]
10. Costello LC, Franklin RB. Prostatic fluid electrolyte composition for the screening of prostate cancer: A potential solution to a major problem. *Prostate Cancer Prostatic Dis*. 2009; 12(1):17–24. [PubMed: 18591961]
11. Costello LC, Franklin RB. Zinc is decreased in prostate cancer: An established relationship of prostate cancer! *J Biol Inorg Chem*. 2011; 16(1):3–8. [PubMed: 21140181]
12. Jaffe EK, Martins J, Li J, Kervinen J, Dunbrack RL Jr. The molecular mechanism of lead inhibition of human uroporphobilinogen synthase. *J Biol Chem*. 2001; 276(2):1531–1537. [PubMed: 11032836]
13. Godwin HA. The biological chemistry of lead. *Curr Opin Chem Biol*. 2001; 5(2):223–227. [PubMed: 11282351]
14. Roels HA, Buchet JP, Lauwerys RR, Sonnet J. Comparison of in vivo effect of inorganic lead and cadmium on glutathione reductase system and delta-aminolevulinic acid dehydratase in human erythrocytes. *Br J Ind Med*. 1975; 32(3):181–192. [PubMed: 1156566]
15. Guo GG, Gu M, Etlinger JD. 240-kDa proteasome inhibitor (CF-2) is identical to delta-aminolevulinic acid dehydratase. *J Biol Chem*. 1994; 269(17):12399–12402. [PubMed: 8175643]
16. Bardag-Gorce F, French SW. Delta-aminolevulinic acid dehydratase is a proteasome interacting protein. *Exp Mol Pathol*. 2011; 91(2):485–489. [PubMed: 21640720]

17. Chen D, Dou QP. The ubiquitin-proteasome system as a prospective molecular target for cancer treatment and prevention. *Curr Protein Pept Sci*. 2010; 11(6):459–470. [PubMed: 20491623]
18. Voutsadakis IA, Papandreou CN. The ubiquitin–proteasome system in prostate cancer and its transition to castration resistance. *Urol Oncol*. 2010
19. Goktas S, Baran Y, Ural AU, Yazici S, Aydur E, Basal S, Avcu F, Pekel A, Dirican B, Beyzadeoglu M. Proteasome inhibitor bortezomib increases radiation sensitivity in androgen independent human prostate cancer cells. *Urology*. 2010; 75(4):793–798. [PubMed: 19800672]
20. Neslund-Dudas C, Mitra B, Kandedgedara A, Chen D, Schmitt S, Shen M, Cui Q, Rybicki BA, Dou QP. Association of metals and proteasome activity in erythrocytes of prostate cancer patients and controls. *Biol Trace Elem Res*. 2012
21. Rybicki BA, Neslund-Dudas C, Nock NL, Schultz LR, Eklund L, Rosbolt J, Bock CH, Monaghan KG. Prostate cancer risk from occupational exposure to polycyclic aromatic hydrocarbons interacting with the GSTP1 Ile105Val polymorphism. *Cancer Detect Prev*. 2006; 30(5):412–422. [PubMed: 17067754]
22. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: The Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol*. 1997; 146(10): 856–869. [PubMed: 9384206]
23. Krieger N, Chen JT, Waterman PD, Soobader MJ, Subramanian SV, Carson R. Choosing area based socioeconomic measures to monitor social inequalities in low birth weight and childhood lead poisoning: The Public Health Disparities Geocoding Project (US). *J Epidemiol Community Health*. 2003; 57(3):186–199. [PubMed: 12594195]
24. Gerin M, Siemiatycki J, Kemper H, Begin D. Obtaining occupational exposure histories in epidemiologic case–control studies. *J Occup Med*. 1985; 27(6):420–426. [PubMed: 4020500]
25. Siemiatycki J, Gerin M, Stewart P, Nadon L, Dewar R, Richardson L. Associations between several sites of cancer and ten types of exhaust and combustion products. Results from a case–referent study in Montreal. *Scand J Work Environ Health*. 1988; 14(2):79–90. [PubMed: 2455336]
26. Xu Z, Kaplan NL, Taylor JA. TAGster: Efficient selection of LD tag SNPs in single or multiple populations. *Bioinformatics*. 2007; 23(23):3254–3255. [PubMed: 17827206]
27. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet*. 2004; 74(1):106–120. [PubMed: 14681826]
28. White PD, Van Leeuwen P, Davis BD, Maddaloni M, Hogan KA, Marcus AH, Elias RW. The conceptual structure of the integrated exposure uptake biokinetic model for lead in children. *Environ Health Perspect*. 1998; 106(Suppl. 6):1513–1530. [PubMed: 9860910]
29. Bock CH, Schwartz AG, Ruterbusch JJ, Levin AM, Neslund-Dudas C, Land SJ, Wenzlaff AS, Reich D, McKeigue P, Chen W, Heath EI, Powell IJ, Kittles RA, Rybicki BA. Results from a prostate cancer admixture mapping study in African-American men. *Hum Genet*. 2009; 126(5): 637–642. [PubMed: 19568772]
30. van Bommel DM, Boffetta P, Liao LM, Berndt SI, Menashe I, Yeager M, Chanock S, Karami S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Slamova A, Rothman N, Han SS, Rosenberg PS, Brennan P, Chow WH, Moore LE. Comprehensive analysis of 5-aminolevulinic acid dehydrogenase (ALAD) variants and renal cell carcinoma risk among individuals exposed to lead. *PLoS ONE*. 2011; 6(7):e20432. [PubMed: 21799727]
31. Muntner P, He J, Vupputuri S, Coresh J, Batuman V. Blood lead and chronic kidney disease in the general United States population: Results from NHANES III. *Kidney Int*. 2003; 63(3):1044–1050. [PubMed: 12631086]
32. Wilker E, Korrick S, Nie LH, Sparrow D, Vokonas P, Coull B, Wright RO, Schwartz J, Hu H. Longitudinal changes in bone lead levels: The VA Normative Aging Study. *J Occup Environ Med*. 2011; 53(8):850–855. [PubMed: 21788910]
33. Leggett RW. An age-specific kinetic model of lead metabolism in humans. *Environ Health Perspect*. 1993; 101(7):598–616. [PubMed: 8143593]

