Increased Influence of Genetic Variation on PON1 Activity in Neonates

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PON1 (paraoxonase-1) detoxifies organophosphates by cleavage of active oxons before they have a chance to inhibit cholinesterases. The corresponding gene PON1 has common polymorphisms in both the promoter (−909, −162, −108) and the coding region (L55M, Q192R). The five PON1 genotypes were determined for maternal blood (n = 402) and cord blood (n = 229) as part of a study of the effects of organophosphate pesticide exposure on infant growth and neurodevelopment. PON1 enzymatic activities were determined for a majority of subjects. The population contained Caucasians, Caribbean Hispanics, and African Americans. PON1 activity was strongly dependent upon the promoter alleles in both maternal and cord blood. For example, PON1 activities for position −108CG, CT, and TT mothers were 146, 128, and 109 arylesterase U/mL (analysis of variance, p < 0.0001), whereas the same PON1 activities for the respective cord bloods were 49.0, 32.4, and 23.2 U/mL (p < 0.0001). Compared with adults, neonates had lower PON1 activity, implying reduced capacity to detoxify organophosphates. In addition there was a larger difference in activity between genotype groups in neonates than in adults. Because the five polymorphisms in PON1 occur in a short stretch of DNA, they were tested for linkage disequilibrium (LD). Significant LD was found among all three promoter polymorphisms as well as between promoter polymorphisms and L55M, with the strongest LD for Caucasians and the weakest for African Americans. The Caribbean Hispanics fall between these two groups. Surprisingly, significant LD also was observed between the promoter polymorphisms and C311S in PON2. LD between the promoter polymorphisms and Q192R was not significant.

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Chlorpyrifos, an insecticide implicated as a developmental neurotoxin in animals (Shih et al. 1998), has been used routinely in the homes of urban populations in the United States. The biochemical contributions of metabolizing enzymes to the neurotoxic potential of chlorpyrifos and other organophosphates remain subjects of active investigation (Sams et al. 2000; Costa et al. 1999; Furlong et al. 2000). Specifically, after activation of chlorpyrifos to the reactive oxon, the oxon may be detoxified by PON1 (paraoxonase-1) before it has the chance to inactivate acetylcholinesterase in the peripheral nervous system (PNS) and central nervous system (CNS). PON1, which is associated with high-density lipoprotein (HDL) and central nervous system (CNS). PON1 also has an arylesterase activity (Gan et al. 1991). HDL levels and PON1 activities are somewhat elevated during pregnancy (Roy et al. 1994). As part of the continuing effort of the Mount Sinai Children’s Environmental Health Center to prospectively assess the neurodevelopmental risks associated with chlorpyrifos exposure in an inner-city population in New York City, we investigated the genetic determinants of chlorpyrifos metabolism in a population of ethnically diverse mothers and their neonates. In this study, we examined the hypothesis that genetic factors may be more crucial in determining risk in neonates than in adults of various ethnicities.

The PON1 gene has been fully characterized (Clendenning et al. 1996) and has been found to be a member of a multigene family of three linked genes, PON1 (MIM 168820), PON3 (MIM 602447), and PON2 (MIM 602447) (OMIM 2002), in that order (Primo-Parmo et al. 1996; Entrez Chromosome Map 2002). Human PON1 had long been recognized to be polymorphic based both on large differences in serum PON1 activity (Geldmacher-von Mallinckrodt et al. 1983) and on the phosphodiesterase/arylesterase activity ratio for different substrates, including organophosphate oxons derived from pesticides and direct-acting cholinesterase inhibitors such as the nerve gas sarin (Davies et al. 1996). The purified isozymes retained these differences in organophosphate hydrolysis activity normalized to arylesterase activity (Smolen et al. 1991). PON1 contains two common polymorphisms in the coding region, Q192R (Adkins et al. 1993; Humbert et al. 1993) and L55M (Garin et al. 1997), and three common polymorphisms in the promoter at −909, −162 and −108 (Levie and James 2000; Brophy et al. 2001a, 2001b). Promoter variants in PON1 affect the level of expression by more than 2-fold (Boright et al. 1998; Brophy et al. 2001a, 2001b). Q192R affects the relative rate of hydrolysis of certain organophosphate substrates compared with phenyl acetate by as much as an order of magnitude but has only a small effect on chlorpyrifos oxon/phenylacetate rate of hydrolysis (Davies et al. 1996). The L55M polymorphism has been found to affect lipid peroxidation (Malin et al. 2001) and PON1 protein stability (Leviev et al. 2001), although the recent work on promoter mutations has suggested that the apparent effect of the L55M polymorphism on enzyme concentration also may be due to linkage disequilibrium (LD) with one of the promoter variants (Brophy et al. 2001b).

