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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEWS

δ-Aminolevulinic Acid Dehydratase Genotype and Lead Toxicity: A HuGE Review

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The *ALAD* gene (chromosome 9q34) codes for δ-aminolevulinic acid dehydratase (ALAD) (E.C. 4.2.1.24). ALAD catalyzes the second step of heme synthesis and is polymorphic. The *ALAD* G177C polymorphism yields two codominant alleles, *ALAD-1* and *ALAD-2*, and it has been implicated in susceptibility to lead toxicity. Genotype frequencies vary by geography and race. The rarer *ALAD-2* allele has been associated with high blood lead levels and has been thought to increase the risk of lead toxicity by generating a protein that binds lead more tightly than the ALAD-1 protein. Other evidence suggests that ALAD-2 may confer resistance to the harmful effects of lead by sequestering lead, making it unavailable for pathophysiologic participation. Recent studies have shown that individuals who are homozygous for the *ALAD-1* allele have higher cortical bone lead levels; this implies that they may have a greater body lead burden and may be at higher risk of the long-term effects of lead. Individuals exposed to lead in occupational settings have been the most frequent subjects of study. Genotype selection bias may limit inferences from these studies. No firm evidence exists for an association between *ALAD* genotype and susceptibility to lead toxicity at background exposure levels; therefore, population testing for the *ALAD* polymorphism is not justified. *Am J Epidemiol* 2001;154:1–13.

ALAD; aminolevulinic acid; epidemiology; genetic predisposition to disease; genetics; lead; lead poisoning; porphobilinogen synthase

GENE AND GENE PRODUCT

The *ALAD* gene is located on chromosome 9q34 and is approximately 16 kilobases long (1). This gene codes for the δ-aminolevulinic acid dehydratase (ALAD) enzyme (E.C. 4.2.1.24), also known as porphobilinogen synthase, a 280-kilodalton protein that is composed of eight identical subunits and requires eight zinc ions as cofactors for full activity (2). The ALAD enzyme catalyzes the second step in heme synthesis, the asymmetric addition of two molecules of aminolevulinic acid to form the monopyrrole porphobilinogen (figure 1), which is the precursor of heme, as well as cytochromes and cobalamins. ALAD is expressed in all tissues, but the highest levels of expression are found in erythrocytes and the liver (3).

GENE VARIANTS

We searched Medline for relevant publications using the headings "ALAD" and "δ-aminolevulinic acid dehydratase." Eight *ALAD* gene variants have been described in the literature. This review focuses on one polymorphism that yields two alleles, designated *ALAD-1* and *ALAD-2*, which exhibit a codominant pattern of inheritance (4). The *ALAD-2* allele contains a G→C transversion at position 177

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Abbreviations: ALAD, aminolevulinic acid dehydratase; DMSA, dimercaptosuccinic acid; *HFE*, hereditary hemochromatosis gene; NHANES, National Health and Nutrition Examination Survey; SD, standard deviation; VDR, vitamin D receptor.

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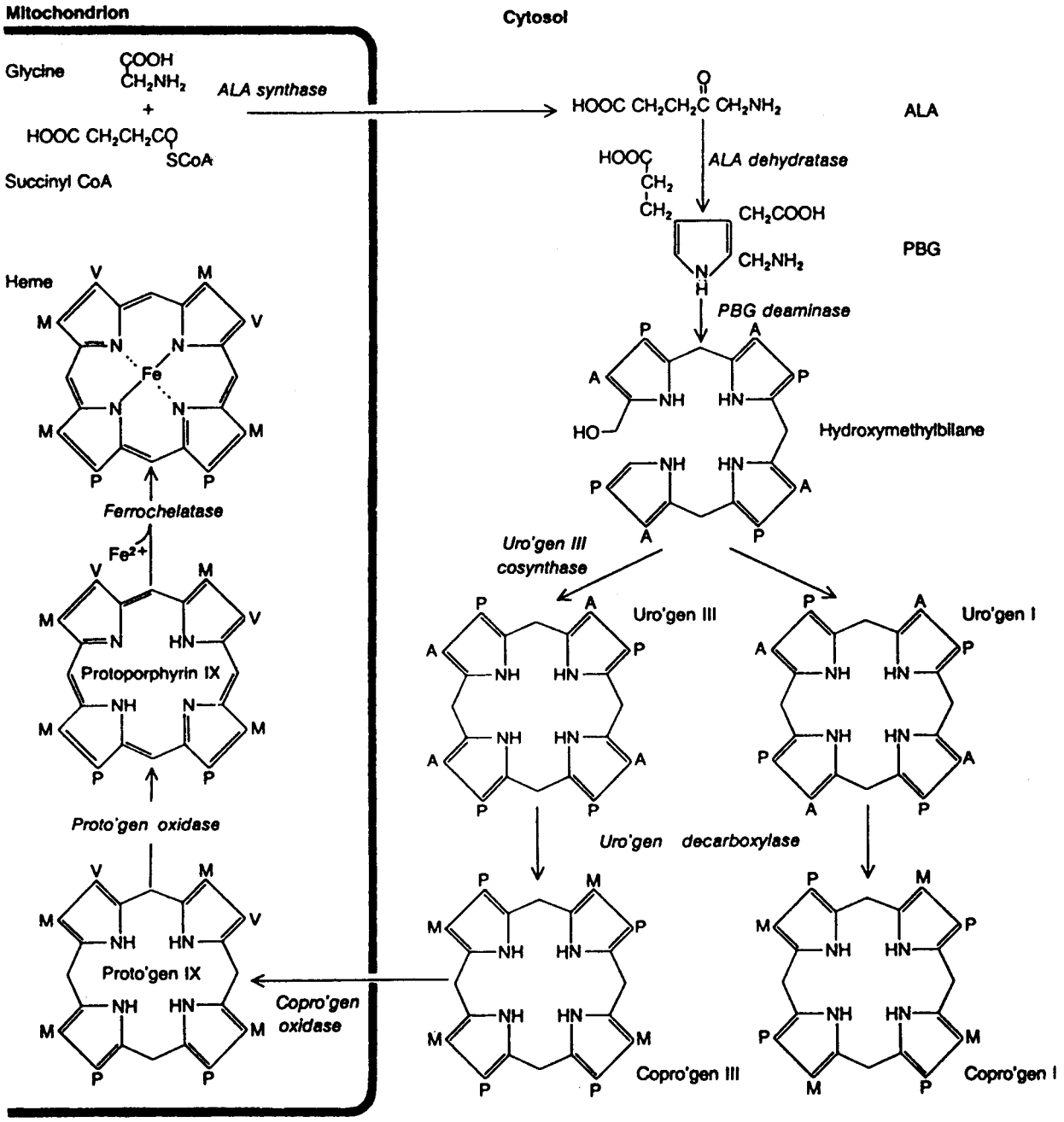

