Title
Developmental changes in PON1 enzyme activity in young children and effects of PON1 polymorphisms.

Permalink
https://escholarship.org/uc/item/53x613w7

Journal
Environmental health perspectives, 117(10)

ISSN
0091-6765

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Publication Date
2009-10-01

DOI
10.1289/ehp.0900870

Peer reviewed
The enzyme paraoxonase 1 (PON1; EC 3.1.8.1) detoxifies activated (oxon) organophosphorus pesticides (OPs), which are neurotoxic (Costa et al. 2005a; Furlong et al. 2002). PON1 also is an antioxidant, and several studies indicate that it inhibits oxidation of low-density lipoprotein, a marker of oxidative stress (James 2006; Li et al. 2003). PON1 genotypes and enzyme activities have been associated with numerous health outcomes related to OP exposure and oxidative stress, including Alzheimer’s disease (Erlich et al. 2006), Parkinson’s disease (Zintzaras and Hadjigeorgiou 2004), cardiovascular disease (Bhattacharyya et al. 2008), and preterm birth (Chen et al. 2004; Lawlor et al. 2006). Studies of in utero OP exposures have also demonstrated potential gene–environment interactions with PON1. Berkowitz et al. (2004) found that among pregnant women with measurable levels of chlorpyrifos in their blood, those with lower PON1 levels had children with smaller infant head circumferences at birth. The same research group also reported that higher maternal OP exposure (measured by urinary metabolites) was significantly associated with decreased child birth weight among mothers with the PON1 192QQ genotype (Wolff et al. 2007). PON1 enzyme activities and levels vary broadly in humans (Costa et al. 2005b), and the determinants of PON1 variation, including genetics and age, may further explain the role this enzyme plays in relation to exposures and diseases.

Several common polymorphisms in the coding and promoter regions of the PON1 gene influence substrate-specific PON1 enzyme activities (Brophy et al. 2001; Ferre et al. 2003), particularly single-nucleotide polymorphisms (SNPs) at positions 192 and –108 (James et al. 2005). The SNP at codon 192 results in an amino acid substitution from the active-site residue glutamine (Q) to arginine (R), and the catalytic efficiency of the PON1 192R allele is greater than that of the PON1 192Q allele. Recent studies have demonstrated that the PON1 192Q genotype accounts for a large portion of the variation in PON1 activity toward the OP substrate paraoxon in Caucasian and African-American (59%) adults (Bhattacharyya et al. 2008) and also in a Mexican-American (48%) population (Rainwater et al. 2008). Of the promoter polymorphisms, the PON1 108 SNP exerts the most noticeable effect on arylesterase (AREase) activity, a measure of PON1 quantity (22.4%), such that the C allele is associated with increased PON1 levels compared with the T allele (Brophy et al. 2001; Deakin et al. 2003). Although genetic polymorphisms account for a large portion of PON1 variation, it is not sufficient in epidemiologic studies to consider PON1 genotypes alone (Jarvik et al. 2000; Richter and Furlong 1999). Interindividuality variability of PON1 phenotypes can range widely even among individuals with the same PON1 genotypes because enzyme levels can also be different. Therefore, studies that measure PON1 activities may be more informative than studies that rely solely on PON1 genotype data.

Several animal studies have demonstrated that low PON1 activity during development results in a reduced metabolic capacity in the young and therefore an increased susceptibility to OP toxicity (Moser et al. 1998; Timchalk et al. 2007). Similarly in humans, PON1 levels in infants are much lower than levels in adults (Chen et al. 2005; Holland et al. 2006). Furthermore, preterm babies have 24% lower activities compared with term babies (Ecobichon and Stephens 1973), suggesting that PON1 levels in the fetus may be even lower. A few studies have measured PON1 activities in healthy young children at single points in time (Garcés et al. 2008; Karikas et al. 2006; Sumegeva et al. 2007; Vincent-Viry et al. 2003), but only one study has described the ontogenetic profile of PON1 activity in the same individuals over time. This study (Cole et al. 2003) of nine children demonstrated high inter-individual variation of PON1 from birth to 2 years of age; PON1 activities increased over time and reached a plateau between 6 months and 2 years of age in most, but not all, individuals. PON1 activity is relatively stable in adults (Costa et al. 2005b); however, in the elderly, it decreases steadily over time (Seres et al. 2004). To date, longitudinal changes in PON1 activity in children after 2 years of age
and potential differences between genotypes have not yet been characterized.