Toxicity of chlorpyrifos is inversely related to serum PON1 activity. Intravenous injection of PON1 before challenge by chlorpyrifos is protective in the rat (Costa et al. 1990). PON1 knockout mice were more susceptible to chlorpyrifos toxicity (Shih et al. 1998), and the toxicity was alleviated by intraperitoneal injection of purified PON1, with the 192RR (homozygous) enzyme providing better protection than 192QQ (Li et al. 2000). Using the rat model, Attenberry et al. (1997) have demonstrated that the age-related decrease in susceptibility to chlorpyrifos CNS toxicity is not due to either the rate of activation to the oxon or to the reactivity of the oxon in the CNS but is entirely due to age-related differences in the detoxification of the oxon. Developing human fetuses have much lower protective PON1 activities than do adults, with the level in cord blood being severalfold lower (Mueller et al. 1983). Furthermore, both chlorpyrifos and chlorpyrifos oxon can cross the placenta. Subcutaneous delivery of a high dose of chlorpyrifos to pregnant rats...
led to significant (30–49%) inhibition of fetal acetylcholinesterase activity (Chanda and Pope 1996). Thus, both fetuses and very young children may be more susceptible to pesticide exposure compared with adults. Lower PON1 activities may reflect increased risk after acute or chronic exposure to chlorpyrifos in utero and in the neonatal period.

Using maternal and cord blood, we have carried out a systematic study of the origin and extent of variation in PON1 activities in our population of three racial/ethnic groups comprising the majority of many inner-city populations: Caucasians, African Americans, and Caribbean Hispanics. Specifically, we address genotype–phenotype relationships for all five polymorphisms and note differences among these three racial/ethnic groups, highlighting the differences between mothers and neonates. We also address LD among the five polymorphisms, noting differences among three racial/ethnic groups that will affect inference of haplotypes.

### Subjects and Methods

#### Study Population

This study is part of an ongoing prospective study of the effect of pesticide exposure on infant growth and neurodevelopment conducted at the Mount Sinai School of Medicine. The study protocol was approved by the institutional review board. A total of 479 pregnant women scheduled to deliver at Mount Sinai Hospital and their neonates were enrolled in the study. Seventy-seven of these women have been excluded because of medical complications, terminations of pregnancy, very premature births, failure to collect specimens from the women before they gave birth, delivery of an infant with birth defects, change of residence, or refusal to continue to participate. A total of 402 maternal blood samples were obtained from individuals self-identified as being Caucasian (n = 82), African American (n = 117), or Hispanic (n = 203), predominantly of Caribbean origin. Maternal blood was collected in heparin-treated Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) at about 26–30 weeks of gestation. At birth, a sample of umbilical cord blood was obtained for 229 infants (Caucasian n = 56, African American n = 66, Caribbean Hispanic n = 107). Cord blood was collected with the same anticoagulant. Although EDTA is a better anticoagulant for long-term DNA storage and polymerase chain reaction (PCR), it cannot be used because it inhibits and destabilizes PON1. Plasma was separated immediately (within 24 hr) by two cycles of centrifugation. Three aliquots of maternal plasma and three aliquots of cord blood plasma were frozen at −70°C, one for determination of PON1. The Buffy coat was separated from the red blood cells, and DNA was extracted and purified using a QIAamp blood kit (Qiagen, Valencia, CA) as described by the manufacturer.

#### PON1 Assay

The hydrolysis of phenylacetate to acetic acid and phenol, measured by absorbance maximum A270, was used to determine human plasma PON1 activity using the standard definition of units (Furlong et al. 1989). The molar extinction coefficient of phenol is 1,310. On the order of 5% of the PON1 activity in plasma may be due to serum cholinesterase and serum albumin (Gan et al. 1991). This contribution was minimized by performing the assays at high pH. A cholinesterase inhibitor, in this case eserine sulfate, is also necessary for specificity. The assay was first performed as described in a Hewlett-Packard diode array spectrophotometer (Agilent, Palo Alto, CA) equipped with a cell holder equipped with a thermostat. The assay was then adapted to 96-well format for high throughput and replication. A buffer solution (1.2x) was prepared as 12 mM Tris–HCl (pH 8.0), 1.2 mM CaCl2, 6 µM EDTA, and 4.8 µM eserine sulfate. Individual plasma samples were diluted 333-fold by addition of 3 µL plasma per milliliter phosphate-buffered saline. Phenylacetate was dissolved in water at 36 mM (10x) immediately before use and mixed with nine parts buffer. Two hundred microliters of this mixture were added to each well. Twenty-microliter aliquots of diluted plasma were placed in individual wells. The plates were incubated at 37°C in a Bio-Tek PowerWaveX microplate reader (Bio-Tek, Winooski, VT). The increase in A270 was followed over time. The slope of the kinetic curve determined by the instrument software was used to calculate the rate of hydrolysis of phenylacetate. Blanks included both samples lacking plasma and samples with plasma but lacking substrate. The measurements of individual samples were reproducible within 10%, mostly limited by the serum dilution step.

#### Statistical Analysis

The genetic influence of PON1 polymorphisms on PON1 activity was analyzed by analysis of variance. Because of limited serum availability for enzymatic activity determinations, a total of 390 maternal and 189 neonate data sets were analyzed. Log transformation of the enzyme activity was performed to improve normality. Linear regression was used to assess age-adjusted trends in genotype effects on PON1 phenotypes. These relationships were examined separately in mothers and neonates as well as by race/ethnicity. LD was calculated as D’ based on Lewontin, which ranges from 0 (no LD) to 1 or −1 (complete LD) (Lewontin 1988). The EH (estimating...
haplotype-frequency) linkage utility program (Terwilliger and Ott 1994) was used to determine chi-square statistics and p-values for tests of allelic association between polymorphic markers. To assess independent and group effects of polymorphisms on PON1 activity, linear and multivariable regression models were used to determine the independent and group contributions of the coding and promoter polymorphisms to phenotypic expression based on Brophy et al. (2001b). All p-values are two-sided. Statistical tests were performed using SAS (SAS Institute 2001).