FIGURE 1. The pathway for heme biosynthesis. ALA, aminolevulinic acid; CoA, coenzyme A; PBG, porphobilinogen. A = $-CH_2COOH$; M = $-CH_2$; P = $-CH_2CH_2COOH$; V = $-CH=CH_2$. The apostrophe (') denotes the "porphyrino-" abbreviation. (Reproduced with permission from Scriver et al. (51).)

of the coding region, resulting in the substitution of asparagine for lysine at amino acid 59 (5). These two alleles determine three isozymes, designated 1-1, 1-2, and 2-2, all of which display similar activities but have different charges (4). Asparagine is a neutral amino acid, whereas lysine is positively charged. Therefore, *ALAD 1-2* heterozygotes produce an enzyme that is more electronegative than that of *ALAD-1* homozygotes, and *ALAD-2* homozygotes produce an enzyme that is more electronegative than that of *1-2* heterozygotes. This forms the basis of the electrophoretic technique originally used to identify the polymorphism and phenotype individuals (4).

The prevalence of the *ALAD-2* allele ranges from 0 to 20 percent depending on the population. Generally, Caucasians have the highest frequency of the *ALAD-2* allele, with approximately 18 percent of the Caucasian population being

ALAD 1-2 heterozygotes and 1 percent being *2-2* homozygotes. In comparison, African and Asian populations have low frequencies of the *ALAD-2* allele, with few or no *ALAD-2* homozygotes being found in such populations. Table 1 lists genotype frequencies from around the world (6–26). All of these frequencies are in Hardy-Weinberg equilibrium. The listed genotype frequencies were determined in the early 1980s by phenotyping. In 1991, Wetmur et al. (5) devised a genotyping technique based on polymerase chain reaction that correctly identified all 93 *ALAD-2* heterozygotes and homozygotes tested; i.e., there was a 100 percent genotype-phenotype correspondence. Most investigators have since used this technique.

Most of the studies presented in table 1 that documented genotype or phenotype frequencies gave little detail about the study population (e.g., age or source of donors), making it hard to rule out any potential biases due to subject selection. These populations are referred to as "general" in the table. Alternatively, some studies used hospital-based study samples. Other studies (e.g., 6, 7, 26) used samples comprising individuals with relatively high levels of lead exposure from occupational studies. This may also promote bias in the results, as persons with "at-risk" genotypes may have been selected against during the course of employment and therefore may not have been represented in the study sample.

BACKGROUND AND EPIDEMIOLOGY OF LEAD POISONING

Lead has been a known toxicant for thousands of years, and it remains a persistent environmental health threat. Exposure to lead can result in significant adverse health effects to mul-

TABLE 1. Frequencies of the δ-aminolevulinic acid dehydratase (*ALAD*) genotype in various studies

Country of study	*ALAD* genotype			No. of study subjects	Method: genotype or phenotype?	Study population	Author(s), year, and reference
	1-1	*1-2*	*2-2*				
Canada	0.817	0.175	0.008	382	Genotype	Lead smelter workers	Fleming et al., 1998 (6)
Canada—British Columbia	0.851	0.149	0.000	134	Genotype	Lead/zinc smelter workers	Alexander et al., 1998 (7)
Chile—Atacameno	0.971	0.029	0.000	175	Phenotype	General	Goedde et al., 1984 (8)
Denmark	0.880	0.115	0.005	1,697	Phenotype	General	Eiberg et al., 1983 (9)
Germany	0.863	0.132	0.005	711	Phenotype	General	Juli et al., 1983 (10)
Germany	0.813	0.153	0.035	144	Phenotype	General	Benkmann et al., 1983 (11)
Germany	0.814	0.177	0.009	220	Phenotype	General	Scheil et al., 1987 (12)
Greece	0.919	0.071	0.010	508	Phenotype	General	Kapotis et al., 1998 (13)
Israel—Ashkenazis	0.645	0.306	0.049	386	Phenotype	Hospital	Ben-Ezzer et al., 1987 (14)
Israel—Arabs	0.779	0.200	0.021	95	Phenotype	Hospital	Ben-Ezzer et al., 1987 (14)
Italy	0.803	0.182	0.015	798	Phenotype	General	Battistuzzi et al., 1981 (4)
Italy	0.814	0.167	0.020	762	Phenotype	General	Petrucci et al., 1982 (15)
Japan	0.841	0.154	0.006	527	Phenotype	General	Komatsu et al., 1987 (16)
Japan	0.901	0.083	0.017	121	Phenotype	General	Benkmann et al., 1983 (11)
Japan	0.836	0.155	0.009	317	Genotype	Lead workers and unexposed controls	Sakai et al., 2000 (17)
Liberia	1.000	0.000	0.000	296	Phenotype	General	Benkmann et al., 1983 (11)
Poland	0.887	0.110	0.003	300	Phenotype	General	Raczek et al., 1994 (18)
Portugal	0.827	0.165	0.008	1,043	Phenotype	General	Amorim et al., 1994 (19)
South Korea	0.939	0.060	0.001	146	Phenotype	General	Roychoudhury & Nei, 1988 (20)
Spain—Basques	0.850	0.145	0.006	339	Phenotype	General	Garcia-Orad et al., 1987 (21)
Spain—Galicia	0.842	0.150	0.008	500	Phenotype	General	Caeiro and Rey, 1985 (22)
Taiwan	0.955	0.044	0.002	660	Genotype	General	Hsieh et al., 2000 (23)
Thailand	0.941	0.058	0.001	117	Phenotype	General	Roychoudhury & Nei, 1988 (20)
United States—New Jersey	0.860	0.137	0.003	691	Genotype	Carpenters	Smith et al., 1995 (24)
United States—New York	0.786	0.198	0.016	1,074	Phenotype	Lead-exposed individuals of several ethnicities	Astrin et al., 1987 (25)
United States—New York	0.889	0.100	0.011	1,278	Phenotype	Lead-exposed children	Wetmur et al., 1991 (26)

tiple organ systems. Toxic effects to the nervous, hematologic, renal, and reproductive systems have been studied extensively and are well documented (27, 28). Since lead was phased out as a gasoline additive (tetraethyl lead) in the 1970s and its use in paint and food containers (e.g., ceramic ware and tin cans) was curtailed, blood lead concentrations have decreased significantly; however, other sources of lead and its unknown threshold of subclinical toxicity continue to make lead an issue of public health concern.