Previously, we reported a relatively high level of OP exposure in the Salinas Valley, California, the location of our longitudinal birth cohort (Bradman et al. 2005; 2007). Additionally, we observed associations between in utero OP exposure and decreases in gestational duration (Eskenazi et al. 2004), abnormal reflexes in neonates (Young et al. 2005), poorer mental development at 2 years of age (Eskenazi et al. 2008), and increases in maternal report of pervasive development disorder (Eskenazi et al. 2008) in these children. Initially, we reported PON1 activity in 130 mothers and their newborns and demonstrated much lower activities in the newborns (Furlong et al. 2006; Holland et al. 2006). In the present study, we measured PON1 activity in 458 children at several time points spanning birth through 7 years of age to determine the ontogeny of PON1 phenotypes. In the same children, we also genotyped two PON1 SNPs (–108 and 192) known to influence phenotypic variation and examined how PON1 genotypes affect children’s PON1 activities at different ages.

Materials and Methods

Study subjects. The Center for Health Assessment in Mothers and Children of Salinas (CHAMACOS) study is a longitudinal birth cohort study of the effects of pesticide and other environmental exposures on neurodevelopment, growth, and respiratory disease in children from primarily Mexican-American families (Eskenazi et al. 2003). Located in Monterey County, California, the Salinas Valley is an area of heavy agricultural production where approximately 200,000 kg of OPs are applied annually (Department of Pesticide Regulation 2005). Women and children in this community are at risk for higher exposures to pesticides through direct exposure if they live near agricultural fields or if the mother works in the field, and through the take-home pathway. A total of 601 pregnant women were enrolled in the CHAMACOS study, and 528 delivered newborns. Mothers in the CHAMACOS cohort were primarily young (mean ± SD, 25.6 ± 5.3 years), married, low-income, Mexican-born, Spanish-speaking women who were farmworkers themselves (44%) or lived with farmworkers at the time of enrollment (84%). Ethnicity of children and mothers was based on mothers’ self-report. For this particular study, we limited analyses to include only subjects who were of Mexican descent (97%). More than half the women in CHAMACOS came from three states in Mexico: Michoacán (23%), Guanajuato (21%), and Jalisco (11%). Study protocols were approved by the University of California—Berkeley human subjects review committee. Written informed consent was obtained from all mothers, which included consent for their children to participate.

Blood collection and processing. Blood specimens were collected from 458 CHAMACOS children (50.1% boys) for the measurement of PON1 enzyme activity. Umbilical cord blood samples (n = 336) were collected by delivery room staff once the baby was safely delivered. Blood specimens were also collected from children at approximately 1 year of age (n = 52; mean ± SD, 1.07 ± 0.19 years), 2 years of age (n = 249; 2.01 ± 0.09 years), 5 years of age (n = 225; 5.10 ± 0.22 years), and 7 years of age (n = 281; 7.10 ± 0.23 years). We were able to measure PON1 enzyme activity at four or more time points for 108 of these children.

Harmonized whole blood was collected in BD Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ), centrifuged, and then divided into plasma, buffy coats, and red blood cells and stored at –80°C. Vacutainers without anticoagulant were used to collect serum and clot. DNA was isolated from blood clots as described previously (Holland et al. 2006).

Determination of PON1 levels and activities. We measured PON1 enzyme activity toward three substrates [paraoxon (PO), phenyl acetate (aryl ester; ARE), and chlorpyrifos-oxon (CPO)] in plasma samples using spectrophotometric methods as described previously (Richter and Furlong 1999). The AREase assay is considered a measure of PON1 enzyme quantity because rates of phenyl acetate hydrolysis do not differ between PON1 homozygous Q and R alleles as they do for paraaxon hydrolysis (P0ase). Methods based on ELISA and Western blot using PON1 antibodies confirm a high correlation (r > 0.85) between measured PON1 quantity and AREase activity (Connelly et al. 2008; Kujiraoka et al. 2000). In contrast, P0ase and chlorpyrifos-oxonase (CPOase) substrate-specific assays reflect both quantity and catalytic efficiency of the enzyme. In this article, we refer to enzyme quantity measured by AREase activity as PON1 levels. CPOase and POase activities (quantity and substrate-specific efficiency) are referred to as enzyme activity. All assays were performed in triplicate. Quality assurance, described in more detail by Huen et al. (2009), included assessment of repeat samples (separate aliquots of the same sample run on different days), internal controls (aliquots of the same sample run on all assay plates), and concurrent analyses of specimens from different time points (samples from different time points run on the same plates). Repeated analysis of 3% of samples showed a high degree of concordance. For the three substrate-specific assays, the average coefficient of variation (CV) for repeated samples ranged from 6% to 9%, and the correlation coefficients between repeated runs ranged from 0.91 to 0.98. Interassay variability, as measured by the average CV for internal controls samples, was between 7% and 9%.

