HEMOCHROMATOSIS AND TRANSFERRIN GENOTYPE MODIFICATION OF LEAD BIOMARKER EFFECTS UPON INFANT NEURODEVELOPMENT

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Introduction:

Environmental events leading to disruption during critical windows of neurodevelopment can permanently impact cognition. (Grandjean, 2006) Reduction in the number of viable neurons in critical areas of a developing brain, coupled with the slow neural destruction associated with advanced aging, provides a plausible neurodegenerative disease mechanism for a variety of environmental contaminants. But only now being explored is how individual genetic susceptibility can modify environmental exposure to the fetus by either enhancing or diminishing neurodevelopment. Recent research has linked common genetic polymorphisms in cholesterol metabolism (APOE ε4) and iron metabolism (hemochromatosis C282Y/H63D) to increased risk for neurodegenerative diseases but evidence is lacking for how these polymorphisms affect fetal neurodevelopment. Previous research from our group has shown an enhanced effect from the APOE ε4 genotype upon 24 month mental development index scores (MDI) and a separate protective effect against the negative impact of lead exposure upon infant cognition. (Wright, 2003)

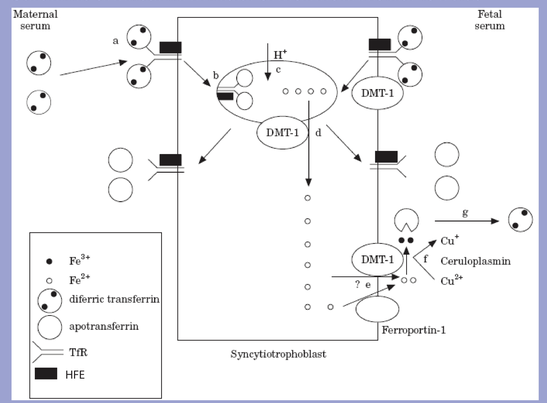
Hypothesis:

In this exploratory study, we examined the potential modifying effect of the hemochromatosis gene (HFE) variants C282Y and/or H63D and transferrin gene (Tf) variant C2 on the impact of lead exposure to infant neurodevelopment. Due to increased iron absorption and unbound iron in HFE/Tf variants we hypothesized that infant variant genotypes would have improved 24-month MDI scores. Furthermore, we hypothesized that lead's negative effects upon children's cognitive ability would be decreased by the presence of at least one variant genotype (H63D, C282Y and/or TfC2) since lead and iron compete for common absorption/transportation receptors.

Materials and Methods:

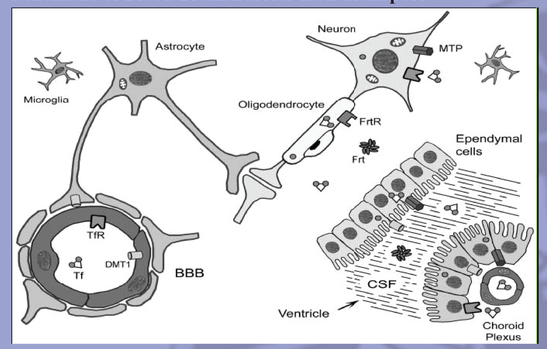
- Sample Population: Mexico City Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) Study
  - Maternal/infant pairs were recruited from three maternity hospitals serving low to moderate income populations in Mexico City from 1994 to 1995
  - 617 eligible mother/infant pairs, 423 infants had archived blood available for genotyping, and 301 infants with available genotypes had 24-month Mental Development Index (MDI) scores.
- Assessment of Child Development:
  - Infant MDI score at 24 months was assessed using the Bayley's Scale of Infant Development (BSID-II.)
  - Maternal IQ was assessed using the Information, Comprehension, Similarities, and Block Design components of the Wechsler Adult Intelligence Score.
- Blood lead measurements
  - Blood samples: Graphite furnace atomic absorption spectrometry (Perkin-Elmer 3000, Chelmsford, MA)
  - Bone lead: K-x-ray fluorescence (KXRF) at mid-tibia (cortical) and patella (trabecular) within 1month of delivery
- Genotyping Methods:
  - Umbilical cord blood: Puregene DNA isolation kits (Gentra Systems, Inc., Minneapolis, MN)
  - Multiplex PCR assays