There are many risk factors for lead poisoning. Generally, living in a home built before 1950 is considered a risk factor because of the presence of multiple avenues of exposure to lead. Old pipes with lead solder can contaminate the water supply, and lead-based paint is still a notorious source of lead in these houses (29). Additionally, living in close proximity to lead-emitting industrial facilities can present a significant source of cumulative exposure to lead via air, water, and soil. Occupational exposure to lead is most often encountered at lead smelters and battery manufacturing facilities, as well as in housing renovation projects in which workers inhale and ingest lead-contaminated fumes and dust from lead-based paint.

Children's hand-to-mouth activity, increased respiratory rates, and increased intestinal absorption of lead make them more susceptible than adults to lead exposure (30, 31). Lead-based paint remains the predominant source of high-dose lead poisoning in children. Poor nutrition, particularly inadequate intakes of calcium and iron, is probably an important risk factor for children as well (32).

Blood lead level (μg/dl) is the biologic index most often used by health care providers as an indicator of recent lead exposure (33). Two analytical techniques, anodic stripping voltametry and atomic absorption spectroscopy, are used to measure blood lead level and have detection limits less than 1 μg/dl (34). In addition to blood lead level, other lead exposure indices include free erythrocyte protoporphyrin and zinc protoporphyrin; both are precursors of heme whose levels elevate upon moderate to high exposure to lead. However, free erythrocyte protoporphyrin and zinc protoporphyrin are neither sensitive enough nor specific enough to be used as primary indicators of lead exposure (27, 35). Lead levels in plasma, urine, bone, and teeth (dentin lead) are less commonly used measures of exposure and body burden.

At steady state, 90 percent of body lead is found in the skeleton (27). The association between lead and bone is due to lead's similar valence to calcium. Measurements of lead in trabecular or spongy bone (e.g., patella), in which lead has a relatively short half-life, and lead in cortical bone (e.g., tibia), which represents a site of long-term lead storage, have been used to estimate the distribution of lead in bone and total body burden (24, 27). Reliable, noninvasive techniques such as x-ray fluorescence have been developed to measure bone lead levels. Lead in bone can leach out, and this constitutes a significant long-term source of lead to the blood (27). Chelating agents such as dimercaptosuccinic acid (DMSA) have been used therapeutically to extract lead from tissues (28). It has been shown that chelatable lead correlates well with lead in trabecular bone (36). Administration of chelators has also been used in research studies to estimate body burden.

Subclinical lead toxicity remains a problem for both adults and children (37–39). Blood lead concentrations of 10 μg/dl in children have been associated with cognitive deficits, aggressive behavior, and hearing dysfunction (40–45). Alarmingly, evidence indicates that no detectable threshold exists for the adverse effects of lead exposure on neurodevelopment (45, 46). Using data from the Third National Health and Nutrition Examination Survey (NHANES III), the Centers for Disease Control and Prevention estimated that 890,000 US children aged 1–5 years (4.4 percent) have blood lead concentrations ≥10 μg/dl, the current level of concern (47). The current mean blood lead concentration for children aged 1–5 years is 2.7 μg/dl (48). In the US adult population, blood lead levels measured in NHANES II and phase 1 of NHANES III showed a decrease from 13.1 μg/dl to 3.0 μg/dl, and currently more than 90 percent of adults have blood lead levels less than 10 μg/dl (49). With respect to the occupational arena, the current goal of the Department of Health and Human Services is to eliminate all occupational exposures resulting in blood lead levels greater than 25 μg/dl (33). The National Institute for Occupational Safety and Health formerly maintained the Adult Blood Lead Epidemiology and Surveillance Program, which reported the prevalence of elevated blood lead levels among adults in 28 US states. At last report, in the third quarter of 1998, 3,322 (16 percent) of the 20,511 adults for whom blood lead levels were reported had levels ≥25 μg/dl; of these, 182 (6 percent) had levels ≥50 μg/dl (50). Both of these prevalence statistics represent declines from previous quarters of reporting.

LEAD AND ALAD

One of lead's primary effects is hematotoxicity, specifically inhibition of heme synthesis. Lead inhibits three enzymes in the heme biosynthesis pathway (figure 1)—ALAD, coporphyrinogen oxidase, and ferrochelatase—but its effects on ALAD are most profound (51). Lead inhibits ALAD stoichiometrically (52–54), and the degree of erythrocyte ALAD inhibition has been used clinically to gauge the degree of lead poisoning. At the molecular level, lead displaces a zinc ion at the metal binding site, not the active site (55), producing inhibition through a change in the enzyme's quaternary structure. ALAD inhibition results in the buildup of aminolevulinic acid, detectable in the plasma and urine at blood lead levels less than 10 μg/dl. Aminolevulinic acid resembles γ-aminobutyric acid and can stimulate γ-aminobutyric acid receptors in the nervous system; this is thought to be one of the primary mechanisms of lead-induced neurotoxicity (55–57).