Determination of PON1 genotypes. The coding polymorphism, PON1_192, was genotyped using the TaqMan real-time polymerase chain reaction (PCR) method. Briefly, primers for the nucleotide sequence flanking the SNP and probes specific for the SNP were custom designed by Applied Biosystems, Inc. (Foster City, CA). The promoter SNP, PON1_108, was genotyped using a fluorogenic allele-specific genotyping assay (Amplitagfluor; Flowgen Biosciences Ltd., Nottingham, UK). This assay required a two-part nested PCR strategy, where the region surrounding the SNP was preamplified using nonallelic flanking primers. The amplicon was then diluted and used as the template for the Amplifluor assay. Quality assurance procedures for genotyping both PON1 SNPs included assessment of randomly distributed blank samples in each plate and duplicates of randomly selected samples with independently isolated DNA from the same subjects. Repeated analysis (4% of samples) in several runs showed a high degree (> 99%) of concordance. All discrepancies were resolved with additional genotyping.

Statistical analysis. To model longitudinal changes of PON1 enzyme activity, we used generalized estimating equations (GEEs) with an exchangeable correlation structure because we had repeated measures on individual subjects. This method is often advantageous in longitudinal models because inferences based on robust estimators are less dependent on the assumption that the data are normally distributed (Hox 2002). Correlations of measurements on individuals at various ages were similar. We created separate models for each of the three outcomes, AREase, CPOase, and POase activity. Independent variables in the model included age (years), PON1 genotype (either PON1_192 or PON1_108), and an interaction term between age and PON1 genotype (age × PON1_192 and age × PON1_108). Because the number of specimens collected at 1 year of age was much smaller than at other time points, we also ran GEE models without this time point. However, because results did not change significantly, we show only the GEE models using all time points.

We previously established that storage duration can affect PON1 activity, particularly in specimens that had been stored for > 2 years (Huen et al. 2009). PON1 activity decreased on average by 17.1%, 39.4%, and 37.6% for AREase, CPOase, and POase, respectively after 5 years of storage (< 0.001 for all three assays). This decline of PON1 enzymatic activity over time has also been reported in other studies (Brackley et al. 1983; Stenzel et al. 2007). In this study, specimens collected at earlier ages were sometimes stored for > 2 years, so age and storage duration were...
Table 1. PON1 polymorphisms and AREase (U/mL) enzyme activity in children of various age groups.

| Frequency* (%) | Birth (n = 336) | 1 year (n = 52) | 2 years (n = 249) | 5 years (n = 225) | 7 years (n = 281) | Mothers# (n = 130) |
|----------------|----------------|----------------|-------------------|-------------------|------------------|------------------|
|                | Mean ± SD     | Mean ± SD      | Mean ± SD         | Mean ± SD         | Mean ± SD        | Mean ± SD        |
| 192            |               |                |                   |                   |                  |                  |
| QQ             | 23.2          | 21.7 ± 15.5*   | 3.9–70.9          | 36.5–126.6        | 36.6–126.6       | 129.5 ± 36.3     |
| QR             | 53.1          | 52.2 ± 14.4    | 3.6–98.0          | 84.6 ± 25.8       | 85–151.2         | 119.8 ± 29.1     |
| RR             | 23.7          | 36.7 ± 13.0    | 9.7–73.6          | 84.6 ± 30.3       | 77.9 ± 16.9      | 61.1–190.4       |
| –108           |               |                |                   |                   |                  |                  |
| CC             | 28.3          | 42.1 ± 13.0**  | 3.9–90.3          | 95.8 ± 23.8**     | 138.5 ± 30.3     | 163.6 ± 51.0     |
| CT             | 53.1          | 52.0 ± 13.5    | 3.8–94.0          | 83.6 ± 22.7       | 154.3 ± 22.7     | 147.1 ± 43.3     |
| TT             | 18.4          | 23.2 ± 11.1    | 3.9–54.7          | 79.7 ± 13.7       | 126.5 ± 20.4     | 136.3 ± 35.9     |

*Genotype frequency was reported for children with enzyme activity measured at birth. Other time points had similar frequencies. #Measurements of PON1 enzymatic activity were taken in pregnant mothers at 28 weeks’ gestation (Holland et al. 2008). #p < 0.05, **p < 0.01, determined by Cuzick’s test for trend.