Schematic of Placental Transfer of Iron from Mother to Fetus



Source:Georgieff, 2000

Schematic of Blood Brain Barrier Iron Transport



Source: Beard J. 2003

Table 1: Infant Genotype Frequency*

	Infant Tf wildtype	Infant Tf Variant	Total (%)
Infant HFE wildtype	200 (66.5)	50 (16.6)	250 (83.1)
Infant HFE variant	42 (13.9)	9 (3.0)	51 (16.9)
Total (%)	242 (80.4)	59 (19.6)	301 (100.0)

*HFE variant (C282Y and/or H63D carrier status)

Table 2: Demographic Characteristics of Population by HFE and Tf Genotype

	HFE Wildtype/ Tf Wildtype (N=200)	HFE Variant/ Tf Wildtype (N=42)	HFE Wildtype/ TfC2 Variant (N=50)	HFE Variant/ TfC2 Variant (N=9)
Umbilical Cord Blood Lead (ug/dl)	6.82 (3.69) (N=173)	6.78 (3.67) (N=44)	6.51 (2.72) (N=37)	5.57 (2.33) (N=8)
Maternal Blood Lead (ug/dl)	8.69 (4.25)	8.68 (4.18)	8.33 (3.77)	7.56 (3.87)
Infant Blood Lead (12 months)	6.60 (2.62) (N=91)	6.88 (3.10)* (N=17)	9.20 (5.69) (N=23)	7.96 (4.25) (N=5)
Infant Blood Lead (24 months)	8.02 (4.58) (N=172)	7.76 (2.78) (N=37)	7.89 (3.62) (N=43)	12.96 (5.99)** (N=9)
Patella Bone Lead (ug/g)	15.3 (15.7) (N=184)	17.1 (14.0) (N=49)	18.2 (13.5) (N=36)	15.8 (13.6) (N=9)
Tibia Bone Lead (ug/g)	11.4 (9.78) (N=192)	9.06 (14.6) (N=48)	9.91 (9.82)** (N=42)	11.3 (5.18) (N=9)
MDI Outcome (24 months)	90.5 (14.2)	92.9 (11.9)*	94.3 (14.7)	93.6 (16.6)
Maternal IQ	86.1 (23.1)	84.5 (21.1)	82.1 (25.3)	79.7 (17.5)
Maternal Age (years)	24.8 (5.2)	24.4 (5.7)	25.4 (5.2)	23.8 (5.7)
Maternal Education (years)	9.8 (3.1)	9.1 (3.1)**	8.9 (2.8)	9.0 (1.7)
Gestational Age (wks, est.)	39.3 (1.46)	39.0 (1.58)	39.1 (1.58)	39.4 (0.73)
Infant birth weight	3169 (427)	3089 (448)	3118 (374)	3406 (365)*
Infant Gender (Male %)	56.0%	69.1%	38%	67%

Values are means (std. dev.) or percentages

* p-value<0.1, ** p-value<0.05 compared to HFE wildtype/Tf wildtype (Student's t-test)

Table 3: Linear regression modeling HFE & Tf genotype and 24 month MDI score adjusting for covariates