THE *ALAD-1/2* POLYMORPHISM AS A MODIFIER OF LEAD'S EFFECTS

Initial studies

Early studies conducted on the *ALAD* polymorphism and lead poisoning focused on differences in blood lead levels by genotype in populations with relatively high levels of lead exposure, either from the home or from occupation

(table 2). Ziemsen et al. (58) were the first to describe differences in blood lead levels by genotype. They found that lead-exposed workers ($n =$ 202) with the *ALAD 1-2* genotype had higher blood lead levels than *ALAD 1-1* homozygotes (44 μg/dl vs. 38 μg/dl) and that *ALAD 2-2* homozygotes had higher blood lead levels at 56 μg/dl. (Phenotyping was actually performed in this study; because the correspondence between genotype and phenotype has been shown to be 100 percent (5), genotype has been inferred from all studies that documented phenotypes.) Astrin et al. (25) subsequently found a higher-than-expected proportion of individuals with the *ALAD 1-2* or *2-2* genotype among persons with lead poisoning screened by blood lead levels greater than 50 μg/dl or free erythrocyte protoporphyrin levels greater than 30 μg/dl ($n =$ 1,074). The ascertainment bias in the sampling technique was noted in the published article (25). Astrin et al. also reported that the *ALAD-2* allele was associated with a fourfold increase in the ability to retain blood lead levels above 30 μg/dl. Furthermore, Wetmur et al. (26) found significant differences in blood lead levels in a group of lead-exposed workers ($n =$ 202) and in New York City children ($n =$ 1,278) screened by elevated free erythrocyte protoporphyrin. They found median blood lead levels that were 9 μg/dl and 11 μg/dl higher, respectively, among *ALAD-2* carriers in these two populations. All three of these studies examined populations with exposure levels higher than normal whose blood lead levels were often greater than 30 μg/dl, a previously designated cutoff used as evidence of lead poisoning.

Hypotheses generated to support these results were based on the charge of the ALAD-2 isozyme (3, 25, 26). Since *ALAD-2* codes for a more electronegative enzyme, the ALAD-2 protein is thought to be able to bind positively charged lead ion more tightly than the ALAD-1 protein. Carriers of the *ALAD-2* allele who are exposed to lead might then retain it in their blood and tissues longer, increasing the chance of an adverse effect due to inhibition of ALAD and consequent buildup of aminolevulinic acid or perhaps due to lead itself, which can initiate oxidative damage and change the structure of cellular components (27). From these initial studies, it is safe to conclude that the kinetics of lead in blood are modified by *ALAD* genotype, although perhaps only at relatively high levels of exposure. These studies also imply that the *ALAD 1-2* and *2-2* genotypes are the "at-risk genotypes" at high exposure levels.

Further studies

Subsequent studies (table 2) were again primarily occupational epidemiologic studies, but they often used new sets of measures for lead exposure and body burden. Bone lead measurements, in particular, began to be used as measures of outcome. In 1995, Schwartz et al. (59) used an occupational cohort of employees from three lead storage battery factories ($n =$ 307). They found that the *ALAD-2* allele was not clearly associated with higher blood levels (i.e., there was no difference in blood lead level by genotype), but individuals with the *1-2* genotype (there were no *2-2* subjects) were 2.3 times more likely to have blood lead levels ≥40 μg/dl, although the 95 percent confidence interval contained 1.0. No relation was found between genotype and zinc protoporphyrin. However, Schwartz et al. did find that the *1-2* genotype was associated with occupational exposures of more than 6 years (odds ratio = 2.6; 95 percent confidence interval: 1.2, 5.8) (59), which suggests that the *ALAD-2* allele conferred a protective effect. In support of this finding, *ALAD 1-2* heterozygote workers with high exposure histories had lower zinc protoporphyrin levels than *ALAD-1* homozygotes with equivalent exposure histories. The authors cited this as a possible genotype-selection factor and proposed that the ALAD-2 subunit of the protein keeps lead in a nonbioavailable form, such that these individuals ($n_i =$ 4) were protected from lead's effects and could tolerate longer exposures to lead than *ALAD 1-1* subjects (59).

Using a group of 122 carpenters with relatively low blood lead levels (average level = 7.8 μg/dl) for study, Smith et al. (24) avoided the bias of previous studies that used individuals with high blood lead levels. They found no association between *ALAD* genotype and blood lead level, which implies that *ALAD* genotype may be a modifier of blood lead level only at high blood lead concentrations. Smith et al. also found no association between genotype and tibial or patellar bone lead levels, which were measured using x-ray fluorescence. However, using the difference between lead levels in patellar bone and tibial bone as an indicator of effect of the genotype on the distribution of lead in bone, they found a difference of borderline significance between the *1-1* and *1-2/2-2* genotypes ($p =$ 0.06) (24). *ALAD-1* homozygotes had a smaller difference in patella-tibia bone lead levels than *1-2/2-2* individuals (3.4 μg lead/g bone mineral (standard deviation (SD) 12.0) vs. 8.6 μg lead/g bone mineral (SD 9.5)). This indicates that *1-1* individuals have increased uptake of lead into cortical bone, the long-term storage depot, relative to *1-2/2-2* individuals. It was hypothesized that *1-2/2-2* individuals partition less lead into cortical bone because of the increased affinity of the ALAD-2 subunit for lead. Hence, *ALAD-1* homozygotes would be at increased long-term risk as they built up higher levels of cortical bone lead that could leach out at times of bone lead redistribution (e.g., during pregnancy). These investigators also observed a relation between *ALAD-2* and subclinical renal toxicity, as evidenced by elevated blood urea nitrogen, uric acid, and creatinine levels in *ALAD-2* subjects.

In contrast, in a study of 89 lead-exposed workers and 34 unexposed workers in Sweden, Bergdahl et al. (60) found lower levels of urinary creatinine and calcium among combined *1-2/2-2* genotype subjects. No association between genotype and lead in blood, bone, or urine in the exposed group was observed in this study. The frequency of *ALAD-2* was less than that expected among lead workers (χ^2 test: $p =$ 0.0025), and the authors cited this finding as potential evidence of a genetic healthy worker effect, in which *ALAD-2* individuals who reached high blood lead levels would be removed from the workplace (by Swedish occupational health standards) and therefore would not be represented in the study sample.