Table 2. Summary of PON1 enzymatic activity in children and mothers.

| Age (years) | No. | AREase (U/mL) | Mean ± SD | Range | CPOase (U/mL) | Mean ± SD | Range | POase (U/mL) | Mean ± SD | Range |
|-------------|-----|---------------|-----------|-------|---------------|-----------|-------|--------------|-----------|-------|
| 0           | 336 | 33 ± 14.5     | 3.8–88.0  |       | 2020.2 ± 860.7| 153.9–5252.2|       | 267.1 ± 162.4| 7.5–1017.5|       |
| 1           | 52  | 66.5 ± 30.4   | 3.9–126.6 |       | 3995.1 ± 1885.7| 195–2706.7 |       | 570.0 ± 417.1 | 37.3–1502.7|       |
| 2           | 249 | 87.0 ± 26.1   | 8.5–161.2 |       | 5310.3 ± 1549.8| 4907.9–9474.5|       | 667.3 ± 395.3 | 37.5–1913.1|       |
| 3           | 225 | 84.0 ± 22.3   | 22.4–157.9|       | 5502.4 ± 1424.1| 1433.0–9080.1|       | 732.0 ± 417.1 | 97.6–1855.6|       |
| 7           | 281 | 121.5 ± 30.3  | 14.5–246.3|       | 7035.8 ± 1700.6| 904.2–11576.7|       | 894.2 ± 477.0 | 119.5–2504.5|       |
| Mothers*    | 130 | 149.2 ± 26.1  | 19.8–281.4|       | 9058.3 ± 2794.4| 1661.7–17098.0|       | 1024.2 ± 656.4 | 66.1–3014.2|       |

*Measurements of PON1 enzymatic activity were taken in pregnant mothers at 28 weeks’ gestation as described previously (Holland et al. 2006).
activity at different ages (1.4–3.6%), whereas the promoter polymorphism PON1-108 accounted for 8.2–24.4% of AREase activity [see Supplemental Material, Table 1 (available online at doi:10.1289/ehp.0908087.S1 via http://dx.doi.org)]. Although 4.4% of the variance of CPOase activity was explained by the PON1-192 SNP at birth, the coding region SNP made a seemingly smaller contribution at other time points (0.1–1.2%), and the role of PON1-108 seemed much larger (4.1–27.3%). Both SNPs accounted for a significant portion of PON1 activity variation. The contribution of PON1-192, which was 39.8% at birth, was generally higher in older children and highest at 7 years of age (70.7%).

Effects of age and PON1 genotype on enzyme activity. We used GEE augmentation of linear models to determine the effects of age and genotype on PON1 activity in children (Table 3). Point estimates in the model using storage duration–corrected data were not markedly different from those generated using raw data, although 95% confidence intervals (CIs) were slightly wider in storage duration–corrected models. Because the results for both models were similar, only results from the storage duration–corrected models are shown. Regression lines were generated for AREase, CPOase, and POase activity by age and are plotted with respect to PON1-192 (Figure 1A–C) and PON1-108 (Figure 1D–F) genotypes. All three substrate-specific PON1 enzyme activities were positively associated with age ($p < 0.0001$ for all models). It appeared that PON1 activities continued to increase past the age of five because paired t-tests showed that AREase, CPOase, and POase were all significantly higher in 7-year-olds than in 5-year-olds ($p < 0.0005$).

For AREase activity, there was a small but statistically significant interaction ($p = 0.016$) between age and PON1-192 genotype in which the slopes for age were lowest (8.3 U/mL per year) for RR children and highest (10.3 U/mL per year) for QQ children (Figure 1A). The promoter polymorphism, PON1-108, had a more pronounced effect on AREase activity than did PON1-192. Furthermore, we observed a small but significant interaction ($p = 0.027$) between age and PON1-108 genotype in which mean AREase activity increased 10.0, 9.1, and 8.2 U/mL per year increase in age for CC, CT, and TT children, respectively.

Similar to AREase, CPOase activity was modestly associated ($p = 0.017$) with PON1-192 genotype (Table 4, Figure 1B). Mean CPOase activity was 591.0 U/mL (95% CI, 104.6–1,077.4 U/mL) higher in RR children and 295.5 U/mL (95% CI, 52.3–538.7 U/mL) higher in QR children than in QQ children. There was no interaction between age and PON1-192 genotype. In contrast, we did observe a small but significant interaction between age and PON1-108 genotype ($\beta$-estimate for age $\times$ PON1-108 genotype $= -54.6, p = 0.022$).