### Results

#### PON1 Allele Frequencies

Single nucleotide polymorphism (SNP) frequencies were obtained for the five common PON1 polymorphisms and for one common PON2 polymorphism (Table 1). The promoter alleles are named in the sense direction. Although the allele frequencies varied substantially among race/ethnicity groups, each of the six polymorphisms has a minor allele frequency of at least 11% in all of the ethnic groups.

#### PON1 Genotypes and PON1 Activity

The maternal geometric mean PON1 activity was independent of race/ethnicity, with mean values of 123, 131, and 130 U/mL for African Americans, Caucasians, and Caribbean Hispanics, respectively (p = 0.08). Effects of maternal genotypes and race/ethnicity on maternal PON1 activity are summarized in Table 2. Overall, a statistically significant (p ≤ 0.02) difference in PON1 activity was found among genotypes of each of the three promoter polymorphisms as well as L55M. The Q192R polymorphism did not influence PON1 activity with phenylacetate. When examined by race/ethnicity independently, L55M was not a significant predictor (p = 0.14) in Caucasian mothers, whereas the promoter polymorphisms were not significant predictors in African-American mothers (p = 0.16). All four polymorphisms remained significant predictors of PON1 activity in Caribbean-Hispanic mothers (p ≤ 0.05).

For neonates, the geometric mean PON1 activity was dependent on race/ethnicity: 44.4 (referent), 33.3 (p < 0.002), and 25.4 (p < 0.0001) U/mL for African Americans, Caribbean Hispanics, and Caucasians, respectively. Effects of neonate genotypes and race/ethnicity on neonate PON1 activity are summarized in Table 3. Similar to the mothers, the three promoter polymorphisms as well as L55M (but not Q192R) were significantly associated with PON1 activity (p < 0.0001). Unlike the mothers, these associations were

### Table 1. Allele frequencies of PON1 genotypes.

| Gene, position and allele | Caucasian (n = 82) | African American (n = 117) | Hispanic (n = 203) | Caucasian (n = 56) | African American (n = 66) | Hispanic (n = 107) |
|--------------------------|-------------------|--------------------------|-------------------|-------------------|--------------------------|-------------------|
| **PON1**                 |                   |                          |                   |                   |                          |                   |
| −909                     |                   |                          |                   |                   |                          |                   |
| G                        | 0.38              | 0.84                     | 0.61              | 0.42              | 0.88                     | 0.65              |
| C                        | 0.62              | 0.16                     | 0.39              | 0.58              | 0.12                     | 0.35              |
| −162                     |                   |                          |                   |                   |                          |                   |
| A                        | 0.20              | 0.42                     | 0.26              | 0.18              | 0.42                     | 0.25              |
| G                        | 0.80              | 0.58                     | 0.74              | 0.82              | 0.58                     | 0.75              |
| −108                     |                   |                          |                   |                   |                          |                   |
| C                        | 0.38              | 0.85                     | 0.65              | 0.44              | 0.89                     | 0.67              |
| T                        | 0.62              | 0.15                     | 0.35              | 0.56              | 0.11                     | 0.33              |
| **PON2**                 |                   |                          |                   |                   |                          |                   |
| 55                       |                   |                          |                   |                   |                          |                   |
| L                        | 0.54              | 0.79                     | 0.71              | 0.58              | 0.81                     | 0.78              |
| M                        | 0.46              | 0.21                     | 0.29              | 0.42              | 0.19                     | 0.22              |
| 192                      |                   |                          |                   |                   |                          |                   |
| Q                        | 0.73              | 0.37                     | 0.54              | 0.76              | 0.30                     | 0.50              |
| R                        | 0.27              | 0.63                     | 0.46              | 0.24              | 0.70                     | 0.50              |
| **PON2 311**             |                   |                          |                   |                   |                          |                   |
| S                        | 0.80              | 0.79                     | 0.77              | 0.75              | 0.73                     | 0.77              |
| C                        | 0.20              | 0.21                     | 0.23              | 0.25              | 0.27                     | 0.23              |

