A Dopamine Receptor (DRD2) but Not Dopamine Transporter (DAT1) Gene Polymorphism is Associated with Neurocognitive Development of Mexican Preschool Children with Lead Exposure

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Objective To investigate the effects of prenatal and postnatal lead exposure and polymorphisms in dopamine metabolism genes on neurocognitive development of Mexican children at 24 months (n = 220) and 48 months (n = 186) of age.

Study design We genotyped the dopamine transporter gene (DAT1; SLC6A3) variable nucleotide tandem repeat and the dopamine receptor D2 (DRD2) Taq1A single nucleotide polymorphism. Children were assessed at 24 months with Bayley Scales of Infant Development (Mental Development Index and Psychomotor Development Index) and at 48 months with McCarthy Scales of Children's Abilities.

Results Blood lead concentration (BLL) in umbilical cord was 6.6 ± 3.3 μg/dL (measured in 1995-96), 8.1 ± 4.4 μg/dL at 24 months, and 8.1 ± 3.6 μg/dL at 48 months. Cord BLL was negatively associated with Mental Development Index ($P < .01$) and Psychomotor Development Index ($P < .1$), but not McCarthy scores. The 48-month BLL, but not the 24-month BLL, was negatively associated with children's scores. Children with DRD2 TT genotype (variant) scored higher than children with CC genotype (wild type) on the Mental Development Index and McCarthy memory scale. Neither polymorphism modified the relationship between BLL (either prenatal or postnatal) and neurocognitive development.

Conclusion Lead exposure was adversely associated with neurocognitive measures, whereas the DRD2 Taq1A TT variant was positively associated with neurocognitive measures. We found no evidence of gene-environment interactions on developmental outcomes in early childhood. *(J Pediatr 2011;159:638-43)*.

Lead exposure is associated with behavioral and cognitive deficits in children.[1] Impairments in executive function are one of the hallmark effects, even at low levels,[2,3] and suggest the involvement of dopamine neurotransmission.[4-7] Altered dopamine metabolism and polymorphisms in dopamine-related genes have also been associated with behavioral manifestations in children.[8,9] Although the contribution of these polymorphisms to attention deficit hyperactivity disorder is established,[8] less is known about their relationship to cognition, particularly in children exposed to environmental toxicants.

Several genes mediate dopaminergic neurotransmission, and at least 2 have putatively functional variants, including genes encoding the dopamine transporter (DAT1) and dopamine receptor-2 (DRD2). The DAT1 clears dopamine from synapses[10] and limits the duration of synaptic activity.[11] A variable number tandem repeat (VNTR) polymorphism of the DAT1 gene (SLC6A3) is well studied,[12] and its length is thought to relate to DAT expression[13] and availability,[14] with higher expression and availability being found in individuals with 10- versus 9-repeat VNTR. In turn, the DRD2 regulates neurotransmission via a feedback loop, and a polymorphism in the DRD2 Taq1A gene (specifically the variant carrier versus wild type genotype) is linked with reduced D2 receptor density and availability.[15,16]

The existing studies on the relationship between DAT1 and cognition are inconsistent: two showed an association with selective attention[17] and IQ in children with attention-deficit/hyperactivity disorder,[18] and one found no relationship.[19] Conversely, Taq1A was not independently associated with IQ.[20-22] None of these studies were designed to evaluate gene-environment interactions. However, there is growing evidence[3,23] that dopamine metabolism is related to the effects of environmental exposures, and specifically lead,[24] suggesting that dopamine gene polymorphisms could modify an individual's vulnerability to lead exposure. This potential interaction between lead

BLL	Blood lead concentration
DAT1	Dopamine transporter
DRD2	Dopamine receptor-2
GCI	General Cognitive Index
MDI	Mental Development Index
PDI	Psychomotor Development Index
VNTR	Variable number tandem repeat

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exposure and dopamine gene polymorphisms needs to be addressed in population studies.

Our group has shown that prenatal exposure to lead was associated with global indices of development in young children.[25] In this study, we investigated whether the association between lead exposure (prenatal and postnatal) and neurocognitive development of Mexican children at 24 and 48 months of age is modified by polymorphisms in DAT1 and DRD2 genes.

Methods

Pregnant women receiving antenatal care between January 1994 and June 1995 at 3 hospitals that serve low-to-middle-income Mexico City populations were invited to participate. The study population, eligibility criteria, and recruitment have been described.[26] A total of 617 women were enrolled, and their infants were eligible to participate in a child development study. The study was approved by ethics review boards at the National Institute of Public Health in Mexico, National Institute of Perinatology in Mexico, Brigham and Women's Hospital, Harvard School of Public Health, and participating hospitals.