We also observed significant effect modification by the PON1-192 genotype ($p < 0.0001$) on the relationship between age and POase activity (Figure 1C). POase activity increased with age for all PON1-192 genotypes, but the slopes for age were much steeper for RR children. For example, mean activity increased by only 21.8 U/L (95% CI, 15.3–28.3 U/L) per year in QQ children, whereas in RR children mean activity increased 119.2 U/L or almost 5.5-fold more per year (95% CI, 97.7–140.9 U/L). The effect is so large that even at 7 years of age the average POase activity in QQ children was lower than the average POase activity in QR and RR children at 2 years of age. Although not as strikingly as the coding polymorphism PON1-192, the promoter SNP, PON1-108, also influenced POase activity significantly (Figure 1F). The effect of age on POase activity was modified by PON1-108 genotype ($p < 0.0001$) such that the slopes for mean POase activity per year were highest in CC children and lowest in TT children.

To determine the effects of combinations of PON1-192 and PON1-108 genotypes on enzyme activity, we performed GEE models comparing children with the PON1-108C/C192R homozygous diplotype (reference group) with those with the PON1-108T/T192Q homozygous genotype. The C192R genotype was significantly associated with all three assays (D–F). CC children had the highest mean activities, whereas TT children had the lowest mean activities. For POase activity, the slopes for CC children were steeper than slopes for CT and TT children (F), demonstrating the significant interaction between age and C192T genotype ($p < 0.0001$).
Children with the PON1<sub>108TT192QQ</sub> diplotype had significantly lower mean AREase (14.1 U/mL; 95% CI, 3.7–24.4 U/mL), CPOase (1525.9 U/L; 95% CI, 835.0–2216.3 U/L), and POase (627.4 U/L; 95% CI, 534.5–720.4 U/L) activities compared with PON1<sub>108CC192RR</sub> children. Additionally, we observed a statistically significant interaction between age and diplotype on POase activity (p < 0.0001). The slope for mean POase activity over age was much steeper for PON1<sub>108CC192RR</sub> children (135.3 U/L per year; 95% CI, 115.0–155.7 U/L) than for PON1<sub>108TT192QQ</sub> children, which was 112.6 U/L per year (95% CI, 91.3–133.8 U/L) lower.

**Effects of age and PON1 genotype on enzyme activity in restricted data sets.** Because the correlation between age and storage duration was appreciably smaller within shorter time intervals, we were able to include a storage duration term (rather than use the storage duration–corrected data) in GEE models where the time interval was restricted to a 1- and 2-year-olds only or b 5- and 7-year-olds only. The results are presented in Table 4. For 1- and 2-year-olds, age was significantly associated with all three substrate-specific measures of PON1 even after adjusting for potential confounding by storage duration (p < 0.001 for AREase and CPOase and p = 0.016 for POase). Further, storage duration was only significantly associated with POase activity (p = 0.04), and the magnitude of the effect (~55.8 U/L) was much smaller than the effect of age (128.0 U/L). Similarly, for 5- and 7-year-olds, age was strongly associated with AREase, CPOase, and POase (p < 0.001) activities, and storage duration was not. For POase, the prominent interaction between age and PON1<sub>192</sub> genotype observed in the storage duration–corrected models was also significant in these models (p = 0.02).

**Correlation analysis.** Correlations of each of the PON1 substrate-specific enzyme activities in children at different time points were highly significant except at 1 year of age, when there was a weak correlation with 7-year-olds and no correlation with cord blood samples at birth. This may be attributable to the relatively small sample size at 1 year of age. Further, because the cord blood measurement accounts for both fetal and placental expression, the correlation between these two time points (birth and 1 year of age) may be weaker than with other age groups. For pairwise comparisons among all other age groups (birth and 2, 5, and 7 years of age), Pearson’s correlation coefficients ranged from 0.37 to 0.62 for AREase and from 0.41 to 0.73 for CPOase assays. POase measurements were even more highly correlated, with correlation coefficients ranging from 0.70 to 0.93 [see Supplemental Material, Table 2 (available online at doi:10.1289/ehp.0900870.S1)]. Within each time point, all three PON1 activities were also significantly correlated with each other (p < 0.001). The correlation coefficients tended to be highest between CPOase and AREase, ranging from 0.84 to 0.97. The pairwise correlation coefficients between POase and CPOase ranged from 0.44 to 0.78. Between POase and AREase, the correlation coefficients were lower, ranging from 0.23 to 0.65 [see Supplemental Material, Table 3 (available online at doi:10.1289/ehp.0900870.S1)].

