Published in final edited form as:

AACN Clin Issues. 2002 November ; 13(4): 550–556.

Allelic Variation and Environmental Lead Exposure in Urban Children

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Abstract

The advent of the Human Genome Project has allowed for increased understanding and sophistication in diagnosis, treatment methods, and overall care planning on the part of healthcare providers for children with genetic disorders. Genetics research dealing with polymorphic changes within a genome has opened the door to awareness of how dormant genetic alleles may become active when coupled with certain environmental insults. Such genetic aberrations may place a child at a higher risk for health disparities when exposed to environmental toxins. It has been posited that such exposure in children with an arylsulfatase-A (ASA) allelic variation is associated with increased risk for neurodevelopmental damage. This initial study contributes to this new field and supports development of finer-tuned methods to prevent ominous outcomes of lead exposure. The purpose of this study was to explore the incidence of children in a representative sample from a Midwest metropolitan city with positive test results for the ASA allelic variation who have been exposed to the environmental toxin lead. In this corollary study of 107 children, part of a parent study on the behavior of African American children prenatally exposed to cocaine, 45% were found to be heterozygous, 11% mutant homozygous, and 44% normal in terms of ASA allele or alleles. Further studies on neurodeficiencies, low-level exposure to environmental toxins, and allelic variations must be conducted before a relation between ASA allelic variance and environmental lead can be determined.

Keywords

genetics; risk factors; child health; environment; lead exposure

Because of the recent and extensive research emanating from the Human Genome Project, nurses and scientists are more knowledgeable about human diseases. The goal of the Human Genome Project, which began in 1990, is to gain knowledge about the effects of genes and to understand how their variations can lead to revolutionary new ways of diagnosing, treating, and someday preventing a multitude of human disorders. Besides providing clues for understanding human biology, knowledge of genetics can lead to an awareness of the diagnostic and research capabilities that may be applied toward solving challenges in healthcare and the environment. Table 1 presents definitions of genetic terminology.

One area of particular interest involves genetic polymorphisms. Genetic polymorphism is simply a term used to describe a gene that may exist or occur in different forms or at different

stages of development, resulting in the natural variations seen in humans, and in some cases in human disease.¹ Environmentally induced allelic variations represent an area of genetic research currently being explored. A human child's very genetic uniqueness may predispose him or her to more ominous health outcomes when exposed to environmental toxins.

Background Information

Environmental lead is a developmental toxin that affects more than 5% of children nationwide, and among low-income children in urban and rural areas, even a greater number are estimated to be affected.² Biologic influences such as allelic variation in genes may intensify a child's overall responses to environmental toxins such as lead.¹ Each person has two arylsulfatase-A (ASA) alleles, one inherited from each parent. The ASA alleles are found at a specific location (locus) in all individuals.

The ASA gene was sequenced in the late 1980s, and by the mid-1990s, 39 different mutations had been identified in the population of people with metachromatic leukodystrophy (MLD). Individuals with an alteration in one ASA allele are potentially at risk for environmental lead effects, and those with a disease-associated alteration in each of their ASA alleles may have some form of MLD.^{3,4} Although there are many examples of environmental toxins that affect the health and genetics of children, this study focused on lead. A few examples of other environmental toxins that can be found in the maternal-child toxicology literature in nursing and medicine are listed in Table 2.

Arylsulfatase Pseudodeficiency

Arylsulfatase-A is a lysosomal enzyme needed to catabolize sulfatides. When ASA is severely deficient or absent, sulfatides progressively accumulate, primarily in the lysosomes, leading to eventual loss of cell function in tissues such as the central nervous system, peripheral nerves, kidney, liver, gall-bladder, and spleen.⁵ This ultimately results in rapid demyelination of the myelin sheath covering of the neurons.

When a genetic aberration is evident in the genetic makeup of a child in the ASA allele or alleles, several scenarios can result. In children afflicted with an ASA pseudodeficiency allele caused by a polymorphism, MLD is diagnosed. People with MLD have one or two of many possible mutations in each ASA allele. With this genetic disease, there is a lack of ASA enzyme or reduced amounts of it. A homozygous inherited genetic disorder of the central nervous system that has its effects on the white matter of the brain, MLD in most cases results in regression of developmental milestones and ends in death by the age of 2 years.^{5,6} Some signs and symptoms of MLD include neurologic impairment, blindness, loss of motor function, rigidity, seizures, and loss of developmental milestones. Individuals with MLD do not catabolize sulfatides and have two true deficiency alleles, which will be referred to as homozygous (aa) for the purposes of this article. Although most of the ASA-deficiency MLD cases are found in the Habbani Jewish community, approximately 12% of cases can be found in the African Americans population, and 2% in the European American population.^{5,6}