Several studies have yielded supporting evidence for the hypothesis that *ALAD* genotype also modifies the kinetics of

TABLE 2. Results of studies on the δ-aminolevulinic acid dehydratase (*ALAD*) genotype and lead exposure

Area of study and recruitment period	Study population	No. of study subjects	*ALAD* genotype (% of subjects)		Mean or median blood lead level (μg/dl)	Measure(s) used	Outcome(s), by *ALAD* genotype		*p* value from statistical testing	Author(s), year, and reference
			1-2	*2-2*			*1-1*	*1-2/2-2*		
Germany	Male lead workers	202	15.8	5.0	40 (17)	Blood lead level (μg/dl)	38 (17)*	44 (17)/56 (18)	NR†	Ziemsen et al., 1986 (58)
						ALAD activity (units/liter)	19 (9)	16 (9)/12 (5)		
						Urine ALA† (ng/ml)	7,000	13,000/11,000		
New York, New York	Individuals with high FEP† levels due to lead exposure; several ethnicities represented	1,074	19.8	1.6	NA†	Blood lead level (μg/dl)			NR	Astrin et al., 1987 (25)
						≥30	95	41		
						<30	841	74		
							Odds ratio = 4.9			
Germany and New York, New York	Lead workers of several ethnicities in Germany	202	16.0	5.0	40.2	Blood lead level (μg/dl) Workers	38.4 (16.8)	47.0 (18.0)	>0.004	Wetmur et al., 1991 (26)
	Environmentally exposed low SES† children with high FEP levels	1,278	10.0	1.0	20.3	Children	19.5 (11.6)	27.1 (15.2)	>0.001	
Somerville, Massachusetts, 1975–1978	Adolescents selected by dentin lead levels (>24 μg/g or <8.7 μg/g)	72	6.9	0.0	NA	Dentin lead level (μg/g)	14.1	8.0	NR	Bellinger et al., 1994 (66)
						Tibial lead level >6 μg/g (%)	19	0		
						Patellar lead >6 μg/g (%)	43	100		
						Neuropsychological test performance	*1-2*'s consistently scored higher than *1-1*'s, as judged by a series of tests			
Korea	Employees from three lead storage battery factories of different exposure levels	307	11.1	0	29.1 (12.5)	Blood lead level (μg/dl)	29.0 (11.7)	30.5 (17.5)	0.50	Schwartz et al., 1995 (59)
						Zinc protoporphyrin (μg/dl)	43.1 (36.3)	46.6 (42.1)	0.61	
						Exposure duration (years)	4.7 (3.1)	5.1 (3.3)		
						No. with >6 years	69	16		
						No. with ≤6 years	204	18		
							Odds ratio = 2.6 (95% CI†: 1.2, 5.8)			
Atlantic City, New Jersey	Carpenters	122 (subset of N_T, N_T = 691)	17.2 (*1-2* and *2-2*)		8.0 (3.9)	Blood lead level (μg/dl)	8.0 (4.1)	8.2 (3.2)	0.85	Smith et al., 1995 (24)
						Patellar lead level (μg/g)	13.1 (14.1)	16.4 (19.2)	0.18	
						Tibial lead level (μg/g)	9.8 (9.1)	7.8 (8.1)	0.36	
						Patellar level minus tibial level (μg/g)	3.4 (12.0)	8.6 (9.5)	0.06	
						Blood urea nitrogen (mg/dl)	18.5 (4.6)	19.6 (4.5)	0.03	
						Uric acid (mg/dl)	6.4 (1.4)	6.7 (1.6)	0.07	
						Creatinine (mg/dl)	1.2 (0.2)	1.3 (0.2)	0.11	
Sweden	Lead smelter workers	89	6.7	1.1	Smelter workers: 30.9	Blood lead level (μg/dl)	31.1	28.8	>0.20	Bergdahl et al., 1997 (60)
						Bone lead level (μg/g)	28	28	>0.20	
						Urine lead level (μg/dl)	2,922	2,984	>0.20	
						Urinary creatinine level (mg/dl)	173.1	86.0	0.14	
						Urinary calcium level (mg/liter)	18.8	7.6	0.009	

(continues)

Sweden (continued)	Unexposed controls	34	29.4	0.0	Unexposed controls: 3.7	Blood lead level (μg/dl)	3.7	3.7	>0.20	Bergdahl et al., 1997 (60)
						Bone lead level (μg/g)	4	6	0.18	
						Urine lead level (μg/dl)	684	394	0.04	
						Urinary creatinine level (mg/dl)	158.4	129.0	0.08	
						Urinary calcium level (mg/liter)	12.5	7.8	>0.20	
Korea	Lead battery manufacturing workers	57	33.3	0.0	25.4 (10.2)	Blood lead level (μg/dl)	26.1 (9.8)	24.0 (11.3)	0.48	Schwartz et al., 1997 (61)
						Zinc protoporphyrin (μg/dl)	57.1 (26.8)	48.3 (22.2)	0.23	
						Plasma ALA (ng/ml)	17.3 (9.2)	11.8 (3.3)	0.03	
						Urine ALA (ng/ml)	1,395 (984)	1,022 (679)	0.15	
						4-hour chelated lead‡,§ (μg)	92.9 (45.1)	70.3 (42.1)	0.07	
Korea	Lead battery manufacturing workers	57	33.3	0.0	25.4 (10.1)	Blood lead level (μg/dl)	26.1 (9.8)	24.0 (11.3)	0.48	Schwartz et al., 1997 (62)
						Zinc protoporphyrin (μg/dl)	57.1 (26.8)	48.3 (22.2)	0.23	
						4-hour chelated lead‡ (μg)	92.9 (45.1)	70.3 (42.1)	0.07	
						Hemoglobin A_1 (%)	6.3 (1.0)	5.9 (1.0)	0.08	
						Hemoglobin A_2 (%)	2.8 (0.9)	2.6 (0.5)	0.52	
						Exposure duration (years)	7.3 (3.3)	7.0 (3.3)	0.76	
Korea	Lead battery manufacturing workers	65	32.3	0.0	27.9	Blood lead level (μg/dl)	31.4	26.3	0.41	Sithisarankul et al., 1997 (63)
						Zinc protoporphyrin (μg/dl)	52	43	0.17	
						Plasma ALA (ng/ml)	14.8	11.6	0.012	
						Urine ALA (ng/ml)	1,080	835	0.21	
						Employment duration (years)	7.6	7.7	NR	
Sweden	Lead smelter workers	14	50.0 (matched to *1-1*)	0.0	Exposed workers: 29.1	Blood lead level (μg/dl)	29.4	28.8	NR	Bergdahl et al., 1997 (65)
						Exposure duration (years)	22	11	NR	
						Erythrocyte protein-bound lead (%)	81 (2)	84 (2)	0.03	
	Matched unexposed workers¶	20			Unexposed workers: 3.8	Blood lead level (μg/dl)	3.9	3.7	NR	
						Erythrocyte protein-bound lead (%)	79 (4)	79 (4)	NR	
British Columbia, Canada	Lead smelter workers	134	14.9	0.0	23.9	Blood lead level (μg/dl)	23.1 (12.2)	28.4 (11.7)	0.08	Alexander et al., 1998 (7)
						Zinc protoporphyrin (μg/dl)	40.1 (28.0)	33.8 (14.2)	0.14	
						Coporphyrin (μg/liter)	47.4 (41.6)	41.6 (36.8)	0.59	
						Sperm concentration (10^6 sperm/ml)	60.3 (3.3)	60.3 (2.1)	0.96	
						Total sperm count (millions)	134 (3.7)	148 (2.5)	0.91	
						At blood lead levels ≥40 μg/dl:				
						Zinc protoporphyrin (μg/dl)	86.0 (41.1)	50.0 (18.4)	0.03	
						Coporphyrin (μg/liter)	46.5 (31.6)	13.6 (7.8)	0.01	
						Sperm concentration (10^6 sperm/ml)	32.2 (7.4)	61.8 (1.9)	0.54	
						Total sperm count (millions)	58 (10.0)	116 (3.0)	0.59	