**Discussion**

In this study, we examined the ontogeny of PON1 phenotypes in a longitudinal birth cohort of 458 children who were followed from birth through 7 years of age. Our data demonstrated a dramatic range of PON1 variability in young children, particularly in newborns. We observed an age-dependent increase in measures of PON1 levels and enzymatic activity that continued through 7 years of age. Furthermore, for POase activity, the rates of increase were allele specific, such that children with PON1<sub>192R</sub> alleles experienced a much more pronounced increase in POase activity over time. Similarly, children with the PON1<sub>108CC192RR</sub> diplotype had significantly higher mean PON1 activities and also experienced steeper increases of POase activity over time compared with children with the PON1<sub>108TT192QQ</sub> diplotype.

**Earlier studies** (Chen et al. 2003; Holland et al. 2006) demonstrated that newborns have lower PON1 levels than adults. Cole et al. (2003) reported that PON1 levels (AREase) and activities (POase) increased noticeably in children during the first year of life and appeared to have plateaued by 2 years of age in some but not all of them. In contrast, our study, which followed a much larger number of children for a longer period of time, demonstrated that children’s PON1 levels and activities continue to increase past 2 years of age until at least 7 years of age. These data suggest that children’s PON1 activities remain lower than their expected adult levels longer than previously thought. Although this phenomenon has not yet been reported in other epidemiologic studies, similar observations in mice have also shown that PON1 levels are low at birth and increase significantly with age (Zemke et al. 2009). Because PON1 can detoxify activated OPs and also has antioxidant properties, our findings provide evidence that the time in which infants and young children remain more vulnerable than adults to OP exposures and oxidative stress may extend from birth through 7 years of age and possibly even later.

In addition to the age-dependent increases in PON1 measures, our data in this large Mexican-American cohort corroborate allele and genotype frequencies of the PON1 SNPs at positions –108 and 192 in other studies of Mexican and Mexican-American populations.

**Table 3. Regression analysis (GEE) of the effects of age and PON1 genotype on PON1 enzyme activity (storage duration–corrected data).**

| genotypes | AREase | CPOase | POase |
|-----------|--------|--------|--------|
|           | β-Estimate | 95% CI | p-Value | β-Estimate | 95% CI | p-Value | β-Estimate | 95% CI | p-Value |
| Age       | 55.3 | 50.8 to 59.8 | <0.0001 | 64.8 | 61.0 to 68.7 | <0.0001 | 64.5 | 58.6 to 70.3 | <0.0001 |
| Genotype  | 10.3 | 9.2 to 11.4 | <0.0001 | 10.0 | 9.1 to 11.0 | <0.0001 | 9.0 | 7.8 to 10.3 | <0.0001 |
| Age × genotype | 0.3 | –3.3 to 3.8 | 0.884 | –9.9 | –13.3 to –6.4 | <0.0001 | –14.1 | –24.4 to –3.7 | 0.008 |
| Age × genotype² | –1.0 | –1.9 to –0.2 | 0.016 | –0.9 | –1.7 to –0.1 | 0.027 | 0.04 | –0.1 to 0.2 | 0.648 |

*PON1<sub>108</sub> was coded 0, 1, and 2 for QQ, QR, and RR genotypes, respectively. The total number of children included in this model was 443. PON1<sub>192</sub> was coded 0, 1, and 2 for CC, CT, and TT genotypes, respectively. The total number of children included in this model was 439. Individuals with the PON1<sub>192RR</sub> and PON1<sub>108CC192RR</sub> diplotype were coded 0, and individuals with the PON1<sub>192QQ</sub> and PON1<sub>108TT192QQ</sub> diplotype were coded 1. The number of children included in this model was 84. Age was expressed in years. Age × genotype refers to the interaction term between age and genotype (PON1<sub>108CC192CC</sub> or PON1<sub>108TT192QQ</sub> diplotype).
(Rainwater et al. 2008; Rojas-Garcia et al. 2005). Consistent with other studies, we found that the Q allele of the PON1\textsubscript{192Q} coding polymorphism was associated with decreased POase activities and that the C allele of the PON1\textsubscript{108C} Promoter polymorphism was associated with increased activity in all three PON1 measures (Deakin and James 2004; Furlong et al. 2005). In transgenic mouse models, the PON1\textsubscript{192Q} alleform does not protect against CPO exposure as well as the PON1\textsubscript{192R} alleform (Cole et al. 2005). Furthermore, mice with lower PON1 levels were more sensitive to OP toxicity as measured by cholinesterase inhibition in the brain (Li et al. 2000). These studies demonstrated that because of their influence on PON1 levels and catalytic efficiency, the PON1\textsubscript{108C} and PON1\textsubscript{192R} genotypes are significant determinants of sensitivity to OP exposure. Furlong et al. (2006) used these data to estimate that the range of sensitivity to CPO in CHAMACOS mothers and their newborns is very wide (1.31- to 164-fold). Although age and PON1 genotype appear to affect this variability significantly, it is likely that other factors such as environmental exposures and additional genetic polymorphisms may account for some of this variability.