The heterozygous (Aa) form of ASA allelic variation and the possible interaction with environmental lead in children represent a relatively new area of toxicology research, with relatively little data at this writing. Poretz et al⁴ suggested that the mutant heterozygous form of the allele ASA may produce a compounded or synergistic effect of environmental lead because of the incomplete ASA enzyme activity. It is postulated that even at low levels of lead exposure (15 µg/dL), children who are heterozygous for the ASA allele will exhibit neurodeficiencies similar to those of children exposed to higher levels of lead, such as behavioral aberrations.⁷ One rationale for this hypothesis is that when the ASA enzyme level is not optimal, a buildup of sulfatides occurs, leading to demyelinating neurologic diseases of

the central nervous system such as MLD, neuropathy of unknown etiology, and multiple sclerosis.^{7,8} When coupled with environmental lead, a toxin widely known to have deleterious effects on neurodevelopment (affecting both intelligence quotient [IQ] and behavior), it is plausible that the interaction between ASA enzyme deficiency and lead exposure may exacerbate clinical findings.

Knowledge of ASA enzyme deficiency allows clinicians to intervene appropriately, testing and diagnosing low levels of lead exposure in children who display neurologic symptoms of high lead exposure or ASA enzyme deficiency. Clinically, knowledge of genetic aberrations enhances the clinical nurse's skill in differential diagnosis and proper management (or genetic referral) of families whose children are afflicted with genetic diseases.

Arylsulfatase-A and Lead Physiologic Mechanism

Although the ASA enzyme influences white matter myelin whereas lead affects gray matter myelin, lead damages Schwann cells by demyelination, resulting in decreased nerve conduction velocity.⁹ For example, Trope et al¹⁰ reported a case study that compared and contrasted a non-lead-exposed developmentally delayed child with a cousin who had been exposed to environmental lead. The results of magnetic resonance imaging in this study showed that the lead-exposed child had alterations in brain metabolites, whereas the developmentally delayed child did not, suggesting that brain metabolites are altered under the influence of lead exposure.¹⁰ This information may help to explain some of the observed neurodeficiencies in children with ASA exposed to low levels of lead.

Arylsulfatase-A and Induced Diseases

Although ASA allelic variation in its heterozygous form does not necessarily lead to MLD, it is quite conceivable that ASA allelic variance may be a contributor to other demyelinating diseases and nervous system disorders such as multiple sclerosis, acute lymphoblastic leukemia (ALL), chronic alcoholism, and neuropathy of unknown etiology (Table 3).

MULTIPLE SCLEROSIS—A chronic autoimmune inflammatory disorder of the central nervous system, multiple sclerosis results in damage to the myelin sheath covering of the axons. This causes visual disturbances, muscle weakness, spasticity, tremors, and urinary retention. A study by Kappler et al¹¹ examined 160 patients with multiple sclerosis to determine whether these patients would exhibit ASA allelic variance on genetic screening using polymerase chain reaction. In this study, the difference in findings between the experimental patients with multiple sclerosis and control subjects was insignificant.¹¹ Although four patients in the experimental group but only one patient in the control group expressed the ASA allele, it was determined that further studies were needed before conclusions could be drawn on this relation.¹¹

ACUTE LYMPHOBLASTIC LEUKEMIA—Acute lymphoblastic leukemia, the most common malignancy among children between the ages of 1 and 5 years, usually is found more frequently in European Americans and males. This disorder is diagnosed by the presence of lymphoblasts in the bone marrow. The literature tends to link functional polymorphisms coupled with environmental factors such as prenatal irradiation as contributing factors to ALL.^{12,13} According to Sinnett et al,¹² the genetic polymorphisms found in ALL partly explain the increased incidence in males. The mainstay of treatment for ALL is bone marrow transplantation, chemotherapy, and steroid therapy. Of the children treated, 70% are free of the disease 5 years after treatment.¹² Overall, in the case of prenatal irradiation, ASA allelic variance coupled with environmental toxins is suggested to be a causative factor in ALL disease expression.^{12,13}

CHRONIC ALCOHOLISM—Abuse of alcohol has become a major health risk in the United States, and alcohol consumption is more prevalent than the use of any illegal drugs.¹⁴ Alcoholism has been related to neuropsychiatric disorders, but it still is undetermined whether alcoholism is a cause or a result of psychiatric disorders.¹⁴ Alvarez-Leal et al¹⁴ studied the relation among ASA enzyme level, chronic alcoholism, and psychiatric disorders. The results of this study showed that the alcoholic psychiatric patients had lower levels of ASA enzyme than the patients without psychiatric disorders who were alcoholics.¹⁴ The researchers posited that the chronic use of alcohol among the patients with low ASA enzyme activity may have led to greater degrees of psychiatric disorders. Alvarez-Leal et al¹⁴ also suggested the potential toxic effect of prenatal alcohol exposure on ASA enzyme activity, especially in children with ASA allelic variance and enzyme pseudodeficiency. pecially in children with ASA allelic variance and enzyme pseudodeficiency.