### Table 2. Maternal PON1 polymorphism frequencies, enzyme activities, and p-values for trend.

| Position and genotype | Caucasian (n = 78) | African American (n = 112) | Hispanic (n = 200) | Combined (n = 390) |
|-----------------------|-------------------|--------------------------|-------------------|-------------------|
|                       | Mean PON1 enzyme activity (SD) | p-Value (trend) | Mean PON1 enzyme activity (SD) | p-Value (trend) | Mean PON1 enzyme activity (SD) | p-Value (trend) | Mean PON1 enzyme activity (SD) | p-Value (trend) |
| −909                  |                   |                          |                   |                   |                          |                   |                   |                          |                   |
| GG                    | 15 (12)           | 160.5 (16.3)             | p = 0.0009        | 72 (80)           | 129.0 (28.5)             | p = 0.28          | 38 (76)           | 148.4 (34.6)             | p = 0.0001        |
| CG                    | 45 (35)           | 135.0 (33.2)             |                   | 24 (27)           | 123.0 (29.0)             |                   | 47 (93)           | 134.1 (32.6)             |                   |
| CC                    | 40 (31)           | 122.5 (29.1)             |                   | 4 (4)             | 120.8 (40.9)             |                   | 16 (31)           | 109.7 (33.8)             |                   |
| −162                  |                   |                          |                   |                   |                          |                   |                   |                          |                   |
| AA                    | 8 (6)             | 159.5 (17.2)             |                   | 21 (23)           | 128.1 (31.4)             |                   | 7 (13)            | 146.6 (42.6)             |                   |
| AG                    | 24 (19)           | 139.5 (26.6)             |                   | 43 (48)           | 130.1 (27.2)             |                   | 39 (76)           | 139.9 (33.1)             |                   |
| GG                    | 48 (53)           | 129.0 (33.5)             |                   | 36 (40)           | 122.5 (29.8)             |                   | 55 (103)          | 131.4 (36.6)             |                   |
| −108                  |                   |                          |                   |                   |                          |                   |                   |                          |                   |
| CC                    | 14 (11)           | 161.6 (16.6)             |                   | 72 (81)           | 129.1 (28.3)             |                   | 44 (88)           | 150.9 (34.6)             |                   |
| CT                    | 46 (36)           | 134.0 (27.8)             |                   | 25 (28)           | 122.4 (28.8)             |                   | 42 (84)           | 129.9 (28.7)             |                   |
| TT                    | 40 (31)           | 124.0 (35.1)             |                   | 3 (3)             | 113.9 (46.9)             |                   | 14 (28)           | 105.7 (35.8)             |                   |
| 55                    |                   |                          |                   |                   |                          |                   |                   |                          |                   |
| LL                    | 33 (26)           | 139.8 (28.6)             |                   | 58 (65)           | 134.0 (27.5)             |                   | 51 (101)          | 149.3 (31.7)             |                   |
| LM                    | 42 (33)           | 133.3 (45.5)             |                   | 39 (44)           | 118.8 (27.7)             |                   | 43 (85)           | 123.9 (35.2)             |                   |
| MM                    | 24 (19)           | 126.9 (34.3)             |                   | 3 (3)             | 96.5 (32.3)              |                   | 7 (14)            | 110.1 (29.8)             |                   |
| QQ                    | 55 (43)           | 138.1 (33.4)             |                   | 14 (16)           | 116.8 (32.5)             |                   | 29 (57)           | 134.1 (42.1)             |                   |
| QR                    | 35 (27)           | 130.6 (31.0)             |                   | 44 (49)           | 133.2 (31.2)             |                   | 51 (101)          | 137.0 (33.9)             |                   |
| RR                    | 10 (8)            | 122.7 (24.7)             |                   | 42 (47)           | 124.1 (23.7)             |                   | 21 (42)           | 134.8 (31.7)             |                   |

*p-Value for trend on log-transformed values. Least-squares means adjusted for mother’s race/ethnicity.
apparent in all three race/ethnicity groups ($p = 0.02$).

Figure 1 compares the genetic influences of the three promoter and L55M polymorphisms on PON1 activity in mothers and their neonates (all race/ethnicities combined). There was a striking difference in the PON1 activities, with the neonates having a geometric mean 4.1-fold lower. In addition, the genetic influence in neonates appears to be stronger, with no polymorphisms affecting maternal PON1 activity by more than 35% but with both −108 (and −909) and L55M polymorphisms affecting PON1 activity by more than 2-fold in neonates.

**PON Linkage Disequilibrium: Important Alleles Affecting PON1 Activity**

Pairwise LD calculations for the five polymorphisms and three race/ethnicities, reported as $D'$ and chi-square with associated $p$-values, are presented in Table 4. In all three race/ethnicities, the promoter polymorphisms are in nearly complete LD with one another ($D' \geq 0.9, p < 0.0001$). The promoter polymorphisms are also in significant LD with L55M, but more so for Caucasians than for African Americans. L55M and Q192R are in LD in Caucasians but not in African Americans. On the other hand, the promoter polymorphisms −108C→T and −909G→C are in significant LD with Q192R in African-Americans but not in Caucasians. Interestingly, strong LD is observed between PON2 C311S and the PON1 promoter polymorphisms in all race/ethnicities, with the strongest linkage in Caucasians. In all of these cases, values for Caribbean Hispanics fall between those for Caucasians and African Americans.

Analysis of conditional variance (adjusted for the other polymorphisms based on Brophy et al. 2001b) showed that maternal −108C→T and L55M polymorphisms contributed 12 and 4%, 1 and 9%, and 17 and 12% of the total variance, respectively, for Caucasians, African Americans, and Caribbean Hispanics. A similar analysis of conditional variance for neonates showed that the −108C→T and L55M polymorphisms contributed 53 and 44%, 8 and 3%, and 19 and 10% of the total variance, respectively, for Caucasians, African Americans, and Caribbean Hispanics.

Table 5 shows the maternal PON1 activities for the nine possible −108C→T and L55M genotypes. Using −108CC, 55LL as referent, all three L55M genotypes of −108 TT were significantly different, with decreasing PON1 activity trend from 55LL (126.6 U/mL) to 55LM (116.1 U/mL) to 55MM (105.1 U/mL). With the same referent, only the −108 TT genotype of 55MM was significantly different, although the sample sizes were low (7 and 9) for the other −108 genotypes.