Table continues

TABLE 2. Continued

Area of study and recruitment period	Study population	No. of study subjects	*ALAD* genotype (% of subjects)		Mean or median blood lead level (μg/dl)	Measure(s) used	Outcome(s), by *ALAD* genotype		*p* value from statistical testing	Author(s), year, and reference
			1-2	*2-2*			*1-1*	*1-2/2-2*		
New Brunswick, Canada	Lead smelter workers	381	17.5	0.1	23.3	Blood lead level (μg/dl)	22.9 (0.4)	25.2 (1.0)	<0.04	Fleming et al., 1998 (6)
						Serum lead level (μg/liter)	2.8 (0.1)	3.4 (0.3)	<0.06	
						Tibial lead level (μg/g)	41.2 (1.8)	42.7 (3.4)		
						Calcaneus lead (μg/g)	71.6 (3.4)	72.3 (6.2)		
						Bone lead-CBLI† (μg/dl) slope for workers hired after 1977				
						Tibia	0.032 (0.001)	0.026 (0.003)	<0.04	
						Calcaneus	0.053 (0.003)	0.036 (0.003)	<0.001	
Taiwan	General population	660	4.4	0.2	6.57	Blood lead level (μg/dl)	6.51 (5.03)	7.83 (5.95)	0.17	Hsieh et al., 2000 (23)
Japan	Lead workers	192	15.1	1.0	Exposed workers: 22.3	Blood lead level (μg/dl)	21.9 (19.7)	22.9 (21.7)	NR	Sakai et al., 2000 (17)
						ALAD activity (units/liter)	42.5 (22.1)	39.7 (20.1)		
						Plasma ALA (ng/ml)	24.6 (47.6)	19.4 (27.6)		
						Urine ALA (mg/g of creatinine)	1.5 (3.5)	1.6 (3.6)		
						Zinc protoporphyrin (μg/dl of red blood cells)	156 (213)	117 (134)		
	Unexposed controls	125	16.0	0.8	Unexposed controls: 3.1					
Korea, 1997–1999	Lead workers	798	9.9	0.0	Exposed workers: 32.0 (15.0)	Blood lead level (μg/dl)	31.7 (14.9)	34.2 (15.9)	NR	Schwartz et al., 2000 (64)
						Hemoglobin (g/dl)	14.2 (1.4)	14.2 (1.6)		
						Tibial lead level (μg/g)	37.5 (40.6)	31.4 (29.5)		
						4-hour chelated lead (μg) after administration of DMSA† at 10 mg/kg	180.3 (181.2)	161.7 (143.0)		
	Unexposed controls	135	8.1	0.0	Unexposed controls: 5.3 (1.8)					

* Numbers in parentheses, standard deviation.
† NR, not reported; ALA, aminolevulinic acid; FEP, free erythrocyte protoporphyrin; NA, not applicable; SES, socioeconomic status; CI, confidence interval; CBLI, cumulative blood lead index; DMSA, dimercaptosuccinic acid.
‡ Chelated lead level (μg) after oral administration of dimercaptosuccinic acid at 5 mg/kg.
§ Among subjects matched by exposure history and blood lead level.
¶ Matched on employment status and blood lead level.

lead in bone. In a study of 381 lead smelter workers, Fleming et al. (6) observed increased uptake of lead from blood into bone among *ALAD-1* homozygotes, which was seen by the increased slope of the line relating bone lead levels to a cumulative blood level index (μg/dl) in *1-1*'s compared with *1-2/2-2*'s. This effect was most pronounced in the trabecular bone (the calcaneus) of workers hired after the implementation of a lead safety initiative at the plant in 1977 ($p < 0.001$, as opposed to $p < 0.04$ in cortical bone). This study also provided more evidence for the influence of *ALAD* genotype on the kinetics of lead in blood at moderate to high exposure levels (mean blood lead level = 23.3 μg/dl), as *1-2/2-2* genotype individuals had 10 percent greater blood lead levels ($p < 0.04$).

Schwartz et al. (61) used the chelator DMSA to test for differences in bioavailable lead by genotype in a group of 57 lead battery manufacturing workers. The data showed that *1-1* individuals yielded more lead in 4-hour urine samples in response to chelation therapy (5 mg/kg orally) than *1-2/2-2* individuals with the same exposure history (92.9 μg (SD 45.1) vs. 70.3 μg (SD 42.1), respectively; $p = 0.07$). Another study by Schwartz et al. (62) corroborated these findings (table 2). Given that DMSA-chelatable lead is used as a measure of bioavailable lead, these data indicate that *ALAD 1-2* subjects have lower levels of bioavailable lead and therefore may be at decreased risk in comparison with *ALAD-1* homozygotes. Schwartz et al. (61) also saw higher levels of aminolevulinic acid in the plasma of *1-1* individuals (17.3 ng/ml vs. 11.8 ng/ml; $p = 0.03$), a finding that was replicated by Sithisarankul et al. ($p = 0.012$) (63). Similar differences in aminolevulinic acid in plasma were seen in a recent study of 192 male Japanese lead workers by Sakai et al. (17). These findings suggest that *ALAD-1* homozygotes may be at greater neurologic risk because of the buildup of aminolevulinic acid in plasma. Finally, Schwartz et al. (62) noted an elevation in hemoglobin A_1 levels among *1-1* subjects ($n = 38$) versus *1-2* subjects ($n = 19$) (6.3 percent (SD 1.0) vs. 5.9 percent (SD 1.0); $p = 0.08$), which led them to conclude that both ALAD and hemoglobin A_1 are important lead binding sites that influence the excretion of chelated lead.