NEUROPATHY—Early symptoms that suggest diseases of the nerves may forecast the eventual development of diseases such as MLD and multiple sclerosis. Often, individuals present with neuropathy and then are diagnosed later with serious neurologic conditions. A Danish study noted one case of a patient presenting with neurologic symptoms thought to be multiple sclerosis or peripheral neuropathy in whom enzyme deficiency also was diagnosed by magnetic resonance imaging.⁸ Thus, individuals with signs and symptoms of neuropathy of unknown etiology might benefit from assessment for ASA allelic variance and enzyme pseudodeficiency that may induce a progressive neurologic process.

Significance of the Study

Both environmental toxins and genetics play a significant role in health disparities among children. The next step is to determine what happens when allelic variations or polymorphism is activated by environmental exposure to toxins.

The first step in answering this question was to determine the prevalence of ASA allelic variation in a particular population. One of every four children in this urban midwestern study site had some exposure to the environmental toxin lead. The incidence of ASA allelic variation in African American children was assessed as part of a larger parent study.

Once children with ASA allelic variation and enzyme pseudodeficiency have been identified, other studies may be conducted to investigate the relation between ASA allelic variation and the environmental effects of lead exposure in urban children. This information is relevant to nursing practice because it relates to a better understanding of genetic disease and environmental toxins that may induce the phenotypic expression of allelic variance or polymorphisms in children. Nurses need to be aware of genetic aberrations in the pediatric population as well as possible environmental threats that may enhance the expression of these underlying conditions. A knowledge of synergistic effects from genetic predispositions and environmental toxins will aid nurses in guiding the care of these children. The purpose of this study was to explore the incidence of children in a representative sample from a midwestern metropolitan city with positive test results for the ASA allelic variance who had been exposed to the environmental toxin lead.

Methods

Subjects were obtained from a larger study conducted to investigate the early school-age outcomes associated with prenatal substance exposure. The details of the methods used in this parent study are described in Delaney-Black et al.¹⁵ Pediatric families participating in the parent study whose current lead levels had been documented and genotypes evaluated were participants in this study. Whole blood rather than plasma was used to evaluate lead levels

according to the Centers for Disease Control Guidelines.¹⁶ Human subject approval was obtained from the Wayne State University Institutional Review Board.

Clinic visits were made to obtain the whole blood specimen and collect the data for the variables of interest in the current investigation. Deoxyribonucleic acid (DNA) analysis is performed at Rutgers University using the polymerase chain reaction (PCR) technique on buccal swabs obtained from subjects in the study. As the most widely used technique in analyzing genetic material, PCR is a DNA analysis method demonstrated to be highly reliable and consistent.

Results

From the parent study sample of 566 African American first-grade children (age, 5.9–7.9 years), 107 assessed children were genotyped for the ASA allelic variance. This sample represented 19% of the subjects seen during the overall study period. Children assessed when the laboratory was closed and those who refused consent for the blood-drawing phase of the parent project were not tested.

Among the 107 children sampled, 44% ($n = 47$) had a normal genotype, and 45% ($n = 48$) were heterozygous for the ASA allele carrying the allelic variance of the auto-somal recessive trait for MLD. The remaining children (11%; $n = 12$), were found to be homozygous for the alleles aa. Within this sample, the whole blood level mean was 5.016 $\mu\text{g/dL}$ (range, 0.2–16.1 $\mu\text{g/dL}$).

Discussion

The data reported here reflect a convenience sample and therefore cannot be considered a representative portion of the population. However, the results suggest that a significant portion of the population sampled had carrier (Aa) status of the ASA allelic variation. Although carriers of the ASA allele may not exhibit any of the symptoms that homozygous ASA children have (ie, MLD), it is quite plausible that the heterozygous form of ASA allele may have some of the deleterious neurobehavioral effects when exposed to the additive environmental insult of lead exposure. Clinically, children with MLD have more severe outcomes. An awareness of the signs and symptoms of MLD will allow nurses to provide the most optimal care and interventions for these children and their families (ie, genetic counseling and appropriate referrals to genetic specialists).