**Discussion**

**PON1 Allele Frequencies**

In this study we have investigated the effect of genotype, as reflected by all of these five common PON1 polymorphisms, on phenotype, as reflected by PON1 activity. The subjects were pregnant women and their neonates at risk of prenatal exposure to chlorpyrifos in the home. The subjects included Caucasians, African Americans, and Caribbean Hispanics. The observed frequencies (Table 1) for the 192R allele among Caucasians and African Americans were consistent with previous studies (e.g., 0.28 for Caucasians compared with 0.27–0.31 in several previous studies (Davies et al. 1996; Leviev and James 2000; Brophy et al. 2001b)). The allele frequencies for all five PON1 SNPs and for PON2 S311C among Caribbean Hispanics in New York City fell between the values for Caucasians and African Americans, with allele frequencies consistent with similar Caucasian and African-American derivation. This study was the first to include all of these polymorphisms and these race/ethnicities in a study of PON1 activity in mothers and their neonates.

**PON1 Genotypes and PON1 Activity: Increased Genotypic Influence in Neonates**

It has been known for many years that neonates have much lower PON1 activities than adults (Mueller et al. 1983). We have confirmed this observation for each of the race/ethnicities (Tables 2 and 3, Figure 1). Specifically, the mean PON1 activity in maternal blood was 4.6, 3.6, and 2.6 times greater than the mean PON1 activity in cord blood for Caucasians, Caribbean Hispanics, and African Americans, respectively. Again, the data for Caribbean Hispanics fall between Caucasians and African Americans. For all groups, however, the results suggest a potentially greater

| Position and genotypes | Caucasian ($n = 47$) | African American ($n = 51$) | Hispanic ($n = 91$) | Combined ($n = 198$) |
|------------------------|----------------------|-----------------------------|---------------------|----------------------|
|                       | Mean PON1 activity (SD) | $p$-Value (trend)* | Mean PON1 activity (SD) | $p$-Value (trend)* | Mean PON1 activity (SD) | $p$-Value (trend)* | Mean PON1 activity (SD) | $p$-Value (trend)* |
| −909                   |                       |                         |                     |                       |                         |                     |                       |                     |
| GG                     | 15 (7)                | 41.8 (12.4)             | $p < 0.0001$        | 40 (36)                | 42.8 (14.9)             | $p = 0.006$          | 43 (81)                | 47.1 (2.2)          |
| CG                     | 53 (25)               | 32.2 (18.2)             | $p < 0.0001$        | 51 (46)                | 37.6 (23.9)             | $p = 0.006$          | 44 (84)                | 35.8 (2.1)          |
| CC                     | 32 (15)               | 18.5 (7.7)              | $p < 0.0001$        | 10 (9)                 | 37.6 (23.9)             | $p = 0.006$          | 13 (24)                | 35.8 (2.1)          |
| −108                   |                       |                         |                     |                       |                         |                     |                       |                     |
| AA                     | 2 (1)                 | 63.1                    | $p = 0.0004$        | 7 (7)                  | 45.7                    | $p = 0.02$           | 8 (17)                 | 49.7               |
| AG                     | 32 (15)               | 38.5 (20.5)             | $p = 0.0004$        | 41 (37)                | 41.3 (15.9)             | $p = 0.02$           | 40 (76)                | 44.3 (2.3)          |
| GG                     | 66 (31)               | 23.7 (10.7)             | $p = 0.0004$        | 52 (47)                | 34.9 (24.1)             | $p = 0.0004$         | 50 (95)                | 32.3 (2.1)          |
| −55                    |                       |                         |                     |                       |                         |                     |                       |                     |
| CC                     | 17 (8)                | 50.5 (23.1)             | $p < 0.0001$        | 48 (43)                | 46.3 (23.5)             | $p = 0.0004$         | 48 (90)                | 49.0 (2.0)          |
| CT                     | 51 (24)               | 28.8 (11.0)             | $p < 0.0001$        | 41 (37)                | 33.2 (13.9)             | $p = 0.0004$         | 39 (73)                | 32.4 (2.2)          |
| TT                     | 32 (15)               | 18.5 (7.7)              | $p < 0.0001$        | 11 (10)                | 37.6 (23.9)             | $p < 0.0001$         | 13 (25)                | 35.8 (2.1)          |
| −311                   |                       |                         |                     |                       |                         |                     |                       |                     |
| LL                     | 32 (15)               | 41.0 (21.1)             | $p < 0.0001$        | 71 (36)                | 52.0 (22.5)             | $p = 0.0004$         | 56 (106)               | 45.6 (1.9)          |
| LM                     | 51 (24)               | 27.5 (9.1)              | $p < 0.0001$        | 25 (13)                | 40.7 (21.7)             | $p = 0.0004$         | 35 (69)                | 32.6 (2.4)          |
| MM                     | 17 (8)                | 12.5 (6.5)              | $p < 0.0001$        | 4 (2)                  | 29.6 (15.3)             | $p < 0.0001$         | 7 (14)                 | 17.2 (5.3)          |
| −192                   |                       |                         |                     |                       |                         |                     |                       |                     |
| QQ                     | 64 (30)               | 28.4 (14.9)             | $p = 0.50$          | 8 (4)                  | 47.5 (29.8)             | $p = 0.59$           | 30 (57)                | 39.2 (2.9)          |
| QR                     | 30 (14)               | 30.9 (21.7)             | $p = 0.50$          | 43 (22)                | 48.5 (27.6)             | $p = 0.50$           | 44 (84)                | 39.4 (2.3)          |
| RR                     | 6 (3)                 | 30.0 (8.2)              | $p = 0.50$          | 49 (25)                | 48.2 (17.0)             | $p = 0.50$           | 25 (48)                | 36.6 (3.1)          |

*p*-Value for trend on log-transformed values. *Least-squares means adjusted for neonate’s race/ethnicity.
degree of susceptibility of a fetus or newborn to the toxic effects of organophosphates.