To our knowledge, no other studies have examined the effects of common polymorphisms on the ontogeny of PON1 phenotypes in young children. In our study, children with the PON1\textsubscript{192Q} and PON1\textsubscript{108C} alleles experienced much steeper increases in POase activity with age in a dose-dependent manner (dose of allele), indicating significant effect modification by PON1\textsubscript{192} and PON1\textsubscript{108} genotypes. A similar pattern was reported by Parker-Katracek et al. (2008), who found that PON1 expression in murine liver tissue increased during embryonic development in an allele-specific manner.

These data suggest that PON1 ontogeny is influenced by genetic polymorphisms and children with certain genotypes (PON1\textsubscript{192Q} and PON1\textsubscript{108C} alleles) may be more susceptible to the adverse effects of OP exposure and oxidative stress throughout childhood. In addition to PON1 genotypes, PON1 developmental expression may also be affected by prenatal exposure to OPs via epigenetic mechanisms. It will be interesting to determine whether OP exposure may be associated with PON1 ontogeny in future studies.

In our analyses, we performed GEE models using raw data and also using data corrected for storage duration. Previous studies, including our own, have reported a decline in POase, AREase, and diazoxonase activities over time, particularly in samples stored for several years (Brackley et al. 1983; Huen et al. 2009; Stenzel et al. 2007). To adjust for storage duration, we created a correction factor by constructing linear models from a data set of 95 subjects whose PON1 activities were measured at two time points. Although it is possible that the relationship between storage duration and PON1 activity may not be linear, it was the most reasonable assumption to use with only two time points available. This assumption would tend to overestimate the effect of storage duration, thus biasing the effect of age on PON1 activity toward the null. We also assumed that variance in measurements at the two time points was due primarily to storage duration and not other unmeasured factors. Although the storage duration–corrected data introduces a degree of uncertainty into our models, we were still able to identify highly significant age-dependent increases in all three PON1 measures using this data. In addition to the models using storage duration data, we were able to apply GEE models to subsets of the data (smaller time intervals) in which the correlation between age and storage duration was weaker. In these models, where a term for storage duration was included, we confirmed our findings that age is positively associated with PON1 activity after adjustment for storage duration. Despite the potential limitations of our analysis, this is the largest longitudinal study examining PON1 activity during childhood development. Use of a cross-sectional study design in future studies may help to confirm our results while avoiding the potential bias introduced by storage duration effects on PON1 activity.

From our data, it is not yet apparent when children’s PON1 levels and activities reach mature levels. Furthermore, levels did not increase appreciably between 2 and 5 years of age, but then continued to increase between 5 and 7 years of age. It is possible that the approach to puberty may affect levels of this physiologically important enzyme. Compared with younger children, average PON1 levels and activities in 7-year-olds were much closer to those we previously reported in pregnant CHAMACOS mothers (Holland et al. 2006). Whether children with different genotypes reach mature levels at different ages remains unclear. Because this study was performed in a cohort of Mexican-American children with relatively high levels of OP exposure, generalization of the age-dependent increase of PON1 activity to other populations should be done with caution. Future studies of PON1 ontogeny should include children as they grow older (past 7 years of age) and also additional populations.

Our present study provides additional evidence that children with the PON1\textsubscript{192Q} and PON1\textsubscript{108C} genotypes may represent vulnerable populations. This may be of particular