Note that the studies by Schwartz et al. (59, 61, 62) and Sithisarankul et al. (63) contained overlapping study samples. The degree of overlap is unknown. The studies are presented separately for the purposes of this review, but the results should be interpreted with caution, since they are based on samples that may have contained substantial redundancy.

Most recently, Schwartz et al. (64) reported on a study of 798 Korean lead workers and 135 unexposed controls. *ALAD 1-1* workers yielded substantially more chelated lead after administration of DMSA at 10 mg/kg (see table 2). Logistic regression modeling of chelated lead showed that creatinine clearance was an important predictor ($\beta = 0.006$, $p < 0.001$) and *ALAD* genotype modified this relation (*ALAD*-creatinine interaction: $p = 0.04$). *ALAD-2* subjects had larger increases in chelated lead with increasing creatinine clearance. (The effect of a polymorphism in the vitamin D receptor gene was also investigated and is discussed below under "Gene-gene interactions.")

In a study by Bergdahl et al. (65), the ALAD enzyme was found to be the principal lead-binding site in erythrocytes. The investigators found a higher percentage of lead bound to erythrocyte ALAD in lead-exposed *ALAD 1-2* subjects than in *1-1* subjects (84 percent vs. 81 percent; $p = 0.03$).

One study of a group of 134 lead smelter workers in Canada by Alexander et al. (7) examined differences in sperm count and sperm concentration by genotype, in addition to blood lead level, zinc protoporphyrin, heme, and coporphyrin. In this group, blood lead levels were higher among *ALAD 1-2* subjects (28.4 μg/dl vs. 23.1 μg/dl for *1-1*'s; $p = 0.08$); *ALAD 1-2* subjects had higher sperm counts, but the difference between the two groups was not significant. Focusing on the relations of *ALAD* genotype, zinc protoporphyrin, and coporphyrin at blood lead levels ≥40 μg/dl, Alexander et al. observed that *ALAD-1* homozygotes had significantly higher zinc protoporphyrin levels (86.0 μg/dl (SD 41.1) vs. 50.0 μg/dl (SD 18.4); $p = 0.03$) and higher coporphyrin levels (46.5 μg/liter (SD 31.6) vs. 13.6 μg/liter (SD 7.8); $p = 0.01$) (7). In other words, markers other than blood lead level indicated that *ALAD-1* homozygotes in this study exhibited more inhibition of heme synthesis after exposure to lead. The authors noted that the nonrandom method of study subject ascertainment (solicitation by postal questionnaire) and the lack of women in the sample were limitations of their study.

The sole population-based study was conducted by Hsieh et al. (23) in a Taiwanese population ($n = 660$). Hsieh et al. measured blood lead levels and found 20 percent higher levels in the *1-2/2-2* group (7.83 μg/dl (SD 5.95) vs. 6.51 μg/dl (SD 5.03)), but this result was not statistically significant ($p = 0.17$). The authors suggested that the lack of significance for the difference in blood lead level by genotype was possibly due to the small number of individuals in the *1-2/2-2* group ($n = 30$). They also postulated that blood lead levels in Taiwan may be relatively low because of the rarity of the *ALAD-2* allele in that population.

ALAD genotype and neurologic outcomes

In 1994, Bellinger et al. (66) published a report in which adolescents ($n = 72$) with high (>24 μg/g) and low (<8.7 μg/g) dentin lead levels were studied, and the results suggested that the body burden and effects of lead were worse among *ALAD-1* homozygotes. Although the study contained only five subjects with the *1-2* genotype, these five persons had lower dentin lead levels than subjects with the *1-1* genotype and consistently scored better on neuropsychological tests (no *p* values were given because of small numbers). To our knowledge, this is the only study on the *ALAD* polymorphism to date that has used a neurologic outcome measure. Subjects with the *1-2* genotype were also less likely to have tibial lead concentrations greater than 6 μg/g and more likely to have patellar lead concentrations greater than 6 μg/g.

INTERACTIONS

In addition to *ALAD* genotype, other factors to consider in determining overall susceptibility to lead toxicity include

substances that inhibit the ALAD enzyme and nutritional status, primarily intakes of calcium and iron. The interaction between these factors and *ALAD* genotype may be important when considering the health effects of lead. Additionally, the genes encoding the vitamin D receptor (*VDR*) and the hemochromatosis-major histocompatibility complex class I protein (*HFE*) are both polymorphic and have recently been implicated in lead poisoning susceptibility. Thus, gene-environment and gene-gene interactions may produce enhanced effects and deserve further exploration.

Gene-environment interactions

The ALAD enzyme is inhibited by alcohol and smoking (67). Three of the studies examined in this review measured smoking (24, 61, 66), and one of them controlled for smoking in regression models of outcome (61). Three studies measured alcohol use (24, 61, 66), and one controlled for alcohol use in a model (24). No studies explicitly examined *ALAD*-alcohol or *ALAD*-smoking interactions.

Calcium status has been shown to influence the intake and effects of lead. Lead binds to calcium-binding proteins and may also compete directly for absorption in the intestine. Mahaffey et al. (68) showed that blood lead levels are lower in children with higher calcium intakes. In addition, several studies in experimental animals have clearly demonstrated that prior intake of calcium reduces the absorption of lead and that absorption of lead is higher in calcium-deficient animals than in normal animals. Studies also show that people absorb more lead when fasting than when not fasting (69); therefore, dietary intake in general is an important factor as well. No studies have explored interactions between *ALAD* and any of these factors.