Effects of functional polymorphisms in the coding region of a gene (e.g., L55M and Q192R) normally remain constant throughout the life of an individual. Polymorphisms in the promoter region of a gene (e.g., −909, −168, −108) can affect the level of transcription. We hypothesized that transcription factor interactions with the promoter may vary throughout life. PON1 activity differed among genotypes for promoter polymorphisms, as exemplified by −108 C→T, and the functional polymorphism L55M. A significant effect of Q192R on PON1 activity was found in one large recent study (Brophy et al. 2001b). Our results for all race/ethnicities individually as well as combined agree with a second recent study showing no significant effect (Leviev and James 2000), but this lack of effect may be due to smaller sample size. In our combined maternal population, the −108 CC and −108 TT homozygotes as well as the 55LL and 55MM homozygotes had approximately 15% greater and lesser activities than the corresponding heterozygotes, respectively. The effect of the promoter polymorphisms in African-American mothers did not reach the level of significance, probably because of sample size considering the lower minor allele frequency. Another study found 20 and 12% for the −108 and L55M polymorphisms, respectively (Leviev and James 2000), and a third study found about 27 and 20%, respectively (Brophy et al. 2001b). All of these results are similar and are strikingly different from our results with cord blood.

In particular, in our combined neonate population, the −108 CC and −108 TT homozygotes as well as the 55LL and 55MM homozygotes had approximately 51 and 40% greater and 28 and 47% lesser activities than the corresponding heterozygotes, respectively. Thus, not only is the average potential risk to a fetus or newborn from organophosphate exposure greater than in the adult, the range of protection afforded by PON1 is more dependent on genotype than in the adult, with the −108 TT and the 55MM homozygotes having PON1 activities 5.8- and 7.8-fold lower than the average adult, respectively. **PON1 Linkage Disequilibrium: Important Alleles Affecting PON1 Activity**

As previously observed (Leviev and James 2000; Brophy et al. 2001b), the promoter polymorphisms were in significant LD (Table 4). Examination of maternal PON1 activity and genotypes confirmed that nearly all of the contribution of these polymorphisms to PON1 activity could be determined by analyzing the −108 polymorphism.

The LD extends to L55M and −108 C→T, but is less than among the promoter polymorphisms, with D’ varying from 0.42 to 0.51 depending on race/ethnicity. After correction for LD, Leviev and James (2000) and Brophy et al. (2001b) found that the −108 CT polymorphism accounted for 24.7 and 22.8%, respectively, of the variance in PON1 for Caucasians. They also found that L55M accounted for 4.4–4.6 or 4.1% of the

![Figure 1](image)

**Figure 1.** PON1 activity by genotype, all race/ethnicities combined, for mothers and neonates. Normal refers to the allele with the highest PON1 activity. Genotypes: (A) −909G→C; (B) −162A→G; (C) −108C→T; (D) L55M. (A, C, D) Maternal and neonate p-value for trend < 0.0001. (B) Maternal p-value for trend = 0.02; neonate p-value for trend < 0.0001.

| Position | PON1−909 | −162 | 55 | PON1−192 | PON1−311 |
|----------|----------|------|----|----------|----------|
| Caucasian |          |      |    |          |          |
| −108     | 0.92     | 1.00 | 0.16| 0.40     | −0.82    |
| −909     | 1.00     | 0.50 | 0.02| 0.01     | 0.08     |
| −162     | 1.00     | 0.60 | 0.07| 5.76     | 0.37     |
| 55       |          | 30.98| 0.93| 33.85    | 0.43     |
| 192      |          | 0.00 | 0.02| 0.00     | 0.02     |
| African American |          |      |    |          |          |
| −108   | 0.97     | 0.90 | 0.43| 7.49     | 0.99     |
| −909   | 1.00     | 0.42 | 0.34| 4.98     | 0.96     |
| −162   | 0.44     | 4.71 | 0.11| 1.72     | 0.03     |
| 55     | 0.16     | 1.72 | 0.07| 1.72     | 0.14     |
| 192    | 1.00     | 0.11 | 0.11| 1.72     | 0.14     |
| Hispanic |          |      |    |          |          |
| −108   | 0.90     | 1.00 | 0.29| 8.76     | 0.87     |
| −909   | 1.00     | 0.51 | 0.22| 5.02     | 0.95     |
| −162   | 0.72     | 15.24| 0.05| 15.24    | 0.24     |
| 55     | 0.50     | 15.24| 0.05| 15.24    | 0.24     |
| 192    | 1.00     | 0.15 | 0.15| 15.24    | 0.24     |