Demographic and maternal characteristics were collected with questionnaires. Newborn characteristics were obtained from medical records, whereas anthropometric measurements were collected within 12 hours of delivery. Umbilical cord blood samples were collected at delivery. Blood lead concentration (BLL) was measured by using graphite furnace atomic absorption spectrometry (Model 3000, Perkin Elmer, Wellesley, Massachusetts) at the American British Cowdray Hospital Trace Metal Laboratory in Mexico City according to established techniques.[27] The laboratory participates in the Centers for Disease Control and Prevention blood lead proficiency testing program and maintained acceptable precision and accuracy in the study period. The limit of detection was 0.8 μg/dL.

Archived cord blood samples were used for DNA extraction and genotyping at the Harvard-Partners Center for Genetics and Genomics (Boston, Massachusetts). High-molecular-weight DNA was extracted from white blood cells (PureGene Kits, Gentra Systems, Minneapolis, Minnesota), yielding 20 to 40 μg of DNA per mL of whole blood. After DNA quantification, samples were adjusted to TE buffer and stored at –80°C. TaqMan platform was used to genotype dopamine receptor D2 Taq1A SNP (rs1800497) and the 3' VNTR at the dopamine transporter gene (SLC6A3). Ninety-seven percent of samples with adequate DNA concentration were successfully genotyped.

Children's development was assessed at 24 months of age with the Bayley Scales of Infant Development II and at 48 months of age with the McCarthy Scales of Children's Abilities. The Bayley Scales of Infant Development II yields the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). The McCarthy yields several subscales, including the General Cognitive Index (GCI) and the memory scale, which we used as dependent variables in this study. Assessments were conducted in Spanish by psychologists at the Department of Developmental Neurobiology, National Institute of Perinatology in Mexico City.

Of the 617 children examined at delivery, 62 were excluded from analysis because of missing information on polymorphisms, marital status, mother's IQ, schooling, and smoking, birth weight, and gestational age; 94 children did not have cord BLL data. At 24 months, 211 children did not return for developmental testing, and 30 children did not have a BLL value. At 48 months, another 19 children were missing maternal IQ and polymorphism data, 221 children did not return for developmental testing, and 35 children did not have a BLL value. Complete information was available on 220 and 186 children at 24 months and 48 months, respectively.

Data were analyzed with STATA software version 10.0 (STATA Corp, College Station, Texas). Genotype frequencies were calculated and compared against expected counts with the χ^2 statistic to test adherence to the principles of Hardy–Weinberg equilibrium. For the DAT1, children with 10-repeat VNTRs in both alleles were compared with children with any 9-repeat alleles. For the DRD2, children with CT and TT genotypes were compared with the wild type genotype (CC). In regression models, we investigated: (1) whether prenatal and postnatal lead exposure was associated with child development; (2) whether dopamine gene polymorphisms were associated with child development; and (3) whether dopamine gene polymorphisms modified the relationship between prenatal or postnatal lead exposure on child development. First (model 1), we addressed whether BLLs were independently associated with developmental scores. Ordinary least squares multiple linear regression models examining the association between developmental outcomes and prenatal and postnatal lead exposure were conducted separately for the 24- (MDI and PDI) and 48- (GCI and memory scale) month measures. The models were adjusted for relevant co-variates. Subsequently, the developmental outcomes were modeled as a function of each polymorphism separately, adjusting for relevant co-variates (models 2 and 3).

Finally, we modeled cord and postnatal BLL with each polymorphism to predict child performance at 24 and 48 months, testing for two-way interactions (BLL versus polymorphism). Interactions between dopamine gene polymorphisms and cord BLL were tested in separate models from interactions between the polymorphisms and postnatal BLLs. Non-significant interactions ($P > .10$) were removed and regression models were re-run to test for main effects of lead exposure and polymorphism. All regression models were adjusted for gestational age, birth weight, child sex, mother's IQ, years of education, age, smoking status, and marital status at enrollment, crowding in the house (bedrooms divided by number of people living in the house), and type of floor (wood/mosaic or concrete; used as a proxy for socioeconomic status).

Results

There were no meaningful differences between children included in the analysis and children who were excluded, either at 24 months (Table I) or 48 months (data not shown). At