	Regression Coefficient	Standard Error	P Value
Model 1 (N=301)			
HFE Variant			
(0=wildtype: N=250, 1=C282Y and/or H63D Carrier: N=51)	2.91	2.09	0.17
Model 2 (N=301)			
Tf Variant			
(0=wildtype, N=242, 1=C2 Carrier, N=59)	3.63	1.93	0.06
Model 3 (N=301)			
HFE/Tf Variant			
(0=wildtype: N=200, 1=presence one genotype variant: N=101)	**3.94**	**1.65**	**0.02**
Model 4 (N=266)			
HFE/Tf Variant (0=wildtype, 1=presence of one variant genotype)	**3.92**	**1.72**	**0.02**
Umbilical Cord Blood Lead (μg/dL)	**-4.28**	**1.67**	**0.01**
Model 5 (N=282)			
HFE/Tf Variant (0=wildtype, 1=presence of one variant genotype)	**3.96**	**1.71**	**0.02**
Patella Bone Lead (μg/g)	-0.06	0.06	0.31
Second Quartile (4.93-15.29)	-2.26	2.29	0.32
Third Quartile (15.29-25.83)	-1.30	2.33	0.58
Fourth Quartile (>25.83)	-2.53	2.38	0.29
Model 6 (N=291)			
HFE/Tf Variant (0=wildtype, 1=presence of one variant genotype)	**4.09**	**1.64**	**0.01**
Tibia Bone Lead (μg/g)	-0.11	0.08	0.16
Second Quartile (5.085-10.34)	0.81	2.21	0.71
Third Quartile (10.34-16.635)	-2.17	2.22	0.33
Fourth Quartile (>16.635)	-1.89	2.30	0.41

*All models are adjusted for maternal IQ, infant gender, birth weight, and gestational age.

Table 4: Interaction models of HFE & Tf genotype and lead biomarker upon 24 month MDI score after adjusting for covariates*

	Regression Coefficient	Standard Error	P Value
Model 1			
HFE/Tf Variant	**3.86**	**1.72**	**0.02**
Umbilical Cord Blood Lead (μg/dL)	**-5.43**	**2.01**	**0.007**
HFE/Tf Variant x Cord Blood Lead	3.74	3.61	0.30
Model 2			
HFE/Tf Variant	0.64	2.11	0.76
Umbilical Cord Blood Lead (μg/dL)	**-4.10**	**1.67**	**0.01**
Umbilical Cord Blood Lead^2 (μg/dL)	-3.73	2.78	0.18
HFE/Tf Variant x (Cord Blood Lead)^2	**15.04**	**5.72**	**0.009**
Model 3			
HFE/Tf Variant	3.58	2.74	0.19
Patella Bone Lead (μg/g)	-0.06	0.07	0.34
HFE/Tf Variant x Patella Bone Lead	0.02	0.12	0.86
Model 4			
HFE/Tf Variant	**5.28**	**2.26**	**0.02**
Tibia Bone Lead (μg/g)	-0.06	0.10	0.57
HFE/Tf Variant x Tibia Bone Lead	-0.12	0.15	0.45

* All models are adjusted for maternal IQ, maternal age, infant gender, birth weight, and gestational age.

Figure 1: Lowess-smoothed plot of of MDI score versus umbilical cord lead

Conclusions:

- Presence of at least one HFE and/or Tf variant genotype significantly predicted higher MDI scores. After adjustment for covariates, HFE and/or Tf carrier status was associated with a 3.94 (95% CI 0.70 to 7.18, p=0.02) higher 24-month MDI score.
- In interaction models the negative effects of umbilical cord blood lead was attenuated by presence of at least one HFE and/or Tf variant genotype.
- Lowess-smoothed plots of 24-month MDI scores versus umbilical cord blood lead indicate a non-linear relationship in carriers of an HFE/Tf variant genotype. These results suggest that at higher levels of lead exposure these infants are protected from lead's negative effects upon neural development.
- Effect modification by HFE/Tf variant genotypes was seen in both blood and bone lead biomarkers, but failed to reach statistical significance in bone lead biomarkers.
- In the view of past studies which demonstrated that Pb and Fe compete for a common absorption transporter (DMT-1) and that Fe preferentially binds to DMT-1 it is plausible that increased Fe absorption in HFE variant genotypes create a protective environment against Pb-induced neuronal damage. (Bressler, 2004)
- This exploratory study provides preliminary evidence that while HFE variant genotypes may accelerate neurodegeneration in older adults it can also modify the fetal environment leading to enhanced neurodevelopment in the face of environmental toxins.

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