NS, not significant.
variance, respectively. Our data for
Caucasian mothers were qualitatively con-
sistent, with conditional variances attribu-
table to –108C→T being greater than that
attributable to L55M, at 12 and 4%, respec-
tively, after adjustment for the other
polymorphisms. In Table 5, PON1 activity
for all three L55M genotypes combined
with –108T were significantly different
from the referent (55LL, –108C, p <
0.02) and decreased from 126.6 (55LL) to
116.1 (55ML) to 105.1 (55MM) U/mL.
Although the number of neonates was
smaller than the number of mothers, the
conditional variance attributable to genes
was greater, and both polymorphisms again
contributed significantly. Taken together,
these data suggest that analysis of both
–108C→T and L55M PON1 genotypes is
required to infer phenotype from genotype.
LD for Q192R depended strongly on
race/ethnicity, with D¢ → –108C→T being
–0.93 (p < 0.0001) in Caucasians and
–0.18 (not significant) in African
Americans. The reverse was seen for
L55M and Q192R, with D¢ being –0.43
(p < 0.01) in African Americans and 0.16
(not significant) in Caucasians. As expected,
D¢ values for Caribbean Hispanics fell
between those for Caucasians and African
Americans. Although Q192R had no significant
effect on PON1 activity as measured
using phenyl acetate as the substrate, this
polymorphism affects substrate specificity
and thus affects the enzymatic activity with
other substrates, such as chlorpyrifos oxon,
both in vitro and in vivo (Furlong et al.
2000). Therefore, for other substrates,
Q192R genotype must also be determined
to infer phenotype from genotype, and the
race/ethnicity dependence of LD must be
taken into account.

Linkage disequilibrium depends on
local recombination rate (Taillon-Miller
et al. 2000), population structure, and the
number of generations since the mutation
(polymorphism) arose (Slatkin 1999).
There has been a significant debate over
the size of the DNA blocks exhibiting LD
(Reich et al. 2001; Dunning et al. 2000;
Abecasis et al. 2001). In this study we also
looked at LD between the PON1 polymor-
phisms and PON2 C311S located 97.2 kb
away from Q192R and more distant from the
other PON1 polymorphisms. PON2
C311S exhibits significant LD only in
Caucasians for PON1 L55M (D¢ = –0.43,
p < 0.05) and no linkage for PON1 Q192R.
However, PON2 C311S was highly linked to
PON1 –108C→T in all three race/ethnicities.
It is of interest to note that both PON1
(Imai et al. 2000) and PON2 (Sanghera et al.
1998) polymorphisms have been linked to cardio-
vascular disease, although a mechanism for
PON2 action has not been demonstrated
(Mackness et al. 1999). The LD demon-
strated in this work may facilitate similar
epidemiologic analyses.

REFERENCES

Abecasis GR, Noguchi E, Hainzmann A, Traherne JA, Bhattacharyya S, Leaves NJ, et al. 2001. Extent
distribution of linkage disequilibrium in three
genomic regions. Am J Hum Genet 58:191–197.
Adkins S, Gan KN, Mody M, La Du BN. 1993.
Molecular basis for the polymorphic forms of
human serum paraoxonase/arylesterase: gluta-
mic acid or arginine at position 181, for the respec-
tive A or B allozymes. Am J Hum Genet 52:
598–608.
Atterbury TT, Burnett WT, Chambers JE. 1997.
Age-
related differences in parathion and chlorpyrifos
 toxicity in male rats: target and nontarget
esterase sensitivity and cytochrome P450-
mediated metabolism. Toxicol Appl Pharmacol
147:411–418.
Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, et al. 2000. Human serum paraox-
ons (PON1) Q and R selectively decrease lipid
peroxides in human coronary and carotid athero-
sclerotic lesions: PON1 esterase and paraoxonase-
d-like activity. Circulation 101:2501–2517.
Aviram M, Rosenblat M, Biegaier CL, Newton RS,
Primo-Parmo SL, La Du BN. 1998. Paraoxonase
inhibits high-density lipoprotein oxidation and
preserves its functions. A possible peroxidative
role for paraoxonase. J Clin Invest 101:1581–1590.
Boright AP, Connolly PW, Brunt JH, Scherer SW, Tsui
LC, Hegerle RA. 1998. Genetic variation in paraox-
onase-1 and paraoxonase-2 is associated with
variation in plasma lipoproteins in Alberta
Hutterites. Atherosclerosis 138:131–136.
Brophy VH, Hastings MD, Clendenning JB, Richter
RJ, Jarvik GP, Furlong CE. 2001a. Polymorphisms
in the human paraoxonase (PON1) promoter.
Pharmacogenetcs 11:77–84.
Brophy VH, Jamps RL, Clendenning JB, McKinstry
LA, Jarvik GP, Furlong CE. 2001b. Effects of 5´
regulatory-region polymorphisms on paraox-
onase-1 (PON1) expression. Am J Hum Genet
66:1428–1436.
Chanda SM, Pope CN. 1996. Neurochemical and
neurobehavioral effects of repeated gestational
exposure to chlorpyrifos in maternal and
developing rats. Pharmacol Biochem Behav
52:771–776.
Clendenning JB, Humberst R, Green ED, Wood C,
Traver D, Furlong CE. 1996. Structural organiza-
tion of the human PON1 gene. Genomics
35:586–589.
Costa LG, Li WF, Richter RJ, Shih DM, Lusis A,
Furlong CE. 1999. The role of paraoxonase (PON1)
in the detoxication of organophosphates and its
human polymorphism. Chem-Biol Interact
119–120:429–438.
Costa LG, McDonald BE, Murphy SD, Ommen GS,
Richter RJ, Motulsky AG, et al. 1999. Serum
paraoxonase and its influence on paroxon and
chlorpyrifos-oxon toxicity in rats. Toxicol Appl
Pharmacol 103:86–76.
Davies HG, Richter RJ, Keifer M, Broomfield CA,
Sowalja J, Furlong CE. 1996. The effect of the
human serum paraoxonase polymorphism is
reversed with diazoxan, soman and sarin. Nat
Genet 14:334–336.
Dunning AM, Durocher F, Healey CS, Teare MD,
McBride SE, Carlimagno F, et al. 2000. The
extent of linkage disequilibrium in four popula-
tions with distinct demographic histories. Am
J Hum Genet 67:1544–1554.
Entrez Chromosome Map. 2002. Available:
http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?,
extended to see PON1, PON3, PON2(accessed 5
November 2002).
Furlong CE, Li WF, Richter RJ, Shih DM, Lusis AJ,
Alleva E, et al. 2000. Genetic and temporal deter-
minants of pesticide sensitivity: role of paraox-
onase (PON1). Neurotoxolcogy 21:91–100.
Furlong CE, Richter RJ, Seidel SL, Costa LG, Motulsky
AG. 1989. Spectrophotometric assays for the
enzymatic hydrolysis of the active metabolites
of chlorpyrifos and parathion by plasma paraox-
onase-arylesterase. Anal Biochem 180:242–247.
Gan KN, Smolen A, Eckerson HW, La Du BN.
1991. Purification of human serum paraoxonase/
arylesterase. Evidence for one esterase catalyz-
ing both activities. Drug Metab Dispos 19:100–106.
Garin MC, James RW, Dussoix P, Blanche H, Passa
P, Frigouel P, et al. 1997. Paraoxonase polymor-
phism Met-Leu54 is associated with modified
receptor expression in acute lymphoblastic
leukemia. Haematologica 82:62–66.
Geldmacher-von Mallinckrodt M, Diepgen TL, Duhme
C, Hommel G. 1983. A study of the polymorphism
and ethnic distribution differences of human