Gene-gene interactions

The effects of calcium on lead intake and absorption are mediated through calcium-binding proteins that are, in turn, mediated through the bloodborne form of vitamin D, calcitriol. Calcitriol binds to the vitamin D receptor, and thus genetic variations in the vitamin D receptor are also important in this pathway. A common polymorphism in the *VDR* gene, a restriction fragment length polymorphism detected by digestion with *Bsm*I that results in the *B* and *b* alleles, has already been shown to affect bone mineral density. The *BB* genotype, which signals no *Bsm*I restriction site, exists in approximately 7–32 percent of the population and has been shown by meta-analysis to be associated with lower bone mineral density (70). Two recent studies by Schwartz et al. (64, 71) explored the role of this polymorphism in tibial bone lead levels among lead workers. Schwartz et al. (71) first reported small differences in bone lead levels by *VDR* genotype among a group of former lead workers (13.9 (SD 7.9), 14.3 (SD 9.5), and 15.5 (SD 11.1) μg lead/g bone mineral for the *bb*, *Bb*, and *BB* genotypes, respectively; adjusted p for linear trend = 0.16). The relation between years since last exposure and tibial bone lead concentration was also modified by *VDR* genotype. In their second report (64), Schwartz et al. noted larger differences in blood lead level by *VDR* genotype than by *ALAD* genotype. On average, the *VDR B* allele gave a 4.2 μg/dl increase in blood lead level, while the *ALAD-2* allele yielded an increase of 3.6 μg/dl in blood lead level. Schwartz et al. also explored the role of a possible gene-gene interaction between *VDR* and *ALAD* and found no evidence of an interaction. Interestingly, they did find an association between *ALAD* and *VDR* genotypes which varied by exposure status. Lead workers with the *ALAD 1-1* genotype were less likely to have the *VDR bb* genotype (odds ratio = 0.29; 95 percent confidence interval: 0.06, 0.91), while unexposed controls with the *ALAD 1-1* genotype were more likely to have the *VDR bb* genotype (odds ratio = 2.5; 95 percent confidence interval: 0.23, 14.84). This may be indicative of genotype selection in the occupational environment.

Like calcium status, iron deficiency also increases the absorption and toxic effects of lead (72). Ferritin, the iron transport protein, binds lead, and ferritin levels are increased among persons with iron-deficient anemia. Counterintuitively, people who are homozygous for the *HFE* mutation that induces hemochromatosis, a disease of iron overload, have been shown to have higher blood lead levels than people with wild-type *HFE* (5.6 μg/dl (SD 0.6) vs. 3.6 μg/dl (SD 0.5) (73)), and heterozygotes have intermediate levels (4.1 μg/dl (SD 0.5)), which suggests that carriers of the mutant gene absorb more lead. However, this finding was not replicated in a recent study by Åkesson et al. (74). To date, no studies have evaluated an *HFE-ALAD* interaction.

LABORATORY TESTS

Early studies used the phenotyping technique developed by Battistuzzi et al. (4) to classify individuals as having *ALAD 1-1*, *1-2*, or *2-2*. In this procedure, whole blood samples are taken and the red blood cells are isolated and lysed. Isolation and electrophoresis of the ALAD protein permits distinction between phenotypes because of the charge differences of the isozymes. Wetmur et al. (5) developed the genotyping technique based on polymerase chain reaction that has been used by most investigators. A 916-base-pair sequence containing the *ALAD-1/2* polymorphic site is amplified and then cleaved with *Msp*I. The cleavage products are then analyzed on agarose gel. Studies using this technique should include positive (e.g., a gene encoding an essential enzyme) and negative controls.

SUMMARY

The evidence surrounding the *ALAD* G177C polymorphism and lead poisoning can be summarized as follows. At high levels of exposure and in comparison with *ALAD 1-1* individuals, *ALAD 1-2/2-2* individuals have increased blood lead levels, lower concentrations of aminolevulinic acid in plasma, lower zinc protoporphyrin levels, lower cortical bone lead concentrations, higher concentrations of trabecular (spongy) bone lead, and lower amounts of DMSA-chelatable lead. Thus, *ALAD* genotype modifies the kinetics of lead in both blood and bone. Although people with the *ALAD-2* genotype may achieve higher blood lead levels

when exposed to lead, they may experience less heme synthesis inhibition than *ALAD-1* homozygotes. When lead binds and inhibits the ALAD enzyme, ALAD expression is increased in response (75, 76). Therefore, individuals with the *ALAD-2* genotype may be better able to compensate than *ALAD-1* homozygotes as more lead is bound to ALAD-2 enzyme (65). This hypothesis might help explain the genotype selection observed by Schwartz et al. (59) in which *ALAD-2* subjects seemed to tolerate longer exposures to lead in the occupational setting. The data also suggest that *ALAD-1* homozygotes may be at greater risk of neurotoxicity than *ALAD 1-2* individuals, since *ALAD-1* homozygotes have higher levels of aminolevulinic acid in plasma (61, 63). Finally, a study by Bellinger et al. (66) gave preliminary evidence that people with the *ALAD 1-2* genotype may have better neuropsychological performance than *ALAD-1* homozygotes with similar lead exposure histories.

It is difficult to make a decision as to which genotype is in fact the "at-risk" genotype, because different measures of outcome indicate that each genotype is more susceptible to one or more adverse effects in comparison with the other. This problem is complicated by the use of different measures in studies. Indeed, the question of which measures are most appropriate for estimating body lead burden and health risk is one that remains to be answered and that merits discussion. In particular, the lack of available data on the effect of this polymorphism on endpoints such as cognitive deficits and/or neuropsychological performance is troubling. In addition, most studies have used occupationally exposed individuals with relatively high levels of lead exposure. Bias in study subject selection is often encountered in these studies. Very few studies have used samples from the general population, for whom exposure levels are generally much lower than in occupational settings; and in both these studies and the occupational studies, the percentage of samples including women is unknown. Thus, it is difficult to make inferences for the general population. Results from current research projects investigating these issues using community samples may help to resolve these questions.

POPULATION TESTING

At this time, there is inadequate evidence to support population-based testing.

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APPENDIX

Websites Pertaining to Lead Exposure and Toxicity

http://www.cdc.gov/niosh/leadpg.html
http://www.epa.gov/opptintr/lead/index.html
http://www.hud.gov/lea/leatips.html
http://www.osha-slc.gov/SLTC/lead/index.html
http://www.ohb.org/leadpub.htm?