34. O'Flaherty EJ. Physiologically based models for bone-seeking elements. IV. Kinetics of lead disposition in humans. *Toxicol Appl Pharmacol.* 1993; 118(1):16–29. [PubMed: 8430422]
35. Montenegro MF, Barbosa F Jr, Sandrim VC, Gerlach RF, Tanus-Santos JE. Ethnicity affects the distribution of delta-aminolevulinic acid dehydratase (ALAD) genetic variants. *Clin Chim Acta.* 2006; 367(1–2):192–195. [PubMed: 16445899]
36. Kelada SN, Shelton E, Kaufmann RB, Khoury MJ. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: A HuGE review. *Am J Epidemiol.* 2001; 154(1):1–13. [PubMed: 11427399]
37. Scinicariello F, Murray HE, Moffett DB, Abadin HG, Sexton MJ, Fowler BA. Lead and delta-aminolevulinic acid dehydratase polymorphism: Where does it lead? A meta-analysis. *Environ Health Perspect.* 2007; 115(1):35–41. [PubMed: 17366816]
38. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A, Chinnaiyan AM. ONCOMINE: A cancer microarray database and integrated data-mining platform. *Neoplasia.* 2004; 6(1):1–6. [PubMed: 15068665]
39. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, Wernerus H, Bjorling L, Ponten F. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol.* 2010; 28(12):1248–1250. [PubMed: 21139605]
40. Lee SS, Lee BK, Lee GS, Stewart WF, Simon D, Kelsey K, Todd AC, Schwartz BS. Associations of lead biomarkers and delta-aminolevulinic acid dehydratase and vitamin D receptor genotypes with hematopoietic outcomes in Korean lead workers. *Scand J Work Environ Health.* 2001; 27(6): 402–411. [PubMed: 11800328]
41. Ohwaki K, Endo F, Muraishi O, Hiramatsu S, Yano E. Relationship between prostate-specific antigen and hematocrit: Does hemodilution lead to lower PSA concentrations in men with a higher body mass index? *Urology.* 2010; 75(3):648–652. [PubMed: 19854477]
42. Pizent A, Colak B, Kljakovic Z, Telisman S. Prostate-specific antigen (PSA) in serum in relation to blood lead concentration and alcohol consumption in men. *Arh Hig Rada Toksikol.* 2009; 60(1): 69–78. [PubMed: 19329378]
43. Rishi I, Baidouri H, Abbasi JA, Bullard-Dillard R, Kajdacsy-Balla A, Pestaner JP, Skacel M, Tubbs R, Bagasra O. Prostate cancer in African American men is associated with downregulation of zinc transporters. *Appl Immunohistochem Mol Morphol.* 2003; 11(3):253–260. [PubMed: 12966353]
44. Goering PL, Maronpot RR, Fowler BA. Effect of intratracheal gallium arsenide administration on delta-aminolevulinic acid dehydratase in rats: Relationship to urinary excretion of aminolevulinic acid. *Toxicol Appl Pharmacol.* 1988; 92(2):179–193. [PubMed: 3341032]
45. Conner EA, Yamauchi H, Fowler BA. Alterations in the heme biosynthetic pathway from the III-V semiconductor metal, indium arsenide (InAs). *Chem Biol Interact.* 1995; 96(3):273–285. [PubMed: 7538452]
46. Kannan GM, Tripathi N, Dube SN, Gupta M, Flora SJ. Toxic effects of arsenic (III) on some hematopoietic and central nervous system variables in rats and guinea pigs. *J Toxicol Clin Toxicol.* 2001; 39(7):675–682. [PubMed: 11778665]
47. Davis JR, Avram MJ. A comparison of the stimulatory effects of cadmium and zinc on normal and lead-inhibited human erythrocytic delta-aminolevulinic acid dehydratase activity in vitro. *Toxicol Appl Pharmacol.* 1978; 44(1):181–190. [PubMed: 675689]
48. Goering PL, Fowler BA. Metal constitution of metallothionein influences inhibition of delta-aminolaevulinic acid dehydratase (porphobilinogen synthase) by lead. *Biochem J.* 1987; 245(2): 339–345. [PubMed: 3663161]
49. Whittaker MH, Wang G, Chen XQ, Lipsky M, Smith D, Gwiazda R, Fowler BA. Exposure to Pb, Cd, and As mixtures potentiates the production of oxidative stress precursors: 30-day, 90-day, and 180-day drinking water studies in rats. *Toxicol Appl Pharmacol.* 2011; 254(2):154–166. [PubMed: 21034764]