Table 5. PON1 genotypes and maternal PON1 activity (–108C→T and L55M) all race/ethnicities combined
(n = 390).

| PON1: –108C→T | PON1: L55M | PON2: C311S |
|----------------|----------------|----------------|
| CC             | CC             | CC             |
| SD = 2,409     | SD = 3,086     | SD = 2,836     |
| (n = 128)      | (n = 128)      | (n = 128)      |
| CT             | CT             | CT             |
| SD = 2,439     | SD = 2,148     | SD = 2,014     |
| (n = 40)       | (n = 40)       | (n = 40)       |
| TT             | TT             | TT             |
| SD = 2,308     | SD = 3,422     | SD = 2,500     |
| (n = 16)       | (n = 26)       | (n = 20)       |
| p = 0.02       | p = 0.0001     | p < 0.0001     |

*p-Value for contrasts and estimates comparing to PON1: –108C/55LL genotype. *Schelle’s multiple comparisons
procedure: p < 0.05 compared to PON1: –108C/55LL genotype.

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Polymorphisms of paraoxonase genes and low-density lipoprotein lipid peroxidation. Lancet 352:468–469.
Malin R, Laine S, Rantalaiho V, Wirta O, Pasternack A, Jokela H, et al. 2001. Lipid peroxidation is increased in paraoxonase L55 homozygotes compared with M-allele carriers. Free Radic Res 34:477–484.
Mueller RF, Hornung S, Furlong CE, Anderson J, Giblett ER, Motulsky AG. 1983. Plasma paraoxonase polymorphism: a new enzyme assay, population, family, biochemical, and linkage studies. Am J Hum Genet 35:393–408. OMIM. 2002. Online Mendelian Inheritance in Man. Available: http://www.ncbi.nlm.nih.gov/Omim/ (for PON1 [MIM 168820], PON2 [MIM 602447], PON3 [MIM 602720]) [accessed 5 November 2002].
Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. 1996. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics 33:498–507.
Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, et al. 2001. Linkage disequilibrium in the human genome. Nature 411:199–204.
Roy AC, Loke DF, Saha N, Viegas OA, Tay JS, Ratnam SS. 1994. Interrelationships of serum paraoxonase, serum lipids and apolipoproteins in normal pregnancy. A longitudinal study. Gynecol Obstet Invest 38:10–13.
Sams C, Mason HJ, Rawbone R. 2000. Evidence for the activation of organophosphate pesticides by cytochromes P450 3A4 and 2D6 in human liver microsomes. Toxicol Lett 116:217–221.
Sanghera DK, Aston CE, Saha N, Kamboh MI. 1998. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. Am J Hum Genet 62:36–44.
SAS Institute. 2001. SAS/STAT Guide for Personal Computers, Version 8. Cary, NC:SAS Institute, Inc. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, et al. 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 394:284–287.
Slatkin M. 1999. Disequilibrium mapping of a quantitative-trait locus in an expanding population. Am J Hum Genet 64:1764–1772.
Smolen A, Eckerson HW, Gan KN, Hailat N, La Du BN. 1991. Characteristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase. Drug Metab Dispos 19:107–112.
Taillon-Miller P, Bauer-Sardina I, Saccone NL, Putzel J, Laitinen T, Cao A, et al. 2000. Juxtaposed regions of extensive and minimal linkage disequilibrium in human Xq25 and Xq28. Nat Genet 25:324–328.
Terwilliger J, Ott J. 1994. Handbook of Human Genetic Linkage. Baltimore, MD:Johns Hopkins University Press.