| Table 4. Regression analyses of age, genotype, and storage on PON1 enzyme activity in smaller age intervals. |
| --- |
| 1 vs. 2 years of age\textsuperscript{a} | 5 vs. 7 years of age\textsuperscript{b} | 5 vs. 7 years of age, interaction model |
| **β-Estimate** | 95% CI | p-Value | **β-Estimate** | 95% CI | p-Value | **β-Estimate** | 95% CI | p-Value |
| AREase Intercept | 42.3 | 4.7 to 79.9 | 0.027 | 21.5 | –28.1 to 71.0 | 0.396 | 23.2 | –30.1 to 76.5 | 0.39 |
| Age\textsuperscript{c} | 21.0 | 11.8 to 30.2 | < 0.001 | 14.9 | 8.2 to 21.6 | < 0.001 | 14.6 | 7.1 to 22.1 | < 0.001 |
| PON1\textsubscript{192}\textsuperscript{d} | 4.8 | –8.8 to 0.7 | 0.022 | –5.3 | –9.0 to –1.7 | 0.004 | –7.3 | –27.9 to 13.2 | 0.48 |
| Storage duration\textsuperscript{e} | 1.4 | –4.4 to 7.1 | 0.643 | –3.9 | –12.8 to 4.9 | 0.384 | –3.9 | –12.7 to 5.0 | 0.39 |
| Age × PON1\textsubscript{192} | 0.3 | –3.1 to 3.7 | 0.85 |
| CPDase Intercept | 3197.9 | 879.9 to 5515.9 | 0.007 | 1837.6 | –986.3 to 4605.0 | 0.202 | 2356.9 | –636.1 to 5349.9 | 0.12 |
| Age\textsuperscript{f} | 1288.9 | 712.0 to 1865.9 | < 0.001 | 703.0 | 318.5 to 1087.5 | < 0.001 | 617.4 | 197.9 to 1036.9 | < 0.001 |
| PON1\textsubscript{108} | 130.0 | –582.2 to 438.3 | 0.133 | 188.5 | –13.6 to 393.5 | 0.068 | 412.0 | –1644.2 to 2027.7 | 0.31 |
| Storage duration | -139.5 | –422.2 to 213.3 | 0.438 | –29.4 | –542.0 to 483.2 | 0.911 | –17.4 | –530.5 to 498.8 | 0.95 |
| Age × PON1\textsubscript{108} | 96.7 | –104.0 to 292.8 | 0.34 |
| P0ase Intercept | 280.1 | –804.0 to 460.6 | 0.128 | –133.3 | –528.7 to 262.0 | 0.509 | 76.1 | –332.3 to 485.4 | 0.72 |
| Age\textsuperscript{g} | 128.0 | 23.6 to 232.3 | 0.016 | 67.1 | 13.8 to 120.5 | 0.014 | 32.6 | –23.2 to 88.4 | 0.25 |
| PON1\textsubscript{192} | 412.9 | 366.6 to 459.2 | < 0.001 | 535.3 | 501.6 to 588.9 | < 0.001 | 293.1 | 86.4 to 499.8 | 0.01 |
| Storage duration | –55.8 | –109.0 to –2.6 | 0.04 | 6.8 | 65.3 to 79.0 | 0.853 | 11.7 | –59.9 to 83.2 | 0.75 |
| Age × PON1\textsubscript{192} | 39.0 | 6.7 to 71.3 | 0.02 |

\textsuperscript{a}This model included 52 children 1 year of age and 249 children 2 years of age. Of these children, 35 had measurements at both time points. \textsuperscript{b}This model included 225 children 5 years of age and 281 children 7 years of age. Of these children, 189 had measurements at both time points. \textsuperscript{c}Age was expressed in years. \textsuperscript{d}PON1\textsubscript{192} was coded Q, R, and RR genotypes, respectively. \textsuperscript{e}Storage duration was expressed in years. \textsuperscript{f}Age × PON1\textsubscript{108} refers to the interaction term between age and PON1\textsubscript{108} genotype.
concern in the Mexican-American population, including our CHAMACOS subjects, because both genotypes are relatively common (23.2% QQ, 18.4% TT, and 7.5% QQ and TT). The PON1 QQ, and PON1 QT alleles are also quite frequent in Caucasian populations (73% Q, 62% T) but much less so in African-American populations (27% Q, 15% T) (Chen et al. 2003). Thus, sensitivity to OP toxicity and oxidative stress may vary between populations.

In summary, we followed a cohort of Mexican-American children from a farmworker community from birth through 7 years of age and found that PON1 levels and activities continue to increase past 2 years of age and possibly even past 7 years of age. Additionally, PON1 polymorphisms modify the effect of age on enzyme activity such that some children experience much steeper increases over time than others. These findings suggest that children’s PON1 activities continue to increase until much later than previously believed, and therefore, the window of increased susceptibility to OP exposures and oxidative stress may also last much longer in younger children. Future policies regarding protection from pesticide exposures should take into account that children may be particularly vulnerable to OP exposures and those with certain PON1 genotypes may be at even greater risk because of lower PON1 levels and activities.

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