Fig. 1. Pair-wise linkage disequilibrium among 11 ALAD tagSNPs in black (**top**) and white (**bottom**) prostate cancer cases.

TABLE I**Prostate Cancer Case Characteristics by Race**

	Black cases (N=260)	White cases (N= 343)	P-value
Age (mean years, SE)	61.9 (7.5)	62.8 (6.6)	0.12
Smoking (ever)	172 (66.2)	228 (66.5)	0.96
Dietary zinc intake mg/day (median, SE)	12.42 (0.57)	14.0(0.39)	0.04
Census tract median household income	\$35,319	\$58,207	<0.001
% Housing built before 1950	47.5	9.2	<0.001
Respiratory occupational Pb exposure			
No	86 (33.1)	122 (35.6)	0.14
Low (<median)	94 (36.2)	98 (28.6)	
High (median)	80 (30.8)	123 (35.9)	
Within those exposed to Pb			
Age at first exposure (mean years, SD)	24.9 (9.1)	24.9 (8.6)	0.99
Years of Pb Exposure (mean, SD)	8.4 (9.6)	11.9 (12.6)	0.02
PSA at dx >10	53 (20.4)	50 (14.6)	0.06
Gleason 7 and primary 4	69 (26.8)	82 (24.2)	0.42
Stage T2C	74 (28.5)	93 (27.1)	0.67

SD, standard deviation; SE, standard error; Pb, lead; PSA, prostate specific antigen.

TABLE II
Frequency of *ALAD* tagSNP Allele (Non-Reference) in Prostate Cancer Cases by Race

<i>ALAD</i> tagSNP	Allele ^d	Position ^a	Location	Black	White	P-value ^c
rs818707	G/A	116149967	3'UTR	1.9	13.9	<0.001
rs818708	C/T	116150109	3'UTR	15.9	51.9	<0.001
rs1805313	T/C	116151191	Intron 11	48.9	33.2	<0.001
rs8177812	C/T	116151527	Intron 10	14.6	18.4	0.087
rs2228083	C/T	116152940	Exon 6 ^b	9.6	8.3	0.474
rs1805316	T/C	116153250	Intron 4	8.1	8.9	0.678
rs2761016	T/C	116153352	Intron 4	34.4	47.1	<0.001
rs1139488	T/C	116153900	Exon 4 ^b	33.1	39.8	0.019
rs818684	C/T	116159423	Intron 1	26.9	17.6	<0.001
rs818688	G/C	116162067	Intron 1	59.3	36.9	<0.001
rs818689	C/T	116162760	Intron 1	21.7	17.6	0.090

^a Alleles are listed as "reference/other" allele as indicated in dbSNP build 132 and base pair positions are based on the mapping of these SNPs to the NCBI genome build 37.1.

^b The two SNPs located in exons of *ALAD* code for synonymous (i.e., do not results in amino acid change) to the *ALAD* protein sequence.

^c P-value is for frequency of the "other" allele in African American cases versus white cases.