Furthermore, the data showed that 12 children, all ages 6 to 7 years, were homozygous (aa) for the ASA allele, but did not display symptoms of MLD. Usually, the life expectancy of children with MLD is 6 months to 2 years of age. However, it is important to note that juvenile- and adult-onset forms of MLD exist. An individual's combination of alleles tends to determine the patient's type of MLD. However, families have been described in which the type of MLD among siblings was not the same, so the genotype-pheno-type correlation is not absolute.

In the juvenile form of MLD, the onset of symptoms occurs between the ages of 4 and 16 years, whereas the adult onset of MLD appears after the age of 16.5 years. This variation in expressivity of the phenotype may result from variation in the type and location of mutations within the ASA gene. If the marker A is closely linked to the mutant allele ASA, then the phenotype more than likely will be expressed. However, if an individual does not have the A marker, or if the marker is not in close proximity to the polymorphism, then the phenotype may not be expressed. It is possible that individuals who have later expression or nonexpression of the ASA allele may be affected by a less virulent form of the mutation and therefore produce residual amounts of the enzyme.⁶

It is not clearly understood why some children with low levels of lead ($\leq 20 \mu\text{g/dL}$) show the signs of neurodeficiencies displayed by children with high levels of lead. However, the

interaction between the heterozygous ASA allele and low lead exposure may be one explanation. In the current study, lead levels did not exceed 16.1 µg/dL. Thus the lead to ASA allelic variation status relation could not be adequately evaluated. Further studies based on pediatric samples with higher lead levels are underway.

Summary

Now that children with the ASA allelic variation and enzyme deficiency have been identified, additional research is needed to investigate the potential interaction between ASA allelic variation and lead exposure in urban children. The current findings enhance nursing knowledge of genetic disease and environmental toxins that may induce the expression of heterozygotic allelic variation in children. To provide the most up-to-date care for children, nurses need to remain current in genetics research to increase their awareness of genetic aberrations in the pediatric population, as well as possible environmental threats that may enhance the expression of these underlying conditions. Information from this study regarding the synergistic affects of genetics and environmental toxins in the pediatric population should propel other nurse scientists to explore similar possibilities in genetics research. Further research on neurodeficiencies and neurobehavior must be conducted before an interaction between ASA allelic variation or enzyme deficiency and environmental lead can be determined.

Acknowledgments

Funding for this research was provided in part by NIDA (DA 08524 to Dr Delaney-Black), the National Foundation March of Dimes (12-FY97-0047 to Dr Delaney-Black), and the Helpie Institute for Urban Pediatric Health Research, Children's Research Center of Michigan, Children's Hospital of Michigan.

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TABLE 1

Definitions

Term	Definition
Phenotype	The observable characteristics of an organism. ¹⁷
Genotype	The genetic constitution (gene makeup) of an organism. ¹⁷
Gene	A heredity determinant of a specific biological function; a unit of inheritance (DNA) located in a fixed position on a chromosome. ¹⁷
Polymorphism	Two or more kinds of individuals present in a breeding population. ¹⁷
Allele	One of a pair, or series, of alternative forms of a gene that occur at a given locus in a chromosome. ¹⁷
Mutation	A change in the DNA at a particular locus in an organism. The term is used loosely to include point mutations involving a single gene change as well as a chromosomal change. ¹⁷

TABLE 2**Environmental Risks for Women and Children**

Environmental Risk	Effect of Environmental Insult
Lead	Neurotoxicity, attention deficit disorder, behavioral changes, learning disabilities
Mercury ^{18,19}	Brain damage, kidney damage, tremors, memory loss, damage to fetus
DDT (pesticide)	Risk of cancer, immune system suppression
Dioxin (by-product of bleaching process found in rayon or rayon-cotton blend tampons) ²⁰⁻²²	Risk of cancer, endometriosis, low sperm count in men, and immune system suppression

TABLE 3

Diseases Associated With ASA

Disease/Disorder	Effects of Disease/Disorder
Metachromatic leukodystrophy	Neurological impairment, blindness, loss of motor function, rigidity, seizures, and loss of developmental milestones, ultimately death
Multiple sclerosis	Visual disturbances, muscle weakness, mood changes, spasticity, tremors, and urinary retention
Acute lymphoblastic leukemia	Anemia, fatigue, lethargy, fever, and bone and joint pain
Chronic alcoholism	Mood changes, neuropsychiatric disorders, pancreatitis, cirrhosis