TABLE III
Case-Only Interaction OddsRatios for ALAD tagSNPs and Lifetime Occupational Lead Exposure by Race

ALAD tagSNP	Risk genotype	Low versus no Pb exposure				High versus no Pb exposure			
		N	IOR ^L	95% CI	P-value	N	IOR ^H	95% CI	P-value
Black cases									
rs818707	GA & AA	182	0.39	0.07–2.23	0.29	163	1.17	0.27–4.97	0.84
rs818708	CT & TT	182	0.93	0.48–1.77	0.82	164	0.94	0.48–1.86	0.87
rs1805313	TC & CC	182	0.82	0.42–1.60	0.56	164	1.08	0.53–2.20	0.84
rs8177812	CT & TT	182	0.88	0.45–1.74	0.72	164	0.88	0.43–1.80	0.73
rs2228083	CT & TT	182	0.69	0.31–1.52	0.36	164	1.29	0.60–2.77	0.51
rs1805316	TC & CC	182	1.70	0.72–4.01	0.23	164	1.64	0.66–4.06	0.28
rs2761016	TC & CC	182	2.08	1.13–3.84	0.019	164	1.04	0.56–1.95	0.90
rs1139488	TC & CC	182	1.26	0.69–2.29	0.45	164	1.00	0.53–1.87	0.99
rs818684	CT & TT	182	1.17	0.64–2.15	0.60	164	2.73	1.43–5.22	0.002
rs818688	GC & CC	181	0.96	0.41–2.24	0.92	164	1.29	0.50–3.30	0.60
rs818689	CT & TT	180	1.23	0.66–2.31	0.51	164	2.20	1.15–4.21	0.017
White cases									
rs818707	GA & AA	224	1.07	0.59–1.93	0.83	241	0.82	0.46–1.48	0.51
rs818708	CT & TT	224	1.11	0.59–2.09	0.75	241	1.16	0.63–2.14	0.62
rs1805313	TC & CC	224	1.00	0.59–1.69	0.99	240	1.00	0.60–1.67	0.99
rs8177812*	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs2228083	CT & TT	224	0.84	0.40–1.79	0.65	241	1.18	0.60–2.32	0.62
rs1805316	TC & CC	224	1.78	0.89–3.56	0.10	239	1.04	0.50–2.14	0.92
rs2761016	TC & CC	223	0.80	0.44–1.44	0.45	241	0.90	0.50–1.61	0.72
rs1139488	TC & CC	224	1.16	0.66–2.04	0.60	241	1.01	0.60–1.71	0.96
rs818684	CT & TT	224	1.15	0.64–2.06	0.63	241	1.22	0.71–2.11	0.46
rs818688	GC & CC	224	0.96	0.56–1.65	0.88	241	0.73	0.44–1.23	0.24
rs818689	CT & TT	224	1.09	0.61–1.96	0.76	241	1.22	0.71–2.11	0.46

Lead, Pb; interaction odds ratio, IOR. IOR^L is for low occupational lead exposure versus no occupational lead exposure, IOR^H is for high occupational lead exposure versus no occupational lead exposure. IOR^L and IOR^H adjusted for age.

* Rs8177812 did not meet criteria for Hardy-Weinberg equilibrium in whites.

TABLE IV
Association of *ALAD* tagSNPs and High Gleason Gradeby Lead (Pb) Exposure Status and Race

Pb exposure	Black			White		
	N	OR ^a (CI)	P-value ^b	N	OR ^a (CI)	P-value ^b
		rs2761016 TC and CC versus TT			rs2761016 TC and CC versus TT	
No Pb	86	0.78 (0.29–2.06)	0.61	121	0.54 (0.18–1.65)	0.28
Low Pb	96	1.02 (0.32–3.21)	0.98	102	0.47 (0.17–1.34)	0.16
High Pb	77	1.30 (0.41–4.08)	0.66	118	0.83 (0.32–2.16)	0.70
		rs818684 CT and TT versus CC			rs818684 CT and TT versus CC	
No Pb	86	0.96 (0.36–2.60)	0.94	122	0.46 (0.13–1.68)	0.24
Low Pb	96	0.75 (0.26–2.15)	0.60	102	0.51 (0.15–1.70)	0.27
High Pb	78	3.96 (1.01–15.46)	0.048 ^c	119	2.95 (1.18–7.39)	0.02
		rs818689 CT and TT versus CC			rs818689 CT and TT versus CC	
No Pb	86	1.06 (0.39–2.86)	0.91	122	0.42 (0.11–1.57)	0.20
Low Pb	94	0.54 (0.17–1.75)	0.31	102	0.52 (0.16–1.74)	0.30
High Pb	78	1.90 (0.61–5.96)	0.04	119	2.95 (1.18–7.39)	0.02

^aOR for Gleason of at least 7 with primary score of 4 versus Gleason of 7, primary score 3 or lower. GEE ORs adjusted for age, smoking, log zinc, log Census tract median household income, stage and the other two aggressiveness features.

^bP-value is *ALAD* tagSNP heterozygous and minor allele homozygous versus major allele homozygous.

^cFisher's exact P reported